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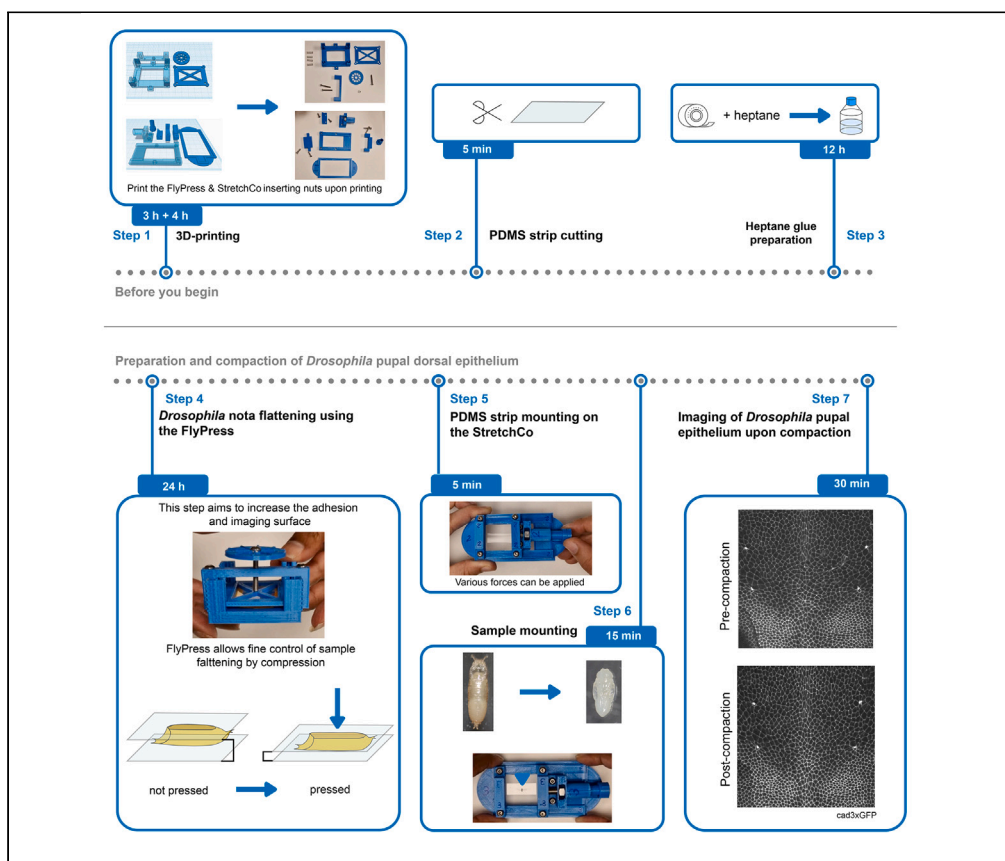
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Protocol

Applying mechanical forces on *Drosophila* tissues *in vivo* using the StretchCo, a 3D-printable device



Applying mechanical forces to tissues helps to understand morphogenesis and homeostasis. Additionally, recording the dynamics of living tissues under mechanical constraints is needed to explore tissue biomechanics. Here, we present a protocol to 3D-print a StretchCo device and use it to apply uniaxial mechanical stress on the *Drosophila* pupal dorsal thorax epithelium. We describe steps for 3D printing, polydimethylsiloxane (PDMS) strip cutting, and glue preparation. We detail procedures for PDMS strip mounting, tissue compaction, and live imaging upon force application.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Mélanie Gracia,
Bénédicte M.
Lefèvre, Raquel
Güell Alonso, ...,
Damien Cuvelier,
Allison J. Bardin,
Yohanns Bellaïche

allison.bardin@curie.fr
(A.J.B.)
yohanns.bellaiche@curie.
fr (Y.B.)

Highlights

Production of
StretchCo and
FlyPress using 3D
printing

Steps for flattening
and mounting of
Drosophila pupae on
the StretchCo

Procedure for dorsal
notum compaction

Live imaging of
epithelial tissue upon
compaction

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Protocol

Applying mechanical forces on *Drosophila* tissues *in vivo* using the StretchCo, a 3D-printable device

Mélanie Gracia,^{1,4,6} Bénédicte M. Lefèvre,^{2,4,6} Raquel Güell Alonso,² Victoire Cachoux,¹ Maria Balakireva,¹ Boris Guirao,¹ Damien Cuvelier,³ Allison J. Bardin,^{2,5,*} and Yohanns Bellaïche^{1,5,7,*}

¹Institut Curie, Université PSL, Sorbonne Université, CNRS UMR3215, INSERM U934, Genetics and Developmental Biology Department, Polarity, Division and Morphogenesis lab, 75005 Paris, France

²Institut Curie, Université PSL, Sorbonne Université, CNRS UMR3215, INSERM U934, Genetics and Developmental Biology Department, Stem Cells and Tissue Homeostasis lab, 75005 Paris, France

³Institut Curie, Université PSL, Sorbonne Université, CNRS UMR144, Cell Biology and Cancer Department, System Biology of Cell Polarity and Cell Division lab, 75005 Paris, France

⁴These authors contributed equally

⁵Senior author

⁶Technical contact

⁷Lead contact

*Correspondence: allison.bardin@curie.fr (A.J.B.), yohanns.bellaiche@curie.fr (Y.B.)
<https://doi.org/10.1016/j.xpro.2024.102851>

SUMMARY

Applying mechanical forces to tissues helps to understand morphogenesis and homeostasis. Additionally, recording the dynamics of living tissues under mechanical constraints is needed to explore tissue biomechanics. Here, we present a protocol to 3D-print a StretchCo device and use it to apply uniaxial mechanical stress on the *Drosophila* pupal dorsal thorax epithelium. We describe steps for 3D printing, polydimethylsiloxane (PDMS) strip cutting, and glue preparation. We detail procedures for PDMS strip mounting, tissue compaction, and live imaging upon force application. For additional details on the use and execution of this protocol, please refer to Cachoux et al. (2023)¹ from which the StretchCo machine has been derived.

