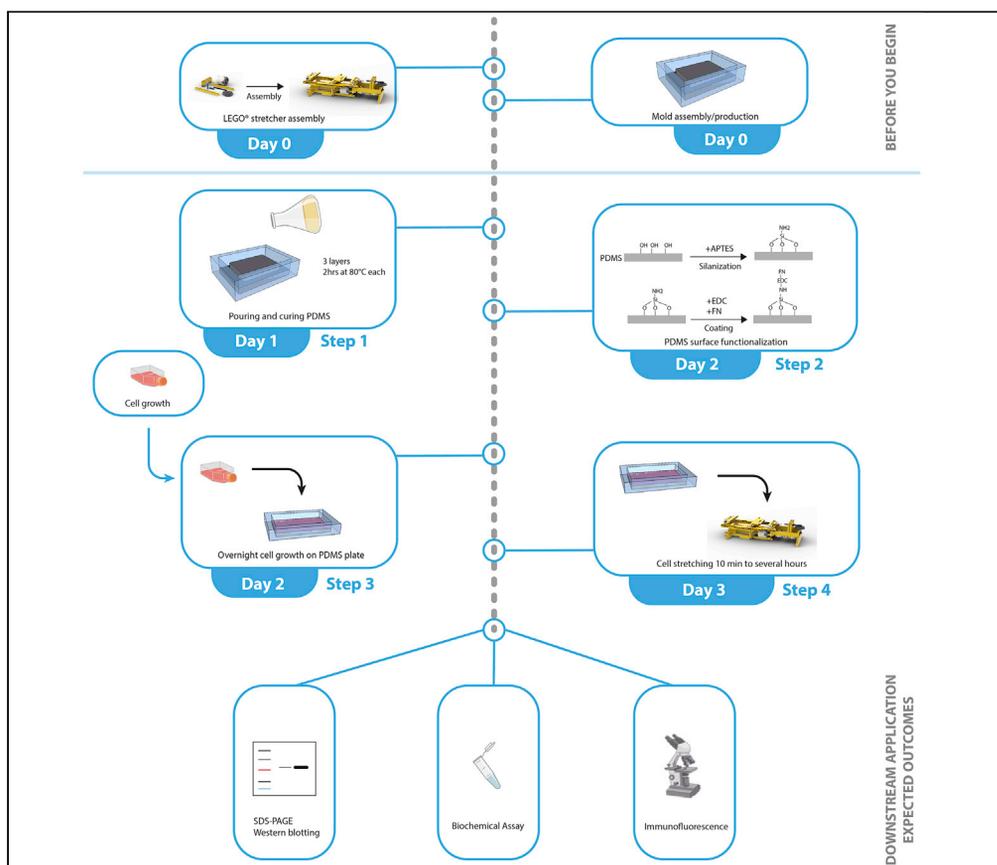


Protocol

Cyclic uniaxial cell stretching in tissue culture using a LEGO®-based mechanical stretcher and a polydimethylsiloxane stretchable vessel



Etienne Boulter,
Chloé C. Féral

etienne.boulter@inserm.fr
(E.B.)
chloe.feral@inserm.fr
(C.C.F.)

HIGHLIGHTS

Protocol to grow cells on a user-generated PDMS vessel

Application of cyclic uniaxial stretch using a LEGO®-based mechanical stretcher

Downstream applications range from fixed cell imaging to protein extraction

Mechanical signals are essential for the regulation of many biological processes. Therefore, it has become paramount to account for these mechanical parameters when exploring biological processes. Here, we describe a protocol to apply cyclic uniaxial stretch on cells in culture using a LEGO®-based mechanical stretcher and a flexible custom-made polydimethylsiloxane culture vessel as well as validated downstream applications. While this system offers an out-of-the-box limited type of simulation, it provides a reliable and low-cost opportunity to perform cell stretching.

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Protocol

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Etienne Boulter^{1,2,*} and Chloé C. Féral^{1,3,*}¹Université Côte d'Azur, INSERM, CNRS, IRCAN, 28 Avenue de Valombrose, 06107 Nice Cédex 2, France²Technical contact³Lead contact*Correspondence: etienne.boulter@inserm.fr (E.B.), chloe.feral@inserm.fr (C.C.F.)
<https://doi.org/10.1016/j.xpro.2021.100437>

SUMMARY

Mechanical signals are essential for the regulation of many biological processes. Therefore, it has become paramount to account for these mechanical parameters when exploring biological processes. Here, we describe a protocol to apply cyclic uniaxial stretch on cells in culture using a LEGO®-based mechanical stretcher and a flexible custom-made polydimethylsiloxane culture vessel as well as validated downstream applications. While this system offers an out-of-the-box limited type of simulation, it provides a reliable and low-cost opportunity to perform cell stretching.

For complete details on the use and execution of this protocol, please refer to Boulter et al. (2020).

BEFORE YOU BEGIN

This protocol provides instructions on how to generate, functionalize, and stretch a PDMS plate associated to a LEGO® based mechanical stretcher. There are a number of cell stretching systems available from vendors and biotech companies or academic investigators (Kah et al., 2020), providing distinctive features but generally built around the same core principles such as the use of PDMS plates or membranes.

