Microfluidics on the fly: Inexpensive rapid fabrication of thermally laminated microfluidic devices for live imaging and multimodal perturbations of multicellular systems

Cite as: Biomicrofluidics 13, 024111 (2019); doi: 10.1063/1.5086671

Submitted: 21 December 2018 · Accepted: 8 April 2019 ·

Published Online: 26 April 2019

Megan Levis,¹ Nilay Kumar,¹ Emily Apakian,¹ Cesar Moreno,¹ Ulises Hernandez,¹ Ana Olivares,¹ Fernando Ontiveros,² and Jeremiah J. Zartman^{1,a)}

AFFILIATIONS

¹Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, Indiana 46556, USA

²Biology Department, St. John Fisher College, Rochester, New York 14618, USA

^{a)}Author to whom correspondence should be addressed: jzartman@nd.edu

ABSTRACT

Microfluidic devices provide a platform for analyzing both natural and synthetic multicellular systems. Currently, substantial capital investment and expertise are required for creating microfluidic devices using standard soft-lithography. These requirements present barriers to entry for many nontraditional users of microfluidics, including developmental biology laboratories. Therefore, fabrication methodologies that enable rapid device iteration and work “out-of-the-box” can accelerate the integration of microfluidics with developmental biology. Here, we have created and characterized low-cost hybrid polyethylene terephthalate laminate (PETL) microfluidic devices that are suitable for cell and micro-organ culture assays. These devices were validated with mammalian cell lines and the *Drosophila* wing imaginal disc as a model micro-organ. First, we developed and tested PETLs that are compatible with both long-term cultures and high-resolution imaging of cells and organs. Further, we achieved spatiotemporal control of chemical gradients across the wing discs with a multilayered microfluidic device. Finally, we created a multilayered device that enables controllable mechanical loading of micro-organs. This mechanical actuation assay was used to characterize the response of larval wing discs at different developmental stages. Interestingly, increased deformation of the older wing discs for the same mechanical loading suggests that the compliance of the organ is increased in preparation for subsequent morphogenesis. Together, these results demonstrate the applicability of hybrid PETL devices for biochemical and mechanobiology studies on micro-organs and provide new insights into the mechanics of organ development.

Published under license by AIP Publishing. <https://doi.org/10.1063/1.5086671>

INTRODUCTION

Microfluidic devices are used across disciplines because of their versatility in providing controllable microenvironments.¹ For example, biomedical researchers continuously adapt microfluidic technologies to study single cells, tissues, and whole organisms.^{2–5} In particular, there has been a significant increase in the number of studies using micro- and mesofluidic devices in multicellular eukaryotic model organisms such as nematodes, zebrafish, and fruit flies.^{3,5–9} Our laboratory and others have shown that the development of embryos and postembryonic organs in model systems can be studied in polydimethylsiloxane (PDMS)-based

devices fabricated using standard lithography.^{4,6,7,10–14} As a particular case, microfluidic devices have enabled investigations into how the microenvironment impacts intercellular signaling in the *Drosophila* wing imaginal disc.⁶ This model organ is useful for studying developmental questions such as morphogen-based pattern formation and morphogenesis and for developing new quantitative assays of multicellular systems.^{14–17}

However, traditional fabrication approaches provide a significant barrier to entry for many biology laboratories, including those focused on developmental biology. The rapid design and validation of devices individualized to specific multicellular systems, including

micro-organs, organoids, and small organisms are needed to significantly enhance new applications ranging from synthetic developmental biology to drug development and 3D tissue-based diagnostics.^{18–23} Consequently, researchers continue to develop methods that are cheaper, faster, and easily customizable for new applications. For example, paper-based²⁴ and 3D printed²⁵ devices have simplified and lowered the cost of microfluidic devices. These approaches, however, are limited in optical transparency or the biocompatibility of materials.²⁴ As one promising technique, xurography (“razor writing”) has been used to create geometrically simple single-channel microfluidic devices and shows promise as an easy-to-use technique for creating geometrically complex microfluidic devices.^{26,27} Xurography of polyethylene terephthalate (PET) and other thin films allow for new designs with channel and chamber structures to be rapidly iterated, giving users the ability to fabricate new prototypes in a matter of minutes without sacrificing reproducibility.²⁸ Recent biological applications of such devices have included hydrodynamic dissociation of cell aggregates and tissues.²⁹ Additionally, xurography of PET laminates (PETLs) can be used to quickly and inexpensively generate multilayered microfluidic devices.³⁰ However, PETL microfluidic devices have not been extensively employed for cell and organ culture applications.

Here, we report an extension of PETL-based microfluidic devices toward cell and organ culture assays. We created and tested a progression of increasingly complicated PETL-based device designs for cell and organ cultures. To overcome limitations in optical transparency, we developed devices combining PETLs with glass and other materials to create a novel hybrid microfluidic platform. These PETLs are composed of layers of PET film bonded with the thermal adhesive ethylene vinyl acetate (EVA). By including a glass layer, we were able to image samples at high resolution (Fig. S1 in the [supplementary material](#)).³¹

We then further developed and characterized specific devices useful for a broad range of organ culture studies. We created a device with a highly complex network of channels to form chemical gradients across cell populations. We also evaluated the permeability of a *Drosophila* wing disc to exogenous molecules treated in another hybrid PETL device. The device delivered control and treated media to different sides of the micro-organ. Our results demonstrate that exogenous (*ex vivo*) gradients formed across the wing disc occur under roughly equivalent timescales to reported metrics of endogenous (*in vivo*) morphogen gradient formation.^{32–34} Finally, we developed a mechano-PETL (M-PETL) device that controllably perturbs organs with mechanical loading. We used the M-PETL to characterize the changes in mechanical properties of wing discs at two different developmental stages. We found that as wing disc ages, the discs increase in compliance as they prepare for subsequent morphogenesis. During morphogenesis, the discs evert and expand.³⁵ These validated devices and biological results demonstrate that the hybrid PETL microfluidic is an extensible platform that enables the analysis and engineering of cellular and multicellular systems. We envision that this method will broaden the use of microfluidic devices within developmental biology, mechanobiology and enable low-cost applications for organs-on-a-chip. In sum, these hybrid PETLs are an inexpensive, customizable, robust platform, enabling new biological insights into developmental systems.