BEFORE YOU BEGIN

In our previous articles,^{1,2} we explored the role of mechanics on tissue development by applying forces on the *Drosophila* notum through a PDMS strip. This step follows an initial flattening of the tissue, which increases the tissue adhesion to the PDMS strip used later to apply the forces.

In our previous set-up, we manually flatten pupae and relied on a metallic device, initially used with cell cultures,^{3–7} to change the dimension of the PDMS strip and thus exert forces on the tissue.

To democratize the use of our protocol and make it more accessible, we designed two new devices: the FlyPress, to gain precision in the flattening and increase survival and reproducibility, and the StretchCo, which is derived from the original metallic device previously used (Table 1). We choose to use 3D-printing to produce these two devices, as it offers cheap, easy-to-produce and versatile solutions. Both devices have been designed using Tinkercad (Autodesk, available online at <https://www.tinkercad.com/>) and optimized for printing on Fused Deposition Modeling (FDM) 3D-printers.

Note: if you do not have 3D-printer in the lab, most FabLabs and various companies offer 3D-printing services.



Table 1. Comparison between the StretchCo and the metallic device used in Cachoux et al., 2023

| Parameter | StretchCo | Metallic device used in Cachoux et al., 2023 | Comment |
|-------------------------------|-----------|--|---|
| Machine design | +++ | +++ | The design of the StretchCo has been improved to ease its use (i.e., the StretchCo is stable when positioned upside-down, and the stage adaptor (SC7) eases its fitting on the microscope stage). |
| Ease to produce | ++ | - | The StretchCo can be printed with FDM printers or can be ordered by various companies and FabLabs. |
| Screws | +++ | ++ | Standard millimetric screws are used in the StretchCo whereas it is not the case in the previous machine. |
| Reproducibility of the device | +++ | ++ | 3D-printing guarantees a good reproducibility, less depending on the person producing the machine. |
| Durability of the device | ++ | +++ | The plastic can delaminate due to aging of the device or printing defects. |
| Resistance to humidity | ++ | ++ | The metallic machine is sensitive to rust whereas the PLA is sensitive to humidity mostly before printing. |
| Versatility | +++ | + | Stl files can be modified as long as they are used following the CC BY-NC-SA 4.0 license. |
| Precision | ++ | +++ | The precision of the imposed compaction was similar with the two machines. The gradation of the metallic device is more precise. The StretchCo cap (SC6) could be redesigned to accommodate more gradation. |
| Stretching / Compaction | +++ | +++ | We can stretch and compact samples in a similar manner with the two machines. |

The protocol below describes the steps to first produce the two devices by 3D-printing and then to uniaxially and ectopically compact the pupal epithelium of the *Drosophila* dorsal thorax using them.

3D-printing of the flattening device, FlyPress

⌚ Timing: 3 h

The FlyPress (Figures 1A–1C) allows a fine controlled compression of a sample, by applying forces on it using a coverslip. We describe here how to print the different parts of the FlyPress device, designated by “FP” and their corresponding number hereinafter, and then demonstrate its use to flatten the dorsal thorax epithelium.

1. Configure your slicer software to obtain the G-code files used for printing the FlyPress device on an FDM printer (Data S1). We used Creality slicer software (version Cxsw3d, available at <https://www.creality.com/pages/download-ender-3>).
 - a. All the parts can be printed at the same time. Orient the middle fixed part (FP3) upside down to ease the printing (Figure 1C).

Note: In our hands, printing with a layer width of 0.16 mm, an infill of 100%, and without a support gives satisfying results with an Ender-3 printer (Creality, Open Source 3D printer) and Er-yone blue polylactic acid (PLA). The temperature of the nozzle and the bed were set at 210°C and 42°C, respectively. The speed was set at 80 mm/s.

- b. Depending on your printer, either set a pause on the slicer, or pause the machine manually at 3.08 mm and 3.84 mm height, in order to insert one M3 hexagonal nuts in the hole of the middle fixed part (FP3), and two M3 hexagonal nuts in the base part (FP1), respectively (Figure 1C, yellow arrows).