Assemble LEGO® mechanical stretcher

⌚ Timing: Estimated one to two hours (Once LEGO® parts are collected)

1. Order LEGO® parts and download assembly booklet
 - a. Order LEGO® parts, using the provided parts list (see supplemental file 1; available at <https://data.mendeley.com/datasets/m594s3s5h7/1>), from the official LEGO® store or an independent retailer (days to weeks).
 - b. Download the assembly booklet (see supplemental file 2; available at <https://data.mendeley.com/datasets/m594s3s5h7/1>) and stretcher model files (see supplemental files 3–5; available at <https://data.mendeley.com/datasets/m594s3s5h7/1>)
2. Assemble LEGO® mechanical stretcher according to instructions from the assembly manual (see supplemental file 2; available at <https://data.mendeley.com/datasets/m594s3s5h7/1>) with help from the Studio 2.0 model files when necessary (see supplemental file 3–5; available at <https://data.mendeley.com/datasets/m594s3s5h7/1>). We estimate the assembly time to be one to two hours per stretcher.



Table 1. Summary of the main features of the LEGO® stretching system as compared to typical commercial/ reported systems

Feature	LEGO® stretcher system	Commercial/other systems
Stimulation type	Uniaxial	Uniaxial or equibiaxial
Stretch frequency	0.2 to 1Hz	0.01 to 5 Hz (uniaxial)
Stretch extent	12%	0.5 to 30% (uniaxial)
Culture Area	40cm ²	Few cm ²
PDMS stiffness	kPa range	MPa range
Cost	150–200€	Up to 50k€

△ **CRITICAL:** During the assembly of the LEGO® stretcher, the critical steps are commented in the assembly booklet. This mostly includes adding the motors at step 80 and gluing the 8-stud axle in the final step (86).

Note: Precise characterization of the mechanical stretcher and presentation of all its features are reported in the original publication. The frequency of the stretch can be adjusted by step increments between 0.2 to 1Hz (Table 1) by changing the combination of wheels of the gear box as indicated in (Boulter et al., 2020). The extent of stretch can also be adjusted by modifying the length of the main arm axle, however, in such case, de novo full characterization of the stretcher motion is required. Troubleshooting 4 and 5

The stretcher itself can be reused indefinitely once assembled. LEGO® parts are made of ABS (acrylonitrile butadiene styrene), a very robust plastic material that can be cleansed and sanitized using cell culture certified cleansing reagents containing ethanol or quaternary ammonium (see KRT). Special attention should be taken not to wet the electrical motors though. Regarding the reliability of the system, we never experienced any failure of any part but, in the unlikely event this would happen, it is possible to replace the defective or broken part. For instance, we have been using the exact same LEGO® motors for the past 6 years.

Prepare and assemble PDMS plate casting mold

⌚ **Timing:** few days

3. Generate PDMS plate casting mold
 - a. The casting mold parts can be generated by milling a plexiglass™ plate upon access to a mechanical workshop (see supplemental file 6–8; available at <https://data.mendeley.com/datasets/m594s3s5h7/1>).
 - b. Alternatively, it is also possible to order or produce casting mold parts by 3D printing using the provided 3D printing templates generated with the CAO App Shapr3D (see supplemental file 9–11; available at <https://data.mendeley.com/datasets/m594s3s5h7/1>)
4. Assemble the casting mold using M2 screws

△ **CRITICAL:** Upon assembly of the casting mold and in order to prevent PDMS leakage, tape the exterior of the casting mold with two rounds of lab tape.

Additionally, the casting molds, at least the plexiglass molds, can be cleaned and re-used indefinitely.

Note: A timing of a few days was indicated but this step can be achieved faster if direct access to a workshop or a 3D printer is available in which case a few hours should be enough. The longer timing takes into account ordering, production, and shipping when ordering from a remote supplier.

The authors only used plexiglass molds and never experienced 3D printing of these molds. Nevertheless, 3D printing now emerges as a very efficient alternative to other methods which, we thought, was worth mentioning. If the end user decides to use 3D printing, we would strongly advise to consider some of the potential drawbacks and limitations of this technology, and seek expert advice regarding choice of printing technology, material, etc. On this note, we would advise against using Fused Deposition Modeling (FDM) which cannot withstand required curing temperature.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Dow SYLGARD™ 184	Farnell	GMID 01673921
Fibronectin from bovine plasma	Sigma-Aldrich	Cat#F1141
3-Aminopropyltriethoxysilane (APTES)	Thermo Fisher Scientific	Cat#80370
1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)	Thermo Fisher Scientific	Cat#22980
DPBS	Thermo Fisher Scientific	Cat#14190-094
Paraformaldehyde 16% EM grade	EMS	Cat#15710
Sucrose	Sigma-Aldrich	Cat#S0389
DMEM	Thermo Fisher Scientific	Cat#21969-035
Penicillin-streptomycin	Thermo Fisher Scientific	Cat#15140-122
L-Glutamine	Thermo Fisher Scientific	Cat#25030-024
FBS	Thermo Fisher Scientific	Cat#10270-106
Experimental models: cell lines		
HeLa	ATCC®	Cat# ATCC® CCL-2™
Software		
Shapr3D (iOS App for CAO)	Shapr3D Zrt.	https://www.shapr3d.com
BrickLink Studio 2.0 (LEGO® design)	BrickLink	https://www.bricklink.com/v2/build/studio.page
Tracker	NA	https://physlets.org/tracker/
Other		
LEGO® cyclic uniaxial cell stretcher	(Boulter et al., 2020)	https://jcs.biologists.org/content/133/1/jcs234666
PDMS casting mold	(Boulter et al., 2020)	
PHAGO'SPRAY DASR	Phagogene	Cat#65049
Autoclave resistant tape	Sigma-Aldrich	Cat#BR61750-1ROLL
MiniVac/ScanVac evaporator system	LaboGene	Cat#7.008.100.011
Mini-PROTEAN Short Plates (x5)	Bio-Rad	Cat#1653308
Mini-PROTEAN Glass Plates (x5)	Bio-Rad	Cat#1653312
22 x 60 mm #1 coverslips	Menzel Glaser	
Supplemental files	Mendeley Data	https://doi.org/10.17632/m594s3s5h7.1