MATERIALS AND METHODS

Device fabrication

Hybrid PETL microfluidic devices were made through an additive, layer-by-layer manufacturing process (Fig. 1). Channel and chamber designs were drawn using commercially available design software (e.g., Adobe Illustrator or Microsoft PowerPoint). A craft cutter (Silhouette CAMEO 2) was used to define channel architectures in PET-EVA film, commercially available as laminating pouches (Scotch, TP3854-100, TP5854-100, Sircle-RL-25-15-1-G). This general method differs from other similar procedures that use self-adherent tapes in that it benefits from the robust bonding resulting from thermal lamination. An additional advantage is that it is possible to reposition the layer as needed before adhesion. Channel height was determined by the thickness of the film used, ranging from 1 to 10 thousandths of an inch (mil), which correspond to layer heights of 25 to 255 μm . Overall channel heights were defined by multiples of any of those dimensions. The number of layers (<10) that can be superimposed and bonded is determined by the total thickness of the device and heat-transfer within the laminator. For imaging purposes, glass coverslips were used as bottom/base layers or added in between film layers by cutting windows to the dimensions of the coverslip. We used VWR coverslips that are size 1, 130–160 μm thick, fitting snugly into a window cut in the 5-mil film. Smaller than size 1.5 coverslips were used here because we imaged three-dimensional micro-organs that are not attached to the glass. Two or more layers can be cut to form a window that accommodates larger sized coverslips. Once cut, the layers were aligned and kept in place using small sections of double-sided tape (Scotch R, 3M-3136) to prevent movement during lamination. Inlets and outlets were outfitted with perforated furniture bumpers (Scotch, various) to create access points compatible with pipettes and microfluidic tubing. A Dremel rotary tool with a 1/32-in. bit was used to perforate the bumpers. Complete equipment and part list are included in Tables 1 and 2 in the [supplementary material](#).

Flow modeling

The effective diffusivity (D_{eff}) of Hoechst and CellMask™ was determined by fitting the solution of a one-dimensional reaction-diffusion equation to the experimental data at multiple time points of 40, 120, 220 and 320 min. Nonlinear regression for the model was carried out using `fitnlm()` function of MATLAB. The results from experiments and corresponding model fits are shown in Fig. 4.

The CFD analysis of mixing of two Newtonian liquids in the corresponding microfluidic device designs was done using OpenFOAM.³⁶ The full description of computational methods and results are presented in the [supplementary material](#).

Cell and organ cultures

Micro-organ studies were conducted using *Drosophila* wing imaginal discs.^{37,38} Wing discs were dissected from wandering third instar larvae grown at 25 °C in standard fly food. DE-Cadherin::GFP expressing flies were used to visualize cell membranes of the wing discs in the SI-PETL and OP-PETL.³⁹ Sqh::mCherry (Myo-II::mCherry) expressing flies—a gift from the

FIG. 1. Design, fabrication, and use of hybrid PETL microfluidic devices. (a) The designs in this paper were drawn using Adobe Illustrator, Microsoft Power Point, Apple Keynote, or Silhouette Design (SD). The image file of the design is transferred (drag and drop) to the freely available SD software, where further modifications and resizing may take place. (b) SD software also controls the Silhouette CAMEO craft cutter, which is commercially available (\$250 at the time of publication). A film with thicknesses ranging 1–10 mil, or thousands of an inch is affixed to adherent, reusable mats (\$5) and loaded into the cutter. (c) Once the polyethylene terephthalate-ethylene vinyl acetate (PET-EVA) film layers are cut and removed from the mat, they are aligned on top of or enclosing a glass coverslip. (d) Aligned layers are thermally bonded using a low-cost commercially available laminator (\$35). Perforated vinyl bumpers (\$0.15) are attached to inlets/outlets before use. From design to use, a simple microfluidic device can be prepared in 15 min. After the initial \$300 investment, cost of materials of any of the devices used in this paper is under \$1. (e) Flow through the channels and tissue loading is facilitated by syringe pumps, with flows ranging from 0.03 to 100 $\mu\text{l}/\text{min}$. Confocal, fluorescence, and bright-field microscopy using inverted microscope setups (to image through the glass floor of the device) takes place over the course of seconds to hours. (f) Images are captured in MetaMorph and processed with FIJI.

Adam Martin Lab—were used to visualize cell areas of the wing discs in the M-PETL.⁴⁰ The organs were dissected and cultured in Grace's *Drosophila* Medium (Sigma, G9771) supplemented with Bis-Tris (Sigma, B9754), Penn-Strep (Gibco, 15140122), and FBS (Gibco, 10438-026).⁴¹ Rat basophil leukemia cells (ATCC, 2256) were grown in RBL-2H3 medium⁴² and were seeded in the cell gradient-PETL (CG-PETL) microfluidic devices at a concentration of $\sim 3 \times 10^6$ cells/ml.

Reagents

Gradients across the micro-organ were formed with a 0.1% solution of CellMask™ Deep Red (ThermoFisher, C10046) and with 10 μM Hoechst (Sigma, bisBenzimide H, B2883) dye dissolved into Graces Medium prepared according to Dye *et al.*⁴¹ Cell gradient experiments were performed with 0.05% v/v CellMask™, 10 μM Hoechst, and 0.1% v/v Sytox (ThermoFisher, S34862), 1% red food coloring (McCormick, 52100071077), dissolved in RBL-2H3 medium.⁴²

Imaging

PETL microfluidic devices were loaded with culture medium according to the specific cell or organ culture methods detailed above. Cells were seeded using syringe pumps (Harvard Apparatus PicoPlus Elite) at a flow rate of 60 $\mu\text{l}/\text{min}$. They were cultured for 4 h without flow at 37 °C/5% CO₂ to allow cells to attach before introducing the chemical gradient. Organs were placed on the

outlet and drawn into the device by negative pressure while suspended in supplemented Graces medium. Media flowing at 20 $\mu\text{l}/\text{h}$ was used throughout imaging in the PDMS based REM-Chip, SI-PETL, and OP-PETL.

Time-lapse confocal imaging was done using a Nikon Eclipse Ti spinning disc confocal microscope (Andor). 10 $\times$ /NA:0.45-air, 20 $\times$ /0.75-air, 40 $\times$ /0.60-air, 60 $\times$ /1.49-oil, and 100 $\times$ /1.49-oil objectives were used for experiments as noted. For gradient experiments, organs were imaged every 5 min for 7 h. Cells in the CG-PETL were imaged once after gradient formation occurs at $t = 5$ min. For viability experiments, we cultured discs in both of the PDMS and PETL microfluidic devices for 12–15 h with 20 $\mu\text{l}/\text{h}$ flow via a pump before imaging at 20 $\times$, 40 $\times$, and 100 $\times$. Images were captured using MetaMorph software. Image processing was performed using FIJI (ImageJ)⁴³ and imported into MATLAB for quantification of gradients.

Organs were imaged before, during, and after loading in the M-PETL microfluidic devices. Z-stacks were taken before compressing. A syringe pump then progressed to pump 50 μl (1.3 psi applied air), at 1 ml/min and held pressure during imaging, which occurred immediately after loading. Images were taken with 20 $\times$ /0.75-air magnification on the Nikon-Andor spinning disc confocal using a 561 nm wavelength laser, exposed for 200 ms. Increments of 50 μl of air were successively compressed until a total 250 μl air was compressed that lead to a totally applied level of 4.8 psi. The pump released the air in the syringe before the final image was taken of the unloaded disc. Total time of compression

was approximately 3 min per z-stack at each pressure and all seven sets of images were taken within 1 h of culture.