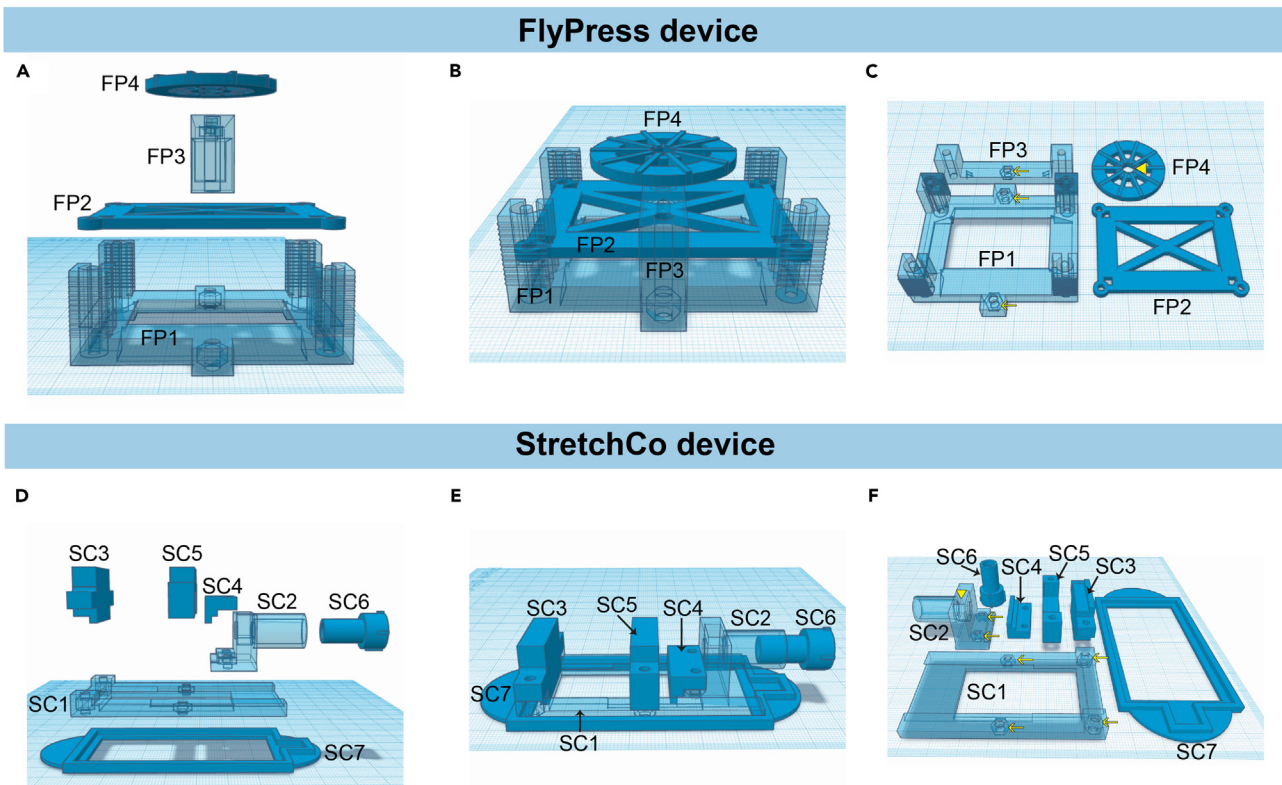


Figure 1. Plans of the FlyPress and StretchCo 3D-printed machines

(A–C) Plans for the FlyPress with 3 different views: disassembled (A) assembled (B) and oriented for 3D-printing on an FDM printer (C). The FlyPress is composed of a base part where the pupae will be attached on a coverslip (FP1), a support associated to a 2nd coverslip to apply pressure on top of the pupae (FP2), a middle fixed part (FP3) and a wheel (FP4). Yellow arrow: holes for the M3 hexagonal nuts inserted during printing; yellow arrowhead: hole of the wheel.

(D–F) Plans for the StretchCo with 3 different views: disassembled (D) assembled (E) and oriented for 3D-printing on a FDM printer (F). The StretchCo is composed of a base part (SC1) associated to a mobile part (bottom: SC2 and top: SC4) and a lateral fixed part (SC3) between which the PDMS strip will be attached, a middle fixed part (SC5), and a stretching / compressing screw cap (SC6). A microscope stage adaptor (SC7) can be added, if needed. Yellow arrow: holes for the M3 hexagonal nuts inserted during printing; yellow arrowhead: hole for M6 hexagonal nut. See also [Methods videos S1](#) and [S2](#).

△ CRITICAL: If the nuts are inserted too early in their holes, a shock with the nozzle can occur, leading to a shift and a failure of the printing. More details are given related to this issue in the [troubleshooting 1](#).

2. The M3 hexagonal nut in the wheel (FP4) can be inserted in the corresponding hole after printing ([Figure 1C](#), yellow arrowhead).

3D-printing of custom-made compaction device, StretchCo

⌚ Timing: 4 h

The StretchCo ([Figures 1D–1F](#)) allows stretching and/or compaction of a PDMS strip, and subsequently, of potentially any sample glued on it. We first describe here how to print the different parts of the StretchCo device, designated by “SC” and their corresponding number hereinafter, and then how to use it to compress epithelial tissue.

3. Configure your slicer software to obtain the G-code file used for printing the custom-made compaction device on a FDM printer ([Data S1](#)).

- a. Orient the middle fixed part (SC5), the lateral fixed part (SC3), and the top mobile part (SC4) upside down to ease the printing of holes for screws (Figure 1F).

Note: Except for the mobile part (SC2), all the pieces can be printed without a support. We found that printing with a layer width of 0.16 mm and an infill of 100% gives good results with an Ender-3 printer (Creality) and Eryone blue PLA.

- b. Depending on your printer, either set a pause on the slicer or alternatively pause the machine manually at 3.7 mm height of the base piece (SC1) and of the mobile part (SC2) to insert four (SC1, yellow arrow, Figure 1F) and two (SC2, yellow arrow, Figure 1F) M3 hexagonal nuts in the corresponding holes, respectively.

△ CRITICAL: If the nuts are inserted too early in their holes, a shock with the nozzle can occur, leading to a shift and a failure of the printing. More details are given related to this issue in the troubleshooting 1.

Note: if the edges of the mobile part and the base part where the PDMS strip will be positioned are too sharp and damage it, sand them slightly or apply a small amount of silicon adhesive to make it smoother.

4. After printing, insert the M6 hexagonal nut (SC2, yellow arrowhead, Figure 1F) holding the stretching / compacting screw in the mobile part (SC2, Figure 1F).

Note: It can additionally be glued in the cavity to avoid it turning upon screwing.