MATERIALS AND EQUIPMENT

Cell complete growth medium		
Reagent	Final concentration	Amount
DMEM Cat#21969-035	NA	450 mL
Penicillin-streptomycin Cat#15140-122	100 U/mL	5 mL
L-glutamine Cat#25030-024	2mM	5 mL
FBS Cat#10270-106	10%	50 mL

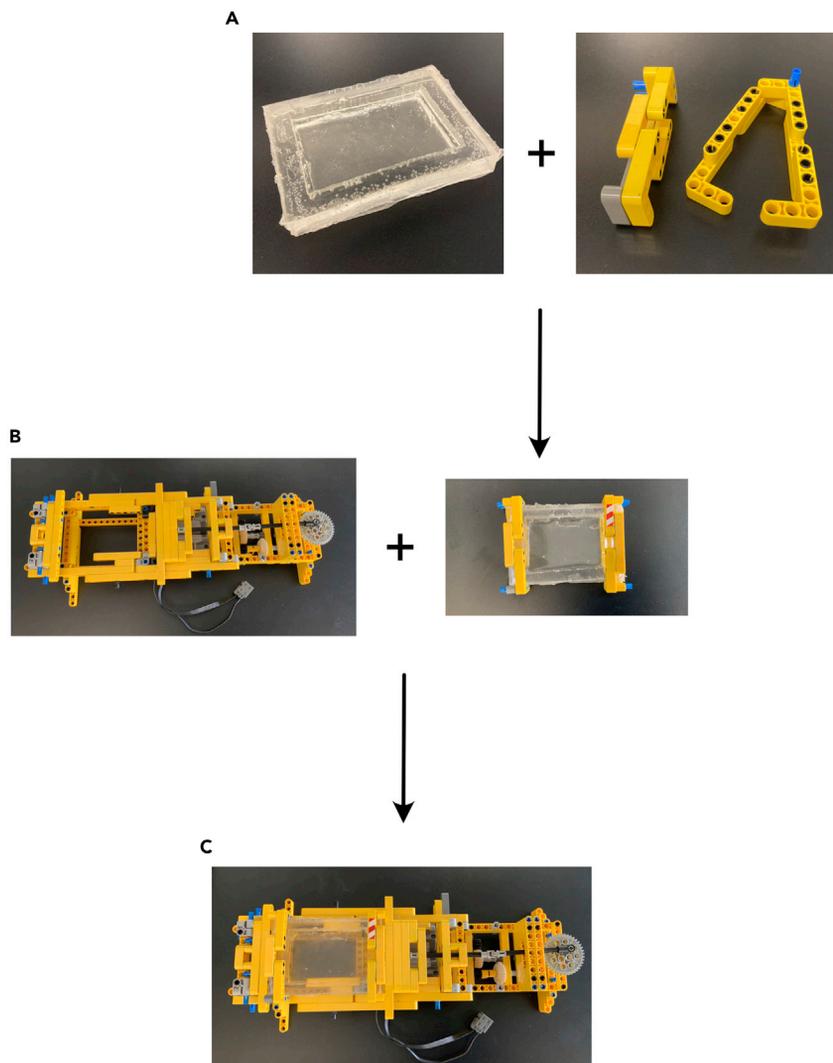


Figure 1. Overview of the cyclic uniaxial cell stretching system

(A) stretchable PDMS plate and its LEGO® adaptors.

(B) Mechanical stretcher and its PDMS plate with adaptors.

(C) System fully assembled.

STEP-BY-STEP METHOD DETAILS

Generation of the PDMS vessel

⌚ Timing: One day

The goal of this step is to generate the PDMS plate that will be fitted on the LEGO® mechanical stretcher (Figure 1). It will be used to grow and stretch cells. The PDMS plate is constituted of three layers of PDMS that are poured and cured successively, upside-down.

1. Pour and cure bottom layer.
 - a. Prepare 15 mL of a 1:20 (w:w) ratio mixture of curing agent to PDMS for each PDMS plate to be cast. Mix extensively either using a magnetic bar and a magnetic stirrer or alternatively, manually using a clean plastic L-shaped bacterial spreader.