Statistical analysis

Populations of dividing cells in wing discs in both the PETL and PDMS microfluidics chips were compared using the nonparametric Mann–Whitney U-test using the MATLAB function `ranksum()`.

RESULTS

Cell gradient PETLs (CG-PETLs) allow for combined patterning of cell culture media and imaging

To our knowledge, PETL-based microfluidic devices with the ability to generate chemical gradients via complex channel designs have not been systematically evaluated for cell or organ culture applications. With this in mind, we tested the use of our microfluidic devices for cell culture and chemical patterning. We fabricated a microfluidic device in which a concentration gradient is formed from two initial solutions in a chip that we termed the Cell Gradient PETL (CG-PETLs). The flows then mixed via diffusion as the channels come together in the device and flowed across cells cultured in five chambers (Fig. 2). The design of this chip was based on the classic mixing microfluidic chip design.⁴⁴ This gradient is highly adaptable and can be customized to find optimal concentration of drugs for treatment of cells. To test the use of the CG-PETL, we created a gradient with CellMask™ across Rat Basophil Leukemia (RBL) cells (Fig. 3). A gradient of CellMask™ intensity was quantified in Fig. 3(e). Finally, we used Sytox to

measure cell death, 93% of cells were viable after adhesion and culturing for 2–4 h in the CG-PETL [Figs. 3(a)–3(d)].

To reach the desired complexity, the gradient design was split into several film layers, and a coverslip was embedded immediately adjacent to the cell chambers (Fig. 2). To prevent leaking between the coverslip and laminate layers, the design was modified to have the chamber “step” over the seam. The biggest design challenge with the CG-PETL chip was to make sure that the layers were aligned correctly at all points because of the complex multilayer design (Fig. 2). The use of a small peg-board and pegs along with perforations in each of the layers allowed them to be aligned and taped to the device to prevent shifting during the lamination process. Another consideration with the CG-PETL was the propagation and trapping of air bubbles in the channels due to the sharp turns in the device, which were minimized by rounding the corners. In the final design (Fig. 2), each channel was seeded with cells delivered using a syringe pump. A reservoir held media on one end, allowing the cells to grow without flow for 4 h as they were attached to the glass coverslip.

Simple imaging PETL (SI-PETL) devices support live imaging of organs at high resolution

We then developed a platform to monitor growth and chemical perturbations of organs and cells in applications requiring high resolution confocal microscopy (Fig. 4). *Drosophila* wing discs are a prototype micro-organ for developing engineering methods to probe cell signaling and biomechanics at the meso-scale (~100 s of μm).^{6,45,46} Recently, investigators have developed a range of culture conditions to investigate the extrinsic organ culture requirements to sustain organ growth and morphogenesis.^{38,47,48}

FIG. 2. Cell gradient PETL (CG-PETL) for cell culture and patterning. (a) Top view of the CG-PETL designed for creating a chemical gradient across cells. (b) Microfluidic device cross section highlighting the PET-EVA and glass layers. (c) The PET-EVA layers are cut before assembly and bonding to the glass coverslip. (d) The final CG-PETL has bumpers attached for inlet and outlet and food coloring highlighting the gradient. (e) Contours of mass fraction of dye within the CG-PETL calculated with CFD analysis.

FIG. 3. RBL-2H3 cells cultured, imaged, and patterned in CG-PETLs. (a) Sytox green dye marking dead RBL-2H3 cells. 40× images were captured using the 488 laser with an exposure time of 200 ms on the Nikon-Andor spinning disc confocal microscope. (b) Hoechst dye marking cell nuclei of all cells. 40× images were captured using the 405 laser with an exposure time of 100 ms. (c) CellMask™ marking cell boundaries. 40× images were captured using the 640 laser with an exposure time of 50 ms. (d) Merged image of cells for each dye demonstrates that cell toxicity is not a concern for these hybrid devices, (~93% alive) and that cells are attached. (e) Quantification of the gradient of CellMask™ across cells normalized to background intensity. Error bars are standard error (SE). (f) Collated images showing gradient (left to right as indicated by increasing height of underlining red bar) of CellMask™ across cells. Lane 1 is on the left and Lane 5 on the right. Scale bars are 20 μm .

Previous studies have shown that PDMS-based devices support the imaging of wing discs for extended periods of time with flow of WM1 Medium.⁶ Similarly, cells and tissues need to be viable for extended periods of time in the device for the new hybrid PETL

microfluidic platform to be useful. To the best of our knowledge, PET-EVA sheets have not been used for this type of closed, gas-impermeable microfluidic device for culturing cells or organs. Thus, we tested the long-term culture to verify that we are not creating

FIG. 4. Simple imaging PETL (SI-PETL) hybrid PETL microfluidic platform for micro-organs. (a) Top view of the SI-PETL designed for culture and imaging of micro-organs. (b) Microfluidic device cross section highlighting the PET-EVA and glass layers. (c) The PET-EVA layers are cut before assembly and bonding to the glass coverslip. (d) The final SI-PETL has bumpers attached for inlet and outlet connections.

FIG. 5. Organ culture and imaging in the SI-PETL chip. (a) Wing imaginal disc expressing DE-Cadherin::GFP inside an SI-PETL chip for culture and imaging. (b) 40× images showing the wing disc pouch region which was used to quantify dividing cells. Images were captured using the 488 laser with a 200 ms exposure. (c) High-resolution imaging at 100×, round mitotic cells are marked with red arrows. (d) Quantification of dividing cells 12–15 h into culture in both PDMS and PETL microfluidic devices. Continued cell division is a sensitive indicator of *ex vivo* organ health.³⁸ The populations of mitotic cells were compared with the Mann–Whitney U test, generating a p-value of 0.6.

excessively hypoxic conditions within the device. Additionally, we compared the viability to PDMS-based devices, which are the current gold standard for microfluidic devices. To test the viability of the hybrid PETL microfluidic devices, *Drosophila* wing imaginal discs were cultured in the microfluidic devices for 12–14 h in supplemented Grace's *Drosophila* Medium (Fig. 5).⁴¹ The cell divisions were quantified as a metric of biocompatibility, since cell divisions are a sensitive indicator of overall organ health. The wing disc organs cultured in the PETLs showed high amounts of cell division at a rate comparable to the standard PDMS microfluidic devices. We compared the viability of wing disc organs in the PETL platform to PDMS microfluidic devices, the current gold standard.⁶ Discs can be cultured in a well-plate for up to 24 h with proliferation peaking at 9–16 h.⁴¹ The organs remained alive and healthy under long-term culture (time >12 h) with a constant flow of media. Dividing cells

were identified by their round shape during mitotic rounding and quantified (Fig. 5). On average, 13.2 ± 8.1 mitotic cells per disc were found in PETLs compared to 14.3 ± 3.9 mitotic cells in a traditional PDMS-based microfluidic device (REM-Chip^{6,49}) after 12–15 h culture. The results show that populations are not significantly different, and organ viability in hybrid PETL compares to that of organs in PDMS. These long-term culture experiments confirmed the biocompatibility of PETL-based devices.