PDMS strips preparation

⌚ Timing: 5 min

5. Cut the 15.2 cm by 15.2 cm PDMS sheets into 3.5 cm by 1 cm strips.
6. Store the cut strips of PDMS in a close box to avoid dust deposition.

Heptane glue preparation

⌚ Timing: 12 h

7. Incubate around 1 m of double-sided tape, cut in small pieces, into 30 mL of heptane, at least 12 h.
8. Remove the tape and conserve the liquid glue in a glass bottle, at 20°C–25°C.

Note: After 6 months the heptane glue will be too thick and will need to be renewed.

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|------------------------------|---|
| Experimental models: Organisms/strains | | |
| <i>D. melanogaster</i> : Ecad:3xGFP | Pinheiro et al. ⁸ | N/A |
| Chemicals, peptides, and recombinant proteins | | |
| Heptane | VWR International S.A.S | 24551290 |
| Silicone adhesive | WPI | Kwik-Sil |
| Software and algorithms | | |
| Creality slicer software for Ender-3 (version Cxsw3d) | Creality | https://www.creality.com/pages/download-ender-3 |

(Continued on next page)

Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|--|-------------------------|---|
| Tinkercad | Autodesk | https://www.tinkercad.com/ |
| Other | | |
| Ender-3 printer (open-source 3D printer) | Creality | Ender-3 |
| Polylactic acid (PLA) 1.75 mm spool | Eryone | Amazon, B075GNKRJPZ |
| 11 x M3 hexagonal nuts | Baban | Amazon, B075CKYJF6 |
| 3 x M3 20 mm screws | Baban | Amazon, B075CKYJF6 |
| 2 x M3 16 mm screws | Baban | Amazon, B075CKYJF6 |
| 2 x M3 12 mm screws | Baban | Amazon, B075CKYJF6 |
| 2 x M3 8 mm screws | Baban | Amazon, B075CKYJF6 |
| 1 x M6 hexagonal nut | PRO Torx | Amazon, B0B39BSZX5 |
| 1 x M6 45 mm screw | PRO Torx | Amazon, B0BRGHGT1M |
| 4 x springs of 0.3 × 4 × 10 mm | Creeya | Amazon, B08KD5Q2P7 |
| Polydimethylsiloxane (PDMS) X4 sheet | Gel-Pak | PF3-60-X4 |
| Double-sided tape | Scotch | 34-8724-1398-3 |
| 24 mm × 40 mm coverslips #1 | Dutscher | 100037 |
| Dumont #5 fine forceps | Dumont | #5 |
| Scissors | FST | 15000-02 |
| 10 μ L pipet tips | VWR International S.A.S | 613-3548 |

STEP-BY-STEP METHOD DETAILS

Flattening of the *Drosophila* pupae

⌚ Timing: 24 h

The flattening of the pupae, using the 3D-printed FlyPress device, will ensure a proper adhesion and visualization of the epithelium by allowing a larger surface contact between the pupae and the PDMS strip.

1. Mounting of the FlyPress (Methods video S1).
 - a. Mounting of the wheel.
 - i. Insert an M3 hexagonal nut into the hole of the wheel (FP4, yellow arrowhead, Figure 1C).
 - ii. Place a 20 mm M3 screw into the M3 hexagonal nut inserted in the wheel (FP4) and tighten it fully.
 - iii. Secure it by screwing a 2nd M3 hexagonal nut onto the M3 screw.
 - b. Screw the M3 screw from the wheel (FP4) into the middle fixed part (FP3, Figure 1C), until the base of the screw comes through the lower part of the middle fixed part.
 - c. Place springs in each of the four corners of the base part (FP1) of the FlyPress.
 - d. Attach a 24 mm × 40 mm glass coverslip on the lower part of the support used to press the sample (FP2, Figure 1C) and another one on the central part of the base part (FP1, Figure 1C), using single-sided tape.
2. Preparation of the pupae.
 - a. Place a strip of double-sided tape on the coverslip attached to the base part (FP1) of the FlyPress.
 - b. Collect multiple, 0 h After Pupa Formation (APF) white pupae and place them on the double-sided tape (Figure 2A), dorsal side facing up. To limit developmental defects caused by non-homogenous flattening, see troubleshooting 2.
3. Proper flattening.
 - a. Gently place the support (FP2, Figure 1C), in the base part, using the four corners as guides. Stop when touching the springs.
 - b. Screw the middle fixed part (FP3) into the base part (FP1, Figure 1C), with two 20 mm M3 screws.
 - c. Screw the wheel (FP4), so that the tip of the screw touches the support part without pushing on it (FP2, Figure 1C).