- b. Degas the mixture for 10 min under vacuum at 190mbar. We routinely use a vacuum concentrator in vacuum mode only, at room temperature.
 - c. Pour the mixture in the casting mold, avoiding bubbles and taking care not to pour over the central plate of the casting mold which will constitute the bottom of the PDMS plate.
 - d. Place horizontally in an oven and cure at 80°C for 2 h.
2. Pour and cure intermediate layer. This layer will constitute the bottom of the plate in contact with cells. The investigator should experimentally assess the stiffness required for its own experimental setup and we provide the protocol for a standard 1:40 ratio mixture.
 - a. Prepare 40 mL of a 1:40 (w:w) ratio mixture of curing agent to PDMS for each PDMS plate to be cast. Mix extensively either using a magnetic bar and a magnetic stirrer or alternatively, manually using a clean plastic L-shaped bacterial spreader.
 - b. Degas the mixture for 10 min under vacuum as previously.
 - c. Pour the mixture in the casting mold over the previous layer avoiding creating bubbles. This layer should cover the central plate.
 - d. Place horizontally in an oven and cure at 80°C for 2 h.
3. Pour and cure top layer. This thin layer is only present to prevent the stickiness of the intermediate layer
 - a. Prepare 5 mL of a 1:20 (w:w) ratio mixture of curing agent to PDMS for each PDMS plate to be cast. Mix extensively either using a magnetic bar and a magnetic stirrer or alternatively, manually using a clean plastic L-shaped bacterial spreader.
 - b. Degas the mixture for 10 min under vacuum as previously.
 - c. Pour the mixture in the casting mold over the previous layer avoiding creating bubbles. This layer should cover the central plate.
 - d. Place horizontally in an oven and cure at 80°C for 2 h.
4. Disassemble the casting mold and peel the PDMS plate off the base plate. Such a thick PDMS plate is quite resistant to tear, however, pay attention and caution to the corners of the PDMS plate when peeling off to prevent tearing (Figure 2G).

⚠ **CRITICAL:** It is critical to mix properly and extensively the PDMS mixture since the two components are quite viscous and do not mix well. Improper mixing will result in heterogeneous mixture that will create zones of different stiffnesses. It is particularly critical to ensure the casting mold is placed horizontal in the oven. Use a bubble level to check horizontality if required. It is also preferable to avoid making bubbles when pouring the liquid mixture in the casting mold. Proceed continuously and slowly when pouring. Finally, as indicated pay extra-caution to the corners of the PDMS plate when peeling off as these may tear upon excessive pulling (Figures 2E–2G).

⏸ **Pause point:** It is possible to pause the protocol at any step of the PDMS plate production.

Note: On the choice of PDMS formulation and associated stiffness, we have tested formulations ranging between 1:40 to 1:60 (w:w) ratios and we can certify that those allow stretching of the PDMS plate by the mechanical stretcher. Softer formulations (1:n>60) would most likely allow stretching as well although we cannot certify them since we have not tested them. Additionally, investigators should figure out which stiffness would be appropriate to conduct their experiment. Parameters include organ of origin of the cells and its physiological stiffness, experimental assessment of the behavior and fate of the cells on various PDMS surfaces, etc.

In case of use of a 3D printed mold, the user should also verify that the material used for the mold will not have any adverse effect on the experiment such as cytotoxicity, autofluorescence, etc.