Organ pattern PETLs (OP-PETL) enable spatiotemporal control of patterning across micro-organs

Next, we designed a third assay to characterize spatiotemporally controlled chemical delivery to geometrically complex wing discs. Our goal was to form an exogenous chemical gradient across

FIG. 6. Organ patterning PETL (OP-PETL), a hybrid PETL microfluidic platform for organ patterning. (a) Top view of the OP-PETL designed for creating a chemical gradient across micro-organs. (b) Microfluidic device cross section highlighting the PVC, PET-EVA, and glass layers. (c) The PVC and PET-EVA layers are cut before assembly and bonding to the glass coverslip. (d) The finished device uses perforated vinyl bumpers as inlets. (e) Streamlines within the OP-PETL calculated with CFD analysis showing a diffusion-controlled region near the wing disc.

the organ to estimate the timescales for diffusion of small molecules to the membranes and nuclei across a micro-organ in culture. Precise gradient formation requires an understanding of the particular diffusivities of chemicals that reach the membrane versus chemicals that reach nuclei of cells within a tissue. We designed a chip in which regions of an organ can be perturbed independently to probe the dynamics of extrinsic chemical gradient formation (Fig. 6). The design contains parallel channels joined by a small connecting channel or window with dimensions $200 \times 500 \mu\text{m}^2$ that allow the positioning of an organoid or micro-organ.

We iterated on multiple designs before determining the optimized form, which included an H-shaped set of channels for spatiotemporal patterning across the micro-organ (Fig. S2 in the supplementary material). This was necessary because of the poor performance for chemically patterning wing discs in a simple, Y-shaped design that was previously used for patterning geometrically simpler *Drosophila* embryos.⁷ Initially, the Y-shaped design was tested by loading a wing disc and flowing colored PBS through the device to determine flow patterns within the device and across the wing disc organ. Wing imaginal discs are shaped like a

flattened “pear” with multiple stereotypical folds (Fig. 7 and Fig. S6 in the supplementary material).⁵⁰ The asymmetry of the micro-organ induced flow along each of its sides to proceed at different rates due to differing lengths along each side. The slowed flow on one side of the organ resulted in engulfment of the whole organ from the stream on the other side, preventing exposure of each treatment to its respective side of the disc. Through iterative design and testing, we determined that an H-shaped channel provided the best performance (Fig. 6). The H-shaped channel prevented direct flow between the two channels and allowed for diffusion led transport of the drug/dye across the tissue. A self-adhesive PVC layer top/sealing layer of the device was added after manually positioning the organ to facilitate positioning of the organ inside the chip (Fig. 6). Because of the ability to rapidly iterate designs, H-shaped OP-PETL microfluidic devices can be made to fit the exact specifications of any asymmetric micro-organ or organoid.

CellMask™ and Hoechst dyes were used to provide a readout of dynamics of dye penetrance into and across wing discs (Fig. 7). CellMask™ is an amphipathic dye that marks the cell membrane via both lipophilic and hydrophilic interactions and works quickly

FIG. 7. Spatial patterning of a wing disc by media flow visualized with fluorescent markers in an OP-PETL. (a) Schematic of OP-PETL showing patterning with drug on one side of the disc. (b) Wing disc imaged at 20× expressing DE-Cadherin::GFP. Image captured with the 488 laser with 200 ms exposure at 20× magnification on the Nikon-Andor spinning disc confocal microscope. (c) Channel showing Hoechst dye after treating for 6.5 h. Image captured with the 405 laser with 200 s exposure at 20× magnification. (d) CellMask™ Deep Red plasma membrane dye after treating for 6.5 h. Image captured using the 640 laser with 50 ms exposure at 20× magnification. (e) Merged image showing Ecad::GFP in green, Hoechst in blue, and Cell Mask in red. (f) Image showing axes for quantification of the gradient formation. (g) Experimental (E) quantification of the CellMask™ patterning as compared to a diffusion model (M) of the system. (h) Experimental quantification of the Hoechst patterning as compared to a diffusion model of the system.

to show any membrane area that the media has reached.⁵¹ Hoechst dye intercalates with DNA in the nuclei of live cells and shows the dynamics of permeation of the dye into the cells.⁵² Our method was capable of treating half of the micro-organ, creating a more accurate internal control for one disc. This allows for comparison between one side of the disc and the other. In the future, this device can be used for pharmacological testing of drugs with the untreated side of the organ included as an internal control.

The effective diffusivities (D_{eff}) of Hoechst and CellMask™ through the *Drosophila* wing imaginal disc were found to be $0.031 \pm 0.008 \mu\text{m}^2/\text{s}$ and $0.11 \pm 0.02 \mu\text{m}^2/\text{s}$, respectively. This D_{eff} was then used to test the validity of these models at multiple time points of 120, 220, and 320 min. The results of the model and experiment have been shown in Fig. 7. A qualitative agreement between the experimental data and model predictions for Hoechst and CellMask™ was observed [Figs. 7(g) and 7(h)]. The effective diffusivities obtained for the dyes are very close to the range of diffusivities for morphogen transport that would be expected to occur due to transcytosis ($\sim 0.1 \mu\text{m}^2/\text{s}$).^{32–34} This provides confirmation of the time scale for exogenous signals such as insulin and ecdysone from outside of the organ to be transported past the extracellular matrix surrounding the organ.^{53,54}

Mechano-PETLs (M-PETLs) enable low-cost mechanobiology studies of micro-organs

Microfluidic devices that modulate the mechanical environment of cells and tissues enable investigation into the interplay between cell mechanics and cell signaling.^{6,55} To further advance the hybrid PETL platform, we created a new device that mechanically compresses micro-organs (Fig. 8). This design uses pneumatic pressure to deflect a flexible membrane from above the imaging chamber to apply uniaxial compression to the organ inside the chamber. This low-cost system enables mechanical actuation of samples inside of the PETL devices. In addition to the glass and PET-EVA layers, two additional materials were added to create the Mechano-PETL (M-PETL) (Fig. 8). A layer made of flexible PVC (Saran™ Wrap) was included to form the deflectable membrane. PVC was chosen for the membrane due to being cost effective and readily available. The final layer was a PET film with EVA coating on both sides. These double adhesive layers allowed the PVC layer to be included in the device.