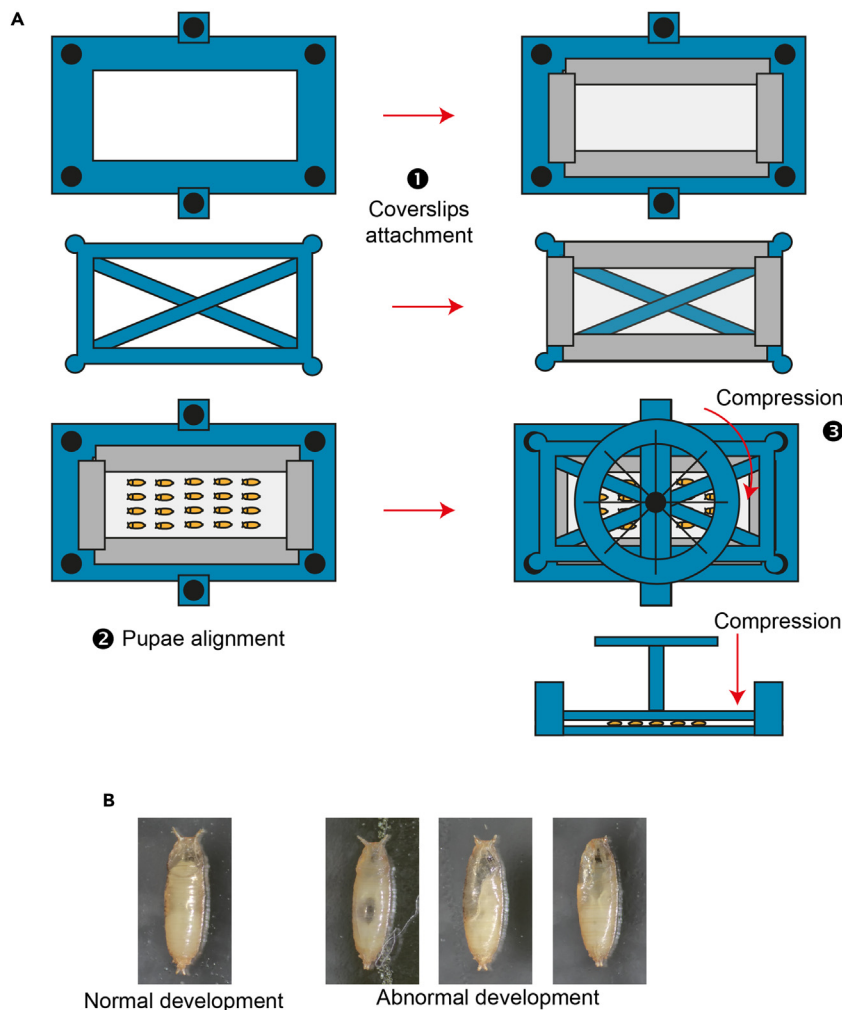


Figure 2. Flattening of the developing *Drosophila* pupae

(A) Scheme showing the flattening of multiple pupae using the FlyPress. 1) Coverslip attachment: first, coverslips are attached to the base part (FP1) and support (FP2) of the FlyPress with single-sided tape. 2) Pupae alignment: then, pupae are aligned on a double-sided piece of tape placed on the base part (FP1) of the FlyPress. 3) Compression: finally, the support part (FP2) is applied on top of the align pupae and the wheel (FP4) rotation allow the compression of the pupae as shown on the lateral view.

(B) Images of different pupae after flattening, presenting normal (left) or abnormal development (right). See also [Methods video S1](#).

- d. Starting from now, count 7.5 turn of the wheel (FP4) to allow a proper flattening of the *Drosophila nota*.

Note: On its top the middle fixed part (FP3) has two arrow heads that will help you, along with the wheel spokes, to count the turns of the wheel (FP4). Each spoke can be colored differently to further assist this.

- e. Maintain the compression during 24 h at 18°C.

Note: When you do not use the FlyPress, it is better not to keep the springs in a compressed conformation.

Alternatives: To use an alternative method without the 3D-printed device FlyPress, please refer to Cachoux, Balakireva et al., 2023.¹

Table 2. Correspondence between number of turns of the M6 stretching / compaction screw and PDMS strip stretching / compaction for a PDMS strip of 35 mm long

| Compaction or stretching of the PDMS strip (%) | Number of turns with a M6 screw (1 mm / turn) |
|--|---|
| 2.8 | 1.00 |
| 5 | 1.75 |
| 10 | 3.50 |
| 15 | 5.25 |
| 20 | 7.00 |
| 25 | 8.75 |
| 30 | 10.50 |
| 35 | 12.25 |
| 40 | 14.00 |
| 45 | 15.75 |
| 50 | 17.50 |

Mounting of the StretchCo device with a PDMS strip

⌚ Timing: 5 min

4. Mounting of the StretchCo ([Methods video S2](#)).
 - a. Mounting of the main screw: M6 screw, which will drive compaction or stretching.
 - i. Place the 45 mm M6 screw in the screw cap (SC6, [Figure 1F](#)).

Note: The screw cap can additionally be secured with glue.

- ii. Then, screw it in the hexagonal M6 nut of the mobile part (SC2, [Figure 1F](#), yellow arrowhead).

Note: The tip of the M6 screw should be aligned with the edge of the mobile part (SC2).

- b. Slide the mobile part (SC2) in the grooves of the base part (SC1, [Figure 1F](#)).

Note: Depending on the stage of your microscope, the 3D-printed stage adaptor can be used to stabilize the device (SC7, [Figure 1F](#); [Methods Video S2](#)).

5. Attaching a PDMS strip on the StretchCo device.
 - a. Using forceps, carefully remove the two layers protecting the PDMS strip.
 - b. Place the PDMS strip between the lateral part of the base (SC1) and the mobile part (SC2, [Figure 1F](#)).

Note: Make sure to align the PDMS strip between the two parts as much as possible, to position it in the center and at 1 or 2 mm from each edge.

- c. Screw the lateral fixed part (SC3, [Figure 1F](#)) onto the base part (SC1) with two 12 mm M3 screws. If needed, reposition the PDMS layer on the mobile part (SC2) to have it well-aligned.
 - d. Screw the top mobile part (SC4, [Figure 1F](#)) on the mobile part (SC2) with two 8 mm M3 screws.
 - e. Screw the middle fixed part (SC5) in the base part (SC1) with two 16 mm M3 screws ([Figure 1F](#)).
6. Pre-stretch the attached PDMS to 150% by screwing the 45 mm M6 screw to its maximum value (17.5 turns, [Table 2](#)).