Functionalization and coating of the PDMS plate

⌚ **Timing:** 1 h

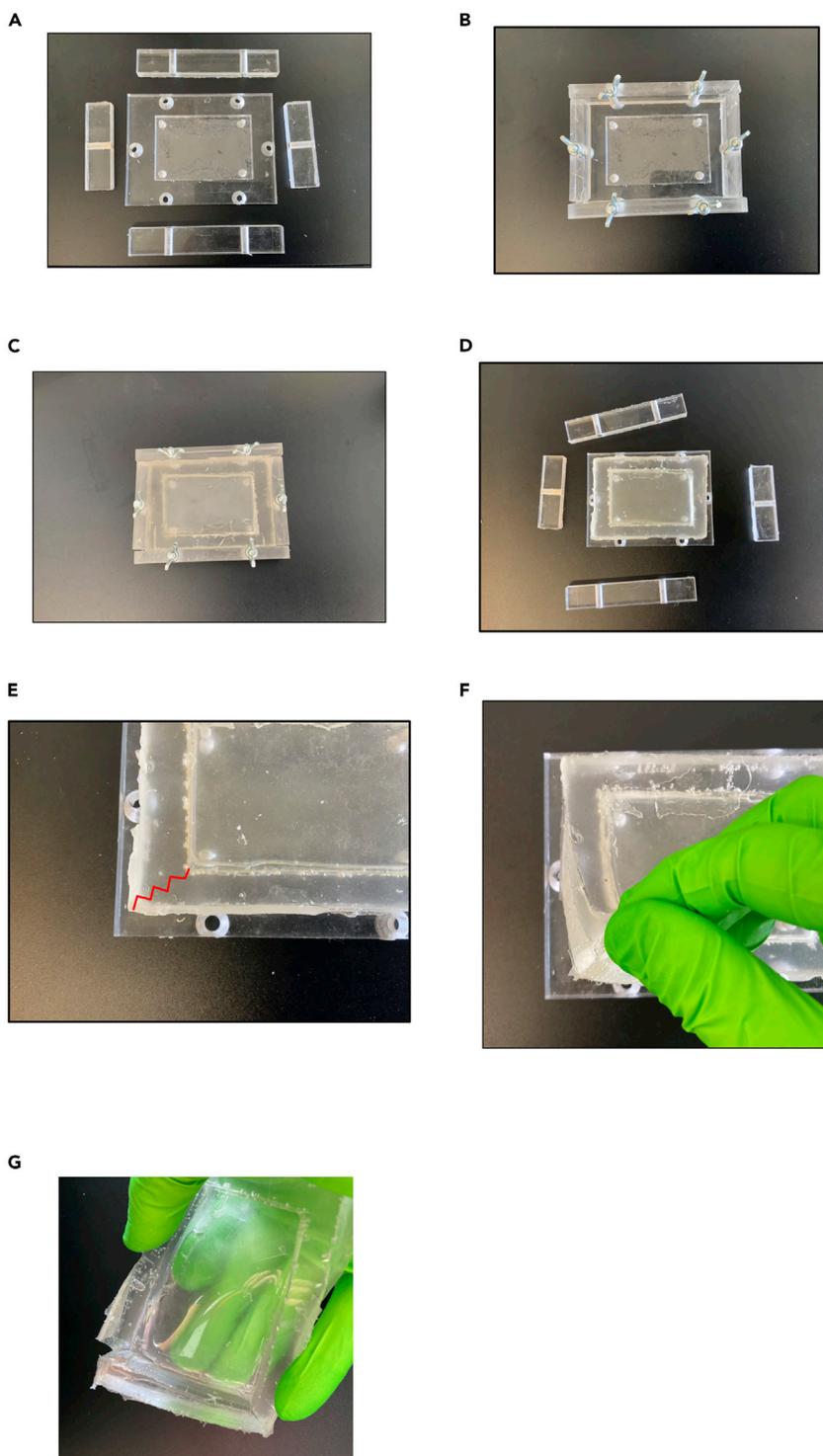


Figure 2. PDMS plate casting mold assembly and PDMS plate casting

(A) PDMS casting mold disassembled.

(B) Casting mold assembled.

(C) PDMS casting mold with fully cured PDMS plate still assembled.

(D) disassembled casting mold with PDMS plate.

(E) zone of potential corner rupture upon excessive pulling during PDMS plate unmolding.

(F) unmolding of the PDMS plate.

(G) example of ruptured PDMS plate.

The goal of this step is to chemically functionalize and coat the surface of the PDMS plate with an extracellular matrix (ECM) protein, fibronectin in this particular case, or any other adhesion molecule for the cells to attach to the culture surface. The functionalization and coating should be carried out just prior to cell seeding.

5. Silanization of the surface of the PDMS plate. All manipulations should be carried out under a chemical hood and waste disposed of properly through appropriate chemical waste.
 - a. Prepare 2 mL of a 20% dilution of APTES in absolute ethanol per plate under a chemical hood. Cover the surface of each PDMS plate with this solution and incubate for 5 min. Remove excess of solution and dispose in chemical waste. Wash twice with 4 mL of absolute ethanol.
 - b. Air dry the PDMS plate in a chemical hood
6. Coating of the surface of the PDMS plate with fibronectin
 - a. Prepare 3 mL of a 10 $\mu\text{g}/\text{mL}$ of fibronectin from bovine plasma in PBS containing 0.05% EDC (w/w).
 - b. Immediately add the solution to the surface of the silanized PDMS plate and incubate for 30 min at room temperature.
 - c. Wash twice with PBS. Cover with PBS until the plate is used.

Optional: At this step, it is possible to irradiate the PDMS plates with germicidal UV for 30 min in order to prevent bacterial contamination.

Note: All manipulations with APTES should be carried out under a chemical hood.

The functionalization of the PDMS plate should be carried out just prior cell plating.

▣▣ Pause point: It is not recommended to pause at this point, the functionalized PDMS plates should be used immediately and should not be stored for future use.

Cell growth on the PDMS plate

⌚ **Timing:** 12 h

The goal of this step is to grow cells on the PDMS plate prior to stretching.

7. Cell splitting
 - a. We routinely use HeLa cells or fibroblasts. Wash cells with PBS on the tissue culture plate.
 - b. Incubate cells with trypsin EDTA (0.05%) for a few minutes at 37°C. Check cell rounding and detachment under a microscope.
 - c. Harvest cells in complete medium (see recipe in Material and Equipment section).
8. Cell seeding on the PDMS plate
 - a. The surface of one PDMS plate is half (40 cm^2) that of a 100 mm petri dish. Plate the required number of cells on the PDMS plate in a maximum of 4 mL of complete growth medium. We recommend cells to be 90% confluent at the time of stretching so seed the cells accordingly. For HeLa cells, we would recommend seeding 4 to 5 million cells 12 h prior to stimulation. For immunofluorescence on individual cells, 500 000 cells would constitute a starting point. [Troubleshooting 1](#) and [2](#)

Note: In this example, we used epithelial HeLa cells but other cell types can be used such as fibroblasts (NIH 3T3 and murine dermal fibroblasts have been used successfully). Some cell types turned out to be more difficult to use such as bovine aortic endothelial cells for instance.