We fit a finite element model to the experimental data to estimate Young's modulus of the PVC membrane [Figs. 8(d) and 8(e), methods in the supplementary material]. The estimated Young's modulus of 3.8–8.6 GPa is comparable to the literature values of

FIG. 8. Mechano-PETLs (M-PETLs) for mechanical actuation of micro-organs. (a) A top view of the M-PETL designed for compressing micro-organs. (b) Cross section highlighting the PET-EVA, PVC and glass layers. (c) The final M-PETL is loaded with red food coloring in the central chamber to highlight functioning of the compression layer. (c') The red dye is pushed out of the way when compression is applied via pneumatic pressure. M-PETL recapitulates compressive functionality from the REM-Chip.⁹ (d) Cross section of the chamber short axis for 0, 3.6, and 9.8 psi applied pneumatically from the top. The yellow dotted line denotes height of the uncompressed chamber (0 psi). The blue dotted line denotes the measurement of deflection—the maximal change in height at each applied pressure. The chamber is dyed using a solution of CellMask™. Chamber compression images were taken at 10× magnification on the Nikon-Andor spinning disc confocal using the 640 nm wavelength laser, exposed for 75 ms and stitched together. (e) Representative images of the simulation of membrane deflection. (f) Displacement of the membrane by applying various pressures. Experimental (black, $n=4$) and simulation results (gray, for average simulated E plus, minus one standard deviation).

PVC.⁵⁶ Through multiple experiments, we found that the M-PETLs were robust and reproducible, thus enabling controlled deformations on wing discs.

Using the M-PETL, we characterized the relative deformations of wing discs from a given mechanical loading at two developmental stages. We used fluorescently tagged nonmuscle Myo II::mCherry (*sqh::mCherry*) as a marker to visualize the shape of the wing discs. We then quantified the deformation of the ellipse-shaped pouch regions of the wing discs. The long axis of the ellipse is parallel to the anterior–posterior axis of the wing disc. The short axis of the pouch is parallel to the dorsal–ventral compartment axis of the wing disc. Notably, this transgenic line is slightly developmentally delayed compared to wild-type flies without the transgene reporter. At 120 h after egg laying (h AEL), the larvae with the *sqh::mCherry* genetic background were still feeding and had smaller wing discs, compared

to wing discs excised from 144 h AEL larvae [Figs. 9(a) and 9(b)]. Release of mechanical loading of wing discs resulted in elastic recovery of the organ's shape. Length changes defined by specific landmarks (see the [supplementary material](#)) along the axes under the applied pressures were used to calculate the observed true strain for each axis according to equation $\epsilon_l = \ln\left(\frac{l}{l_0}\right)$.⁴⁶

The deformation response of wing discs at each developmental stage was then quantified at applied pressures ranging from 0 to 5 psi. During the feeding stages, the wing disc grows exponentially; the growth rate declines as the organ approaches its final size during the wandering stage. Growth brings about increasing anisotropy in organ shapes and increased folding.^{57,58} The measured strain for each of the applied pressures along each axis are plotted in Figs. 9(c)–9(e). Surprisingly, for a given level of mechanical loading, the observed strains along both the short and long axes of

FIG. 9. Mechano-PETLs enable characterization of strain due to uniaxial compression of wing discs two developmental stages. Max intensity z-projections of 120 h AEL (a) and 144 h AEL (b) wing discs expressing myo-II::mCherry (*sqh::mCherry*) under compression from 0 to 5 psi pressure. Initial pouch areas are indicated by the dotted black line, compressed pouch area is denoted by the white blue line. (c) Observed true strain along the short (x) axis of the ellipse-shaped pouch under compression ranging from 0 to 5 psi applied pneumatic pressure. (d) Observed true strain along the major (y) axis of the ellipse-shaped pouch. (e) Magnitude of the observed true strain along the z-axis (tissue height). Error bars are standard deviation (SD). Wing disc organs expressing myo-II::mCherry, images were captured immediately after each pneumatic pressure application ($t < 3$ min). Disc images were taken at 20 $\times$ magnification on the Nikon-Andor spinning disc confocal using the 561 nm wavelength lasers, exposed for 200 ms. Discs at 120 h AEL (feeding), $n = 4$. Discs at 144 h AEL (wandering), $n = 5$.

TABLE I. Summary of Poisson's ratios of the wing disc in the M-PETL.

	ν_{xz}	ν_{yz}
120 h AEL	0.3 ± 0.1	0.39 ± 0.06
144 h AEL	0.5 ± 0.1	0.4 ± 0.1

the older discs were greater than the younger discs [Figs. 9(c)–9(e) and Fig. S6 in the [supplementary material](#)]. This could be attributed to the relative differences in geometry and extent of folding of the soft epithelial tissues within the thin layer of extracellular matrix that surround the organ.⁵⁹ Table I reports the measured Poisson ratios, ν , for the wing discs with respect to the x, y, and z dimensions as denoted in (Fig. 9), where the directional Poisson's Ratios are as follows, $\nu_{xz} = -\frac{\epsilon_x}{\epsilon_z}$ and $\nu_{yz} = -\frac{\epsilon_y}{\epsilon_z}$.

The Poisson ratio for compressions of both feeding (120 h AEL) and wandering (144 h AEL) larvae are in the range of reported values of uniaxial stretching, which ranges from 0.29 to 0.53 for the *Drosophila* wing imaginal disc.⁴⁶ The observed increased anisotropy of the apparent Poisson ratio isotropy along the short axis may be due to increased tissue folding as the late wing disc prepares to undergo buckling and eversion to form the future wing blade.^{35,59} Finally, fitting an approximate model of the 144 h AEL wing pouch undergoing compression provides an estimate of the apparent Young's modulus on the order of 10^5 Pa (see the [supplementary material](#)), in agreement with previous efforts that utilized a uniaxial stretching device.⁶⁰ This result is also consistent with other estimates for embryonic-type tissues.³ Interestingly, as wing discs prepare for pupariation, mechanical compliance appears to increase to robustly facilitate subsequent morphogenesis, which results in dramatic shape changes of the organ.