Mounting of the flatten *Drosophila* pupae

⌚ Timing: 15 min

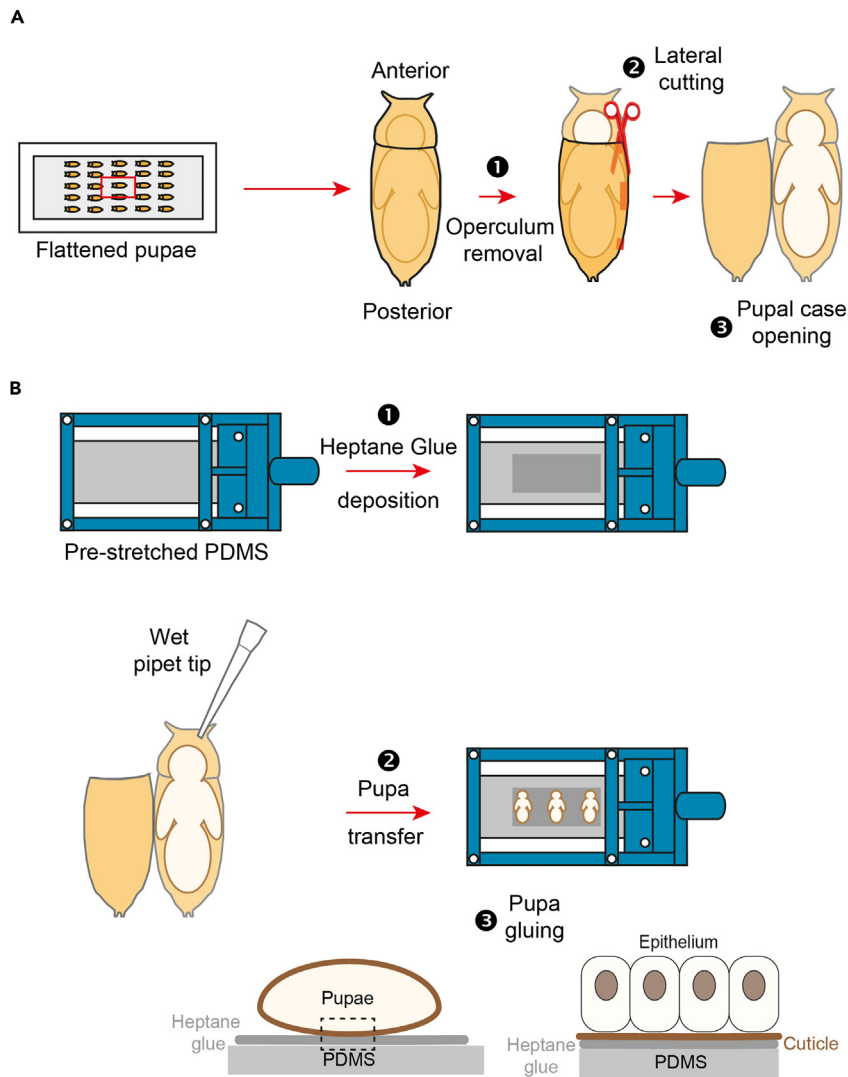


Figure 3. Preparation of *Drosophila* pupae and mounting on the StretchCo

(A) Preparation of *Drosophila* pupae for the mounting on the StretchCo. Right: Scheme of the flattening coverslip, presenting multiple flattened pupae (dark yellow). Left: Removal of the pupal case of one of the flattened pupae (red squared). First, the operculum is removed (1), then the pupal case is cut laterally (2). After that, the upper pupal case can be opened to give access to the pupa (3).

(B) Scheme presenting the different steps of the mounting of pupa on the StretchCo. First, heptane glue is deposited on the pre-stretched PDMS (1). Then, the prepared pupa is moved with a wet pipet tip (2). Then, the cuticle of the pupa will be glued on the PDMS (3). See also [Methods video S2](#).

After the flattening, the pupa will be removed from the pupal case and directly glued on the PDMS layer using heptane glue.

△ **CRITICAL:** Most flattened pupae are properly dorsally oriented and do not display defects in development (75% of the pupae survive to give adults flies; $n = 170$), use these for the live-imaging experiment and discard those showing developmental defects ([Figure 2B](#); [Methods video S2](#)).