It may be challenging to manipulate the PDMS plate out of the incubator. We routinely use SDS-PAGE glass plates to accommodate the PDMS plate. We use one clean large glass plate as a support for the PDMS plate and a small plate as a lid ([Figure 3](#)).

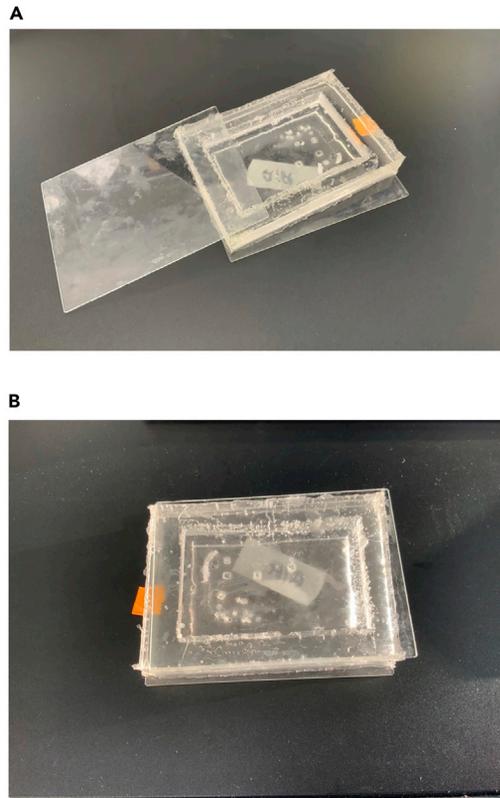


Figure 3. Practical handling of the PDMS plate for cell culture
(A and B) The PDMS plate is handled between two glass plates.

Cell stretching on the mechanical stretcher

⌚ Timing: Variable from several minutes to a few hours

The goal of this step is to mount the PDMS plate on the LEGO® mechanical stretcher and to apply cyclic uniaxial stretch to the cells.

9. Fit the LEGO® adaptors to the PDMS plate ([Methods video S1](#))
 - a. Carefully adjust the jaws of the adaptors on the PDMS plate as shown in [Figure 4](#) and [Methods video S1](#).
 - b. Firmly press the two parts against each other and insert the pins as indicated.
 - c. Repeat the procedure with the other adaptor
10. Mount the PDMS plate on the mechanical stretcher using the LEGO® adaptors ([Methods video S2](#))
11. Perform cyclic uniaxial cell stretching ([Methods video S2](#)).
 - a. Place the stretcher in the incubator ([Figure 4F](#)).
 - b. Plug the battery box.
 - c. Switch the system on to start stretching, monitor time. [Troubleshooting 3](#)

EXPECTED OUTCOMES

The expected outcome of the stretching experiment is a cellular response that includes, depending on the duration of the stimulation, cell reorientation, cell adhesion complexes reorganization and