DISCUSSION

A wide variety of microfluidic devices allow for microscopy-based biological applications, ranging from subcellular positioning⁶¹ to immobilization of whole organisms.¹² Here, we created and tested four PETL-based microfluidic devices to demonstrate their potential to serve as low-cost, reliable, and sophisticated set of research tools for cell and organ cultures, with a specific focus on developmental biology applications. Our results confirm that PETL fabrication methods allow for a range of functionalities. For example, integration of a deflectable, PVC-based membrane component enabled us to reproducibly control mechanical perturbations on organs (Figs. 8 and 9). Further, we used experimental results captured in the M-PETL with finite element analysis to characterize the relative mechanical responses of wing discs at two developmental stages. This mechanical actuation led to the interesting result: wing discs appear to increase in mechanical compliance at the tissue level as they approach the final stages of larval growth. At this stage, the organs are poised to undergo eversion, which results in extreme morphogenetic changes and requires regulation of actomyosin contractility.^{35,62} The M-PETL highlights the multifunctionality of these low-cost PETL microfluidic devices for mechanobiology applications of micro-organs and tissues. Future investigations using the M-PETL will enable systematic

investigations into the interplay between mechanical forces and tissue growth and signal transduction.^{45,50,63–65}

Currently, PDMS-based devices remain the gold standard for growing and perturbing cell and organ systems. PDMS is utilized because of its biocompatibility and rheological properties. An important drawback of PDMS in microfabrication is the need to create a mold or master, which usually requires a clean room and a lengthy lithography process. Currently, microfluidic devices are designed in a variety of design software applications, from AutoCAD to Adobe Illustrator. This is because the design layers have to be printed at ultrahigh resolution for the photolithography process. The printed layer (mask) has to be aligned perfectly to prevent blurring or any distortion of the features. Masks are expensive to print and generally include a lag-time as they are usually printed by a third party.

With the rise in popularity of microfluidic devices, there is growing interest and need for simplifying and reducing the cost of microfluidic devices.^{66–69} Research groups have addressed the fabrication limitations of lithography-based microfluidics with multiple approaches, each with its own set of strengths and limitations. For example, one study created PDMS devices from pasta molds⁶⁸ and another effort developed paper-based microfluidics.^{24,66} The pasta molding process is limited to the sizes and shapes of available pasta. Furthermore, designs cannot be precisely controlled or mass produced as the designs are crafted by hand. Among many, these include paper-based devices as well as devices with features cut out of sticky tape.^{24,66,69} Paper-based microfluidics may require lithography and/or pretreatment of the paper before the device can be assembled, so they may not actually reduce the need for a clean room environment.^{24,66} Additionally, paper is not optically clear or totally liquid resistant. On the other hand, the ability to precisely align layers using sticky tape⁶⁹ is limited because the material is adhesive at all times, if not aligned properly the adhesive can catch the side of an organ and tear it during the loading process.

PET, like PDMS, is biocompatible and has been used in a variety of implantable medical applications.⁷⁰ With PETLs, the device's layers can be positioned precisely before the materials are adhered together. EVA, the adhesive on laminating sheets, is heat activated and cures when it is cooled. The process of thermal lamination can be expanded to include a variety of materials, as we have demonstrated in this work. Here, we introduced glass and PVC layers, but this can be expanded to a broad range of materials that can be fed through a laminator.

In addition to the ease of creating complex multifunction devices with multiple materials, these hybrid PETLs dramatically lower the cost of microfabrication. An advantage of cutting the features directly is that the need for a photo-mask is eliminated, significantly reducing iteration time. It is also notable that the designs can be drawn in any software. With minimal training, researchers and students can design, build, test and modify their designs at a fraction of the time and expense required for PDMS-based devices.

Thus, hybrid PETL microfluidic devices for developmental biology and organoid culture applications hold particular promise because they significantly reduce fabrication time from hours/days to minutes. Furthermore, the capital costs for a laboratory to generate a new device are reduced from >\$20 000 for traditional

lithography in a clean room⁴ to <\$300 (Fig. 1 and Tables 1 and 2 in the [supplementary material](#)) in the PETL platform, with the ability to produce a virtually unlimited number of new designs. Developing devices on the hybrid PETL microfluidic platform offers the advantage of rapid modifications to accommodate the ever-changing needs of laboratory experimentation, since variations of prototypes can be easily built and tested without requiring a new mask for photolithography. Thus, these hybrid PETL devices are a viable, affordable, rapid prototyping alternative to PDMS devices that can be adapted in any research lab or educational setting. A limitation of the PETL platform is that the cutting resolution is $\sim 100\ \mu\text{m}$. We found this acceptable for the size scales of the devices that we made, but this resolution may be limiting for some applications. This paper addresses the need to easily design complex microfluidic devices that are validated for cell and organ cultures. Using a craft cutter allows researchers to create devices easily and quickly, being able to customize devices in a matter of minutes.

CONCLUSIONS

The expanding field of microfluidics for developmental biology applications increasingly requires precisely constructed devices with complex features for a variety of applications. Many engineering and biology laboratories lack ready access to clean rooms with lithography equipment. Here, we introduce a set of thermally laminated PET/EVA microfluidic devices that include other materials such as glass and PVC to greatly reduce financial and temporal cost for researchers who wish to design and fabricate their own microfluidic devices. We tested the range of assays in these sophisticated, multilayer PETL devices for the purpose of culturing and controlling media flow and mechanical loading of micro-organs for microscopy applications. We report mechanical properties including strain, Poisson's ratio and an estimate of the magnitude of the elastic modulus of the wing imaginal disc, a prototypical micro-organ used to understand the biophysics of organ growth and development.¹⁴ This tunable prototyping and fabrication method can be expanded to control the microenvironment of human organoids as well.⁷¹ Our assays highlight the imaging capability, healthy long-term culture *ex vivo*, and mechanical actuation enabled by these devices. Additionally, these systems decrease the start-up cost of equipment and fabrication for biomicrofluidic devices in cell and developmental biology research groups to less than \$300. The work presented here introduces a series of assays in rapidly prototyped, advanced microfluidic devices for bioengineering research. The next generation of PETL-based assays will enable the expansion of multicellular engineered systems to bridge many knowledge gaps, including those related to translational research, educational tools, inexpensive diagnostics, and biosensors.

SUPPLEMENTARY MATERIAL

The [supplementary material](#) includes simulation methods, results and discussion as well as videos of the images collected in each PETL chip. Tables for equipment list for PETL production are also included.

ACKNOWLEDGMENTS

The work in this manuscript was supported in part by National Science Foundation (NSF) (Grant No. CBET-1553826) (and associated ROA supplement) and National Institutes of Health (NIH) (Grant No. R35GM124935) to J.Z., and the Notre Dame Melchor Visiting Faculty fund to F.O. The authors gratefully acknowledge the Notre Dame Integrated Imaging Facility. We would like to thank Nick Contento for early related computational analysis on the related work. We thank members of the Zartman Lab, including Francisco Huizar, Jamison Jangula, Qinfeng Wu, Dharsan Soundarajan, Vijay Velagala, and anonymous reviewers for helpful comments. We also thank Cody Narciso and Franklin Mejia for their critiques, as well as Jonathan Juan and Anthony Kavanagh for initial PETL experiments. We would like to thank Jenna Sjoerdsma and Basar Bilgiçer for providing mammalian cells and culture protocols.