7. Removal of the pupal case under a binocular microscope ([Figure 3A](#); [Methods video S2](#)).

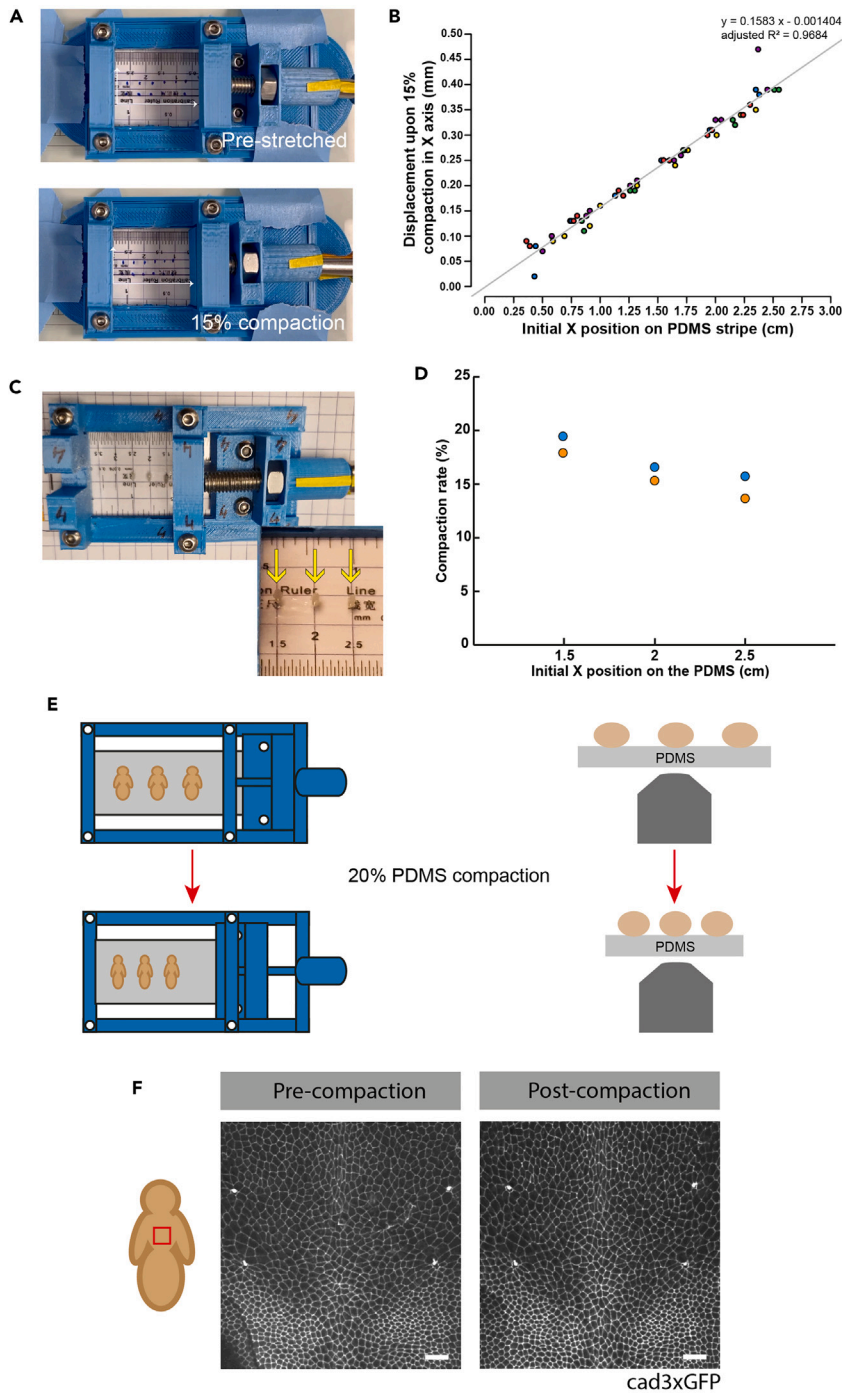


Figure 4. PDMS response and Compaction of the *Drosophila* developing nota

(A) To evaluate force dissipation in the PDMS strip, dots were drawn at various positions and their position was recorded in reference to the indicated referential (white arrows) at the initial stage (pre-stretching of 20%; top panel) and at 15% of compaction (bottom panel), relatively to the initial stage.

(B) Upon 15% compaction, displacement in X in function of the initial X. Dot behavior was recorded from 5 independent experiments indicated by different colors. Gray lines correspond to linear regressions and were obtained using the $\text{lm}(Y \sim X)$ function in R.

(C) Image showing the grid used to assess the positioning of the pupae on the PDMS with pupae positioned at 1.5, 2 and 2.5 cm from the mobile part (SC2) of the StretchCo.

Figure 4. Continued

(D) Upon 20% PDMS compaction, the rate of compaction of the tissue was measured for pupae positioned at 1.5, 2 and 2.5 cm from the edge of the mobile part. Color codes correspond to the number of replicate (n = 2).

(E) Scheme presenting the compaction process on three pupae in upper view (top) and lateral view (bottom), with the objective positioning.

(F) Time-lapse images of a cadherin:3xGFP notum taken before (pre-compaction) and after (post-compaction) 20% compaction of the PDMS. Scale bar: 20 μ m. See also [Methods video S3](#).

Note: To ease this process, work directly on pupae attached to the coverslip on the FlyPress base (FP1).

- a. With fine forceps, remove the operculum to reveal the pupal head.
 - b. With sharp scissors, cut laterally the pupal case from anterior to posterior on one side.
 - c. Open the upper pupal case laterally by grabbing the cut end and pulling it toward the other side to reveal the pupa.
 - d. Repeat the process to be able to mount up to 5 pupae at once.
8. Gluing of the pupa on the PDMS ([Figure 3B](#); [Methods video S2](#)).
- a. Put 1 μ L of heptane glue in a line in the center of the PDMS layer.

⏸ Pause time: Before placing the pupa on the glued PDMS, wait a few seconds for the glue to dry a bit and to avoid the pupae from sliding.

- b. Move the pupa from the flattening slide to the PDMS by grabbing it gently by the head with a small pipet tip.

Note: You can slightly wet the pipet tip to increase its adherence on the pupae head.

⚠ CRITICAL: The deformation of the PDMS strip along its length is not homogenous, resulting in differences of sample movement upon force application ([Figures 4A and 4B](#)). The compaction of the PDMS will be more efficiently applied on the pupae if there are glued closer to the mobile part ([Figures 4C and 4D](#)). For details, see [troubleshooting 3](#).

- c. Place the pupa on the glued PDMS, dorsal side facing the PDMS.

Optional: Gently apply pressure on the ventral side of the pupa to ensure a proper gluing.

Imaging of the *Drosophila* dorsal epithelium upon compaction

⌚ Timing: 30 min

When glued to the PDMS, the *Drosophila* nota will be compacted by releasing the pre-stretching of the PDMS and live imaging can be performed using an inverted confocal microscope to follow the development. We use an Inverted Spinning disk Wide Borealis Confocal Microscope - CSU-W1 (Andor/Roper/Nikon) using a 40x NA 0.95 Air dry PL APO objective, and an inverted Spinning disk Wide Confocal Microscope- CSU-W1 (Roper/Nikon/GATACA) using a 40X dry objective (40x/0,65 DIC M/N2 PLAN NGC).