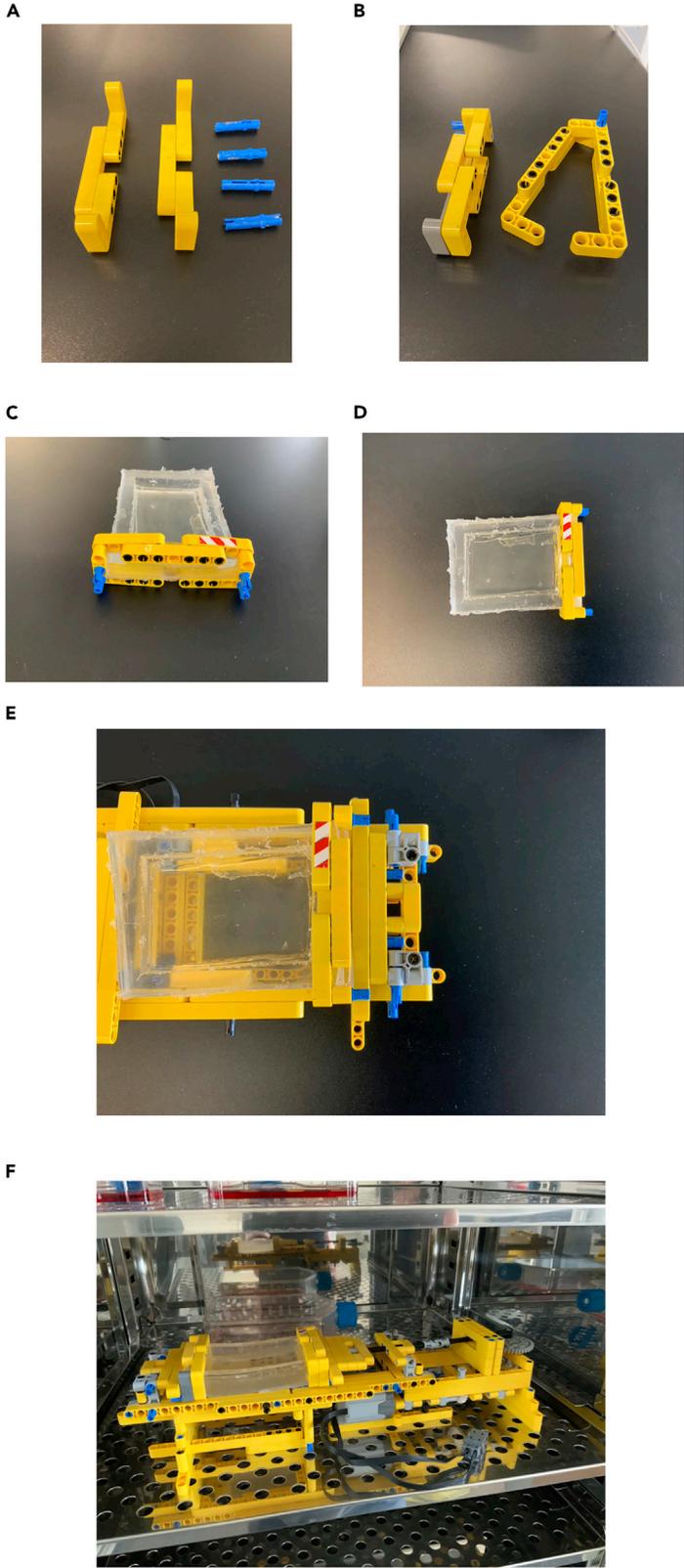


Figure 4. Preparation of the PDMS plate for mechanical stretching

- (A) individual elements required to assemble one adaptor.
- (B) partially assembled adaptor.
- (C and D) sPDMS plate with one adaptor attached to one side.
- (E) fully fitted PDMS plate attached to the mechanical stretcher via the adaptor.
- (F) entire system ready to stretch in a CO₂ incubator.

mechanical signaling pathway activation. Here, we will describe a few assays or techniques that we validated and can be used to monitor the outcome of the experiment.

Cell lysis and biochemical assay or Western blotting

Following cell stretching, cells can be lysed directly on the PDMS plate to either perform a biochemical assay such as a GST-RBD pull-down assay or undergo Western blotting. To monitor the outcome of the experiment we routinely monitor RhoA activity using a GST-RBD pull-down assay (Boulter et al., 2020)

Fixed cell imaging

Alternatively, cells can be fixed directly on the PDMS plate prior to permeabilization and staining. We previously performed cell fixation on the PDMS plate using a solution of paraformaldehyde 3.7%, sucrose 100 mM in PBS for 30 min at 37°C. Other fixation protocols will require to be validated. It is then possible to perform cell staining like on glass coverslips. The bottom of the PDMS plate is cut out from the PDMS plate and can be chopped into smaller pieces for immunostaining. The PDMS piece is turned upside-down and immunostaining is done directly at the surface of the PDMS according to the user's protocol. At the end of the immunostaining, the piece of PDMS is mounted directly on a 22 × 60 mm #1 coverslip using any mounting medium (we routinely use mowiol). The coverslip can then be observed and imaged on an inverted wide-field or confocal microscope.

When performing immunofluorescence staining, we recommend cutting out the whole bottom of the PDMS plate. It is still possible to reuse the PDMS plate by casting a new bottom surface with adequate formulation.

Nucleic acid purification

We did not test DNA or RNA purification at this point, therefore, we cannot advise on this assay.

LIMITATIONS

While there are a number of advantages associated with LEGO® building such as design versatility or parts availability, this type of design also harbors some inherent limitations due to the nature of LEGO® bricks themselves. In addition, the specific design of the stretcher which was intended to be as simple, cheap, and easy to use as possible may also contribute to some of its limitations. Generally speaking, the stimulation parameters of the stretcher are within the range of that of commercially available systems but remain restricted to a fraction of this range. For instance, the form of stimulation remains limited to a sine wave while it is much more versatile in commercial systems, the range of frequency can only be adjusted by step increments between 200 mHz and 1 Hz (as compared to 0.01 to 5 Hz for commercial systems), etc. (Table 1).

Another limitation comes from the PDMS plate which stiffness can only be adjusted within a limited range (1:40 to 1:60 w:w curing agent to PDMS ratio). This is mostly due to the design of the PDMS plate which requires some extensive structural support and a specific stiffness range to both prevent its unintended deformation at rest and allow its extension upon stimulation.

It is however noteworthy that this possibility to slightly adjust the stiffness of the plate remains an exclusivity from our system that is usually unavailable in commercially available systems. [Troubleshooting 4](#) and [5](#)

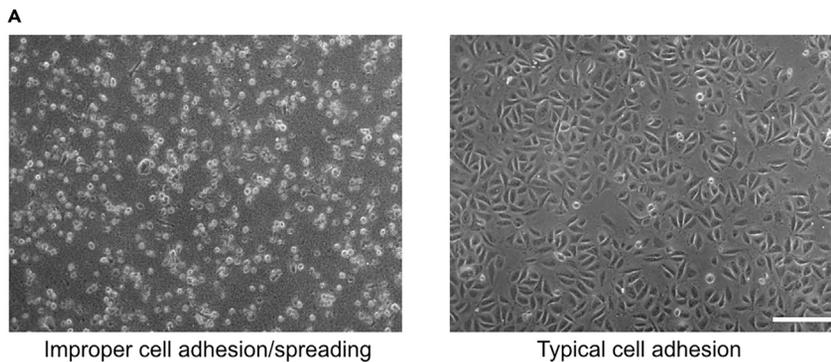


Figure 5. Examples of proper/improper attachment or spreading to the PDMS plate
scale bar is 100 μm .