REFERENCES

- ¹D. A. Porto, T. M. Rouse, A. San-Miguel, and H. Lu, "Microfluidic platforms for quantitative biology studies in model organisms," in *Microfluidic Methods for Molecular Biology*, edited by C. Lu and S. S. Verbridge (Cham Springer International Publishing, 2016), pp. 1–18.
- ²E. K. Sackmann, A. L. Fulton, and D. J. Beebe, "The present and future role of microfluidics in biomedical research," *Nature* **507**(7491), 181–189 (2014).
- ³A. Z. Shorr, U. M. Sönmez, J. S. Minden, and P. R. LeDuc, "High-throughput mechanotransduction in *Drosophila* embryos with mesofluidics," *Lab Chip* **19**, 1141–1152 (Feb. 2019).
- ⁴K. F. Sonnen and C. A. Merten, "Microfluidics as an emerging precision tool in developmental biology," *Dev. Cell* **48**(3), 293–311 (2019).
- ⁵Y. Cho, D. N. Oakland, S. A. Lee, W. R. Schafer, and H. Lu, "On-chip functional neuroimaging with mechanical stimulation in *Caenorhabditis elegans* larvae for studying development and neural circuits," *Lab Chip* **18**(4), 601–609 (2018).
- ⁶C. E. Narciso, N. M. Contento, T. J. Storey, D. J. Hoelzle, and J. J. Zartman, "Release of applied mechanical loading stimulates intercellular calcium waves in *Drosophila* wing discs," *Biophys. J.* **113**(2), 491–501 (2017).
- ⁷E. M. Lucchetta, J. H. Lee, L. A. Fu, N. H. Patel, and R. F. Ismagilov, "Dynamics of *Drosophila* embryonic patterning network perturbed in space and time using microfluidics," *Nature* **434**(7037), 1134–1138 (2005).
- ⁸N. Chronis, "Worm chips: Microtools for *C. elegans* biology," *Lab Chip* **10**(4), 432–437 (2010).
- ⁹S. Mondal, S. Ahlawat, and S. P. Koushika, "Simple microfluidic devices for in vivo imaging of *C. elegans*, *Drosophila* and Zebrafish," *J. Vis. Exp.* **67**, 3780 (2012).
- ¹⁰G. T. Dagani, K. Monzo, J. R. Fakhoury, C. C. Chen, J. C. Sisson, and X. Zhang, "Microfluidic self-assembly of live *Drosophila* embryos for versatile high-throughput analysis of embryonic morphogenesis," *Biomed. Microdevices* **9**(5), 681–694 (2007).
- ¹¹T. J. Levorio, C. Zhao, T. Rouse, S. Y. Shvartsman, and H. Lu, "An integrated platform for large-scale data collection and precise perturbation of live *Drosophila* embryos," *Sci. Rep.* **6**, 21366 (2016).
- ¹²M. Ghannad-Rezaie, X. Wang, B. Mishra, C. Collins, and N. Chronis, "Microfluidic chips for in vivo imaging of cellular responses to neural injury in *Drosophila* larvae," *PLoS ONE* **7**(1), e29869 (2012).
- ¹³D. Qin, Y. Xia, and G. M. Whitesides, "Soft lithography for micro- and nano-scale patterning," *Nat. Protoc.* **5**(3), 491–502 (2010).
- ¹⁴C. Narciso and J. Zartman, "Reverse-engineering organogenesis through feedback loops between model systems," *Curr. Opin. Biotechnol.* **52**, 1–8 (2018).
- ¹⁵D. M. Umulis and H. G. Othmer, "Mechanisms of scaling in pattern formation," *Development* **140**(24), 4830–4843 (2013).