9. Fix the StretchCo device to the stage of an inverted confocal microscope.

Optional: Take a pre-compaction image of the glued pupa before starting the compaction ([Figure 4F](#), left).

10. Progressive compaction of the nota and imaging.
 - a. Screw the 45 mm M6 screw by 1 turn, corresponding to 2.8% ([Table 2](#)).
 - b. Adjust the position of the stage to visualize the pupa after movement of the PDMS and take a new image of the pupa.

- c. Repeat the previous two steps (steps 7a-b) 7 times, to progressively release the pre-stretching of the PDMS, and ensure a 20% PDMS compaction (Table 2; Figures 4E and 4F; Methods video S3).
- d. When the desired level of compaction is reached, launch a time series to follow tissue development under maintained mechanical constraints.

Note: We use as control, pupae flattened and glued on the pre-stretched PDMS without compaction (omitting step 7).

EXPECTED OUTCOMES

Our protocol delineates how to apply a 20% compaction on the *Drosophila* pupal notum with the StretchCo device while monitoring tissue behavior by live-imaging, as illustrated in Figure 4F and Methods video S3. This example, as a proof-of-concept, shows that the StretchCo can be used to apply tissue deformation *in vivo*. Furthermore, the device can be used to stretch samples. Upon tissue deformation, the dynamics of the tissues can then be monitored with high temporal and spatial resolution, over several hours, making it possible to explore the effects of mechanical forces on long timescales as shown in our previous publications.^{1,2} Since it is built on the use of a PDMS layer to deform the tissue, it could be easily adapted to numerous samples. Altogether, the StretchCo device paves the way for a large range of experiments to explore the biomechanics of tissues *in vivo*.

LIMITATIONS

The StretchCo design has been designed to be compatible with inverted microscopes. Its utilization, therefore, might not be possible with upright microscopes without adaptation of the original design.

The set-up imposes the use of objectives with a high working distance as the samples are separated from the objectives by silicon glue and the PDMS layer. In addition, we advise the preferential use of dry objectives: water or oil objectives will cause unwanted displacements of the PDMS along the Z axis. These factors limit the objective magnification used and the kind of sample that can be imaged. Also, due to PDMS optical properties, the refractive index of the PDMS might vary between the pre-compaction and post-compaction leading to optical aberrations.

TROUBLESHOOTING

Problem 1

Premature insertion of nuts in the printing pieces can cause nozzle shift leading to print failure.

Proper insertion of the nuts upon printing is crucial to obtain a working device. This insertion must be done before the deposition of the PLA layer closing the hole, but as late as possible, as the premature insertion will lead to a nut overhanging the printing piece. This overhanging can lead to a clash between the nozzle and the nut, that can cause a shift in the nozzle and a failed print.

Potential solution

If you can set your printer to pause automatically at the correct level, you can therefore adjust this level if the suggested one does not work for you. Indeed, the correct level will depend on the thickness of your first layer.

If your printer cannot be set to pause automatically, then carefully monitor the printing progression. After pausing and inserting the nut, monitor upon resuming printing that the nut does not overhang too much on the top. If it is the case, stop your printer, remove the nut with forceps and resume printing up to the next layer. If you slightly missed the last layer before nut hole closing, you can pause the printer and try to gently remove the plastic closing the hole to insert the

nut in it, and then resume the printing. This last option may cause minor printing defects without impairing the use of the device.

Problem 2

Non-homogeneous flattening of the *Drosophila* can cause developmental defects.

The flattening of the pupae is essential for the compaction to be applied. Pupae need to be flattened enough to have a proper contact surface with the PDMS, but excessive flattening will lead to developmental defects.

Potential solution

As the pupal size can vary between individuals, you can adjust the position of the pupae on the coverslip attached to the base part (FP1) of the FlyPress depending on the size of the pupae. Small ones should be placed more in the center of the coverslip since the flattening will be higher there.

Problem 3

Position-dependent variation in sample compression.

In our set-up, PDMS deformation upon mechanical constraints can be non-homogeneous along its length. Thus, depending on their position along the PDMS strip, the samples undergo slightly different displacement (Figures 4A and 4B).

Potential solution

The range of PDMS deformation increases with the position along the length of the PDMS strip. However, the deformation at a given location is reproducible (Figures 4C and 4D). Monitoring the initial position of the samples on the PDMS strip or mounting the samples at a defined position could thus be a mean to implement the desired PDMS deformation.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yohanns Bellaïche (yohanns.bellaiche@curie.fr).

Technical contact

Further information about technical aspects of the protocol can be addressed to the technical contacts: Mélanie Gracia (melanie.gracia@curie.fr) or Bénédicte Lefèvre (benedicte.lefevre@curie.fr).

Materials availability

All reagents generated in this study are available from the [lead contact](#) without restriction.

Data and code availability

The protocol includes all the datasets generated and analyzed in this study. Data is available from the [lead contact](#) upon request. Stl files (Data S1) have been provided as a zip archive with the submission and are usable following the Creative Commons license CC BY-NC-SA 4.0.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xpro.2024.102851>.

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AUTHOR CONTRIBUTIONS

M.G., B.M.L., V.C., M.B., B.G., A.J.B., and Y.B. designed the project. M.G., B.M.L., and D.C. developed experimental methods. M.G., R.G.A., and B.M.L. performed the experiments and analyzed the data. M.G., B.M.L., A.J.B., and Y.B. wrote the manuscript and designed the figures.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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