TROUBLESHOOTING

Problem 1

Issue with cell adhesion.

The usual symptom is that cells are sitting at the bottom of the plate but are not attached to the surface which can be tested by slightly shaking the plate sideways to observe if cells are immobile (Figure 5). This issue most likely arises from improper surface coating which prevents cells from adhering to the PDMS plate. It is not to be confused with defective cell spreading which may rise from other issues.

Potential solution

If surface coating has previously been successfully achieved at such a concentration of surface protein, this most likely means that this particular coating was faulty. From our experience this is generally due to the decay of the crosslinker which is a highly reactive species. We suggest trying again with a new batch of crosslinker.

Problem 2

Issue with cell spreading.

This is not to be confused with defective cell adhesion although it may initially look the same under the microscope. Here, the cells are attached to the bottom of the plate but fail to spread properly.

Potential solution

This issue may arise from the stiffness of the plate that is incompatible with proper cell spreading. This is a complicated issue to fix since it is highly dependent on the inner properties of the cells and adjustments are limited. We would generally suggest increasing stiffness by decreasing the curing agent to PDMS ratio to 1:40.

Alternatively, it is also possible that the surface coating was carried out with a concentration of coating protein too low resulting in extreme ligand spacing preventing cell spreading. Try increasing protein concentration.

If the ligand concentration has been successfully used before, the issue may arise from the activation of the PDMS surface. It is then possible to activate the PDMS prior to silanization using an O₂ plasma cleaner.

Problem 3

No measurable cellular response to mechanical stretching.

It may happen to have no measurable cellular response at the end of the stretching experiment.

Potential solution

First, we noticed that different cells may have different response amplitude to stretch, for instance, we obtained strong and reliable RhoA activation in HeLa cells while this response was fainter in fibroblasts. There is no definitive guide to these cell-to-cell differences, but this should be taken into consideration and experienced by the end user. Also, the stimulation regimen may require some adjustments in order to obtain the appropriate cellular response, such as stretch frequency or amplitude. This can be achieved by modifying the gearbox or liftarm length.

Problem 4

The stimulation parameters need to be adjusted to fit the user's requirements.

Potential solution

It is possible to adjust both the frequency and potentially the stretch extent of the device. To change the frequency of the stimulation, the gearbox can be modified to adjust the frequency by step increments between 0.2 and 1 Hz (Boulter et al., 2020). It is more complicated to adjust the extent of stretch. This requires modifying the length of the main arm by replacing an axle by a longer or shorter axle. One possibility is to build different stretchers with different axle length. Alternatively, one can change the main arm to replace it with a different pre-assembled arm. This procedure is more intricate because some parts of the main arm need to be glued together, therefore individual parts cannot just be swapped and a full new arm needs to be preassembled. [Methods videos S3](#) and [S4](#) present the procedure to remove and replace the main arm. Please note that while the gearbox modifications are very reproducible, main arm modifications generally require de novo characterization of the stretcher.

Problem 5

Modifications of the stretcher have been carried out and it needs to be characterized or calibrated. Alternatively, the user may want to perform a quality control check on the performances of its device.

Potential solution

The stretcher needs to be calibrated by characterizing its motion. Using a permanent black ink marker, the user needs to add a dot on top of the main arm as a marker (see [Methods video S1](#)). A second dot can be added on a steady part of the stretcher as control for vibrations. Add a PDMS plate on the stretcher and operate the stretcher. Position a ruler by the side of the stretcher to calibrate distances. Video record the stretcher in action from the top in order to record the movement of the dots (such as in [Methods video S1](#)). Download the free Tracker tool (see KRT). Load the movie to the Tracker software. Calibrate distances using Trajectories>New>Calibration Tools>Calibration ribbon, measure the distance on the ruler using the ribbon and enter the actual measurement and unit. Define the tracking dot: Trajectories>New>Point Mass, shift-click on the dot to track. Open the autotracker feature, control-shift-click on the dot to track. Adjust tracking parameters and click search to automatically track the motion of the dot. From the motion curves, determine frequency and stretch extent.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Chloé C. Féral (chloe.feral@inserm.fr).

Materials availability

The LEGO® cyclic uniaxial cell stretcher and PDMS casting molds plans and assembly booklet are provided with this article or available directly from the authors with latest updates.

Data and code availability

No code was developed. Supplementary File are available at <https://data.mendeley.com/datasets/m594s3s5h7/1>.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xpro.2021.100437>.

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AUTHOR CONTRIBUTIONS

Conceptualization, methodology, investigation, writing, resources, and supervision: E.B. and C.C.F.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

Boulter, E., Tissot, F.S., Dilly, J., Pisano, S., and Féral, C.C. (2020). Cyclic uniaxial mechanical stretching of cells using a LEGO® parts-based

mechanical stretcher system. *J. Cell Sci.* 133, jcs234666.

Kah, D., Winterl, A., Přečková, M., Schöler, U., Schneider, W., Friedrich, O., Gregor, M., and Fabry, B. (2020). A low-cost uniaxial cell stretcher for six parallel wells. *HardwareX* 9, e00162.