- ¹⁶S. Restrepo, J. J. Zartman, and K. Basler, "Coordination of patterning and growth by the Morphogen DPP," *Curr. Biol.* **24**(6), R245–R255 (2014).
- ¹⁷A. Buchmann, M. Alber, and J. J. Zartman, "Sizing it up: The mechanical feedback hypothesis of organ growth regulation," *Semin. Cell Dev. Biol.* **35**, 73–81 (2014).
- ¹⁸M. Rimann and U. Graf-Hausner, "Synthetic 3D multicellular systems for drug development," *Curr. Opin. Biotechnol.* **23**(5), 803–809 (2012).
- ¹⁹M. B. Johnson, A. R. March, and L. Morsut, "Engineering multicellular systems: Using synthetic biology to control tissue self-organization," *Curr. Opin. Biomed. Eng.* **4**, 163–173 (2017).
- ²⁰J. Davies, "Using synthetic biology to explore principles of development," *Development* **144**(7), 1146–1158 (2017).
- ²¹M. M. Modena, K. Chawla, P. M. Misun, and A. Hierlemann, "Smart cell culture systems: Integration of sensors and actuators into microphysiological systems," *ACS Chem. Biol.* **13**(7), 1767–1784 (2018).
- ²²C. Narciso *et al.*, "On-chip three-dimensional tissue histology for microbiopsies," *Biomicrofluidics* **10**(2), 021101 (2016).
- ²³Q. Wu, N. Kumar, V. Vegala, and J. Zartman, "Tools to reverse-engineer multicellular systems: Case studies using the fruit fly," *J. Biol. Eng.* (in press).
- ²⁴A. W. Martinez, S. T. Phillips, G. M. Whitesides, and E. Carrilho, "Diagnostics for the developing world: Microfluidic paper-based analytical devices," *Anal. Chem.* **82**(1), 3–10 (2010).
- ²⁵C. M. B. Ho, S. H. Ng, K. H. H. Li, and Y.-J. Yoon, "3D printed microfluidics for biological applications," *Lab Chip* **15**(18), 3627–3637 (2015).
- ²⁶M. Islam, R. Natsu, and R. Martinez-Duarte, "A study on the limits and advantages of using a desktop cutter plotter to fabricate microfluidic networks," *Microfluid. Nanofluidics* **19**(4), 973–985 (2015).
- ²⁷S. Cosson, L. G. Aeberli, N. Brandenberg, and M. P. Lutolf, "Ultra-rapid prototyping of flexible, multi-layered microfluidic devices via razor writing," *Lab Chip* **15**(1), 72–76 (2015).
- ²⁸D. A. Bartholomeusz, R. W. Boutte, and J. D. Andrade, "Xurography: Rapid prototyping of microstructures using a cutting plotter," *J. Microelectromech. Syst.* **14**(6), 1364–1374 (2005).
- ²⁹X. Qiu *et al.*, "Microfluidic channel optimization to improve hydrodynamic dissociation of cell aggregates and tissue," *Sci. Rep.* **8**(1), 2774 (2018).
- ³⁰K. Martínez-Hernández, N. Rovira-Figueroa, and F. Ontiveros, "Implementation and assessment of student-made microfluidic devices in the general chemistry laboratory," *J. Nano Educ.* **8**(2), 84–113 (2016).
- ³¹E. J. New, V. C. Wimmer, and D. J. Hare, "Promises and pitfalls of metal imaging in biology," *Cell Chem. Biol.* **25**(1), 7–18 (2018).
- ³²S. Zhou *et al.*, "Free extracellular diffusion creates the Dpp morphogen gradient of the *Drosophila* wing disc," *Curr. Biol.* **22**(8), 668–675 (2012).
- ³³A. Kicheva *et al.*, "Kinetics of morphogen gradient formation," *Science* **315** (5811), 521–525 (2007).
- ³⁴P. Müller, K. W. Rogers, S. R. Yu, M. Brand, and A. F. Schier, "Morphogen transport," *Development* **140**(8), 1621–1638 (2013).
- ³⁵M. D. de la Loza and B. J. Thompson, "Forces shaping the *Drosophila* wing," *Mech. Dev.* **144**, 23–32 (2017).
- ³⁶H. Jasak, A. Jemcov, and Ž. Tuković, "OpenFOAM: A C++ Library for Complex Physics Simulations," in *International Workshop on Coupled Methods in Numerical Dynamics* (IUC, Dubrovnik, Croatia, 2007), pp. 1–20.
- ³⁷G. Echalié, N. Perrimon, and S. E. Mohr, *Drosophila Cells in Culture* (Academic Press, 2018).
- ³⁸J. Zartman, S. Restrepo, and K. Basler, "A high-throughput template for optimizing *Drosophila* organ culture with response-surface methods," *Development* **140**(3), 667–674 (Feb. 2013).
- ³⁹J. Huang, W. Zhou, W. Dong, A. M. Watson, and Y. Hong, "Directed, efficient, and versatile modifications of the *Drosophila* genome by genomic engineering," *Proc. Natl. Acad. Sci.* **106**(20), 8284–8289 (2009).
- ⁴⁰A. C. Martin, M. Kaschube, and E. F. Wieschaus, "Pulsed contractions of an actin-myosin network drive apical constriction," *Nature* **457**(7228), 495–499 (2009).
- ⁴¹N. A. Dye *et al.*, "Cell dynamics underlying oriented growth of the *Drosophila* wing imaginal disc," *Development* **144**(23), 4406–4421 (2017).
- ⁴²N. Andrews, K. A. Lidke, G. Hsieh, B. S. Wilson, J. M. Oliver, and D. S. Lidke, "High affinity IgE receptor diffusional dynamics measured by single quantum dot tracking in resting and activated cells," *FASEB J.* **21**(5), A184–A184 (2007).
- ⁴³J. Schindelin *et al.*, "Fiji: An open-source platform for biological-image analysis," *Nat. Methods* **9**(7), 676–682 (2012).
- ⁴⁴N. L. Jeon, S. K. W. Dertinger, D. T. Chiu, I. S. Choi, A. D. Stroock, and G. M. Whitesides, "Generation of solution and surface gradients using microfluidic systems," *Langmuir* **16**(22), 8311–8316 (2000).
- ⁴⁵J. Rajagopalan, A. Tofangchi, and M. T. A. Saif, "Drosophila neurons actively regulate axonal tension in vivo," *Biophys. J.* **99**(10), 3208–3215 (2010).
- ⁴⁶T. Schluck, U. Nienhaus, T. Aegerter-Wilmsen, and C. M. Aegerter, "Mechanical control of organ size in the development of the *Drosophila* wing disc," *PLoS ONE* **8**(10), e76171 (2013).
- ⁴⁷S. Restrepo, J. J. Zartman, and K. Basler, "Cultivation and live imaging of *Drosophila* imaginal discs," in *Drosophila Methods and Protocols*, edited by C. Dahmann (Springer New York, New York, NY, 2016), pp. 203–213.
- ⁴⁸J. Zartman, "Organ culture methods for the *Drosophila* wing imaginal disc," in *Drosophila Cells in Culture* (Elsevier, 2018), pp. 145–164.
- ⁴⁹M. Lake *et al.*, "Microfluidic device design, fabrication, and testing protocols," *Protoc. Exch.* (published online).
- ⁵⁰D. Eder, C. Aegerter, and K. Basler, "Forces controlling organ growth and size," *Mech. Dev.* **144**, 53–61 (2017).
- ⁵¹"CellMask Deep Red Plasma membrane Stain—Thermo Fisher Scientific," see <https://www.thermofisher.com/order/catalog/product/C10046>.
- ⁵²S. A. Latt, G. Stetten, L. A. Juergens, H. F. Willard, and C. D. Scher, "Recent developments in the detection of deoxyribonucleic acid synthesis by 33258 Hoechst fluorescence," *J. Histochem. Cytochem.* **23**(7), 493–505 (1975).
- ⁵³M. Ma, X. Cao, J. Dai, and J. C. Pastor-Pareja, "Basement membrane manipulation in *Drosophila* wing discs affects Dpp retention but not growth mechanoregulation," *Dev. Cell* **42**(1), 97–106.e4 (2017).
- ⁵⁴J. M. Tennesen and C. S. Thummel, "Coordinating growth and maturation—Insights from *Drosophila*," *Curr. Biol.* **21**(18), R750–R757 (2011).
- ⁵⁵D. Huh, B. D. Matthews, A. Mammoto, M. Montoya-Zavala, H. Y. Hsin, and D. E. Ingber, "Reconstituting organ-level lung functions on a chip," *Science* **328** (5986), 1662–1668 (2010).
- ⁵⁶M. V. Titov, *PVC Technology* (Springer Science & Business Media, 2012).
- ⁵⁷C. Narciso *et al.*, "Patterning of wound-induced intercellular Ca²⁺ flashes in a developing epithelium," *Phys. Biol.* **12**(5), 056005 (2015).
- ⁵⁸M. Tozluoglu *et al.*, "Planar differential growth rates determine the position of folds in complex epithelia," e-print [bioRxiv:515528](https://arxiv.org/abs/1905.11528) (2019).
- ⁵⁹A. Keller, F. Lanfranconi, and C. M. Aegerter, "The influence of geometry on the elastic properties of the *Drosophila* wing disc," *Phys. Stat. Mech. Appl.* **510**, 208–218 (2018).
- ⁶⁰T. Schluck and C. M. Aegerter, "Photo-elastic properties of the wing imaginal disc of *Drosophila*," *Eur. Phys. J. E. Soft Matter* **33**(2), 111–115 (2010).
- ⁶¹S. Takayama, E. Ostuni, P. Leduc, K. Naruse, D. E. Ingber, and G. M. Whitesides, "Laminar flows—Subcellular positioning of small molecules," *Nature* **411**, 1016 (2001).
- ⁶²S. Aldaz, L. M. Escudero, and M. Freeman, "Dual role of myosin II during *Drosophila* imaginal disc metamorphosis," *Nat. Commun.* **4**, 1761 (2013).
- ⁶³T. Aegerter-Wilmsen, C. M. Aegerter, E. Hafen, and K. Basler, "Model for the regulation of size in the wing imaginal disc of *Drosophila*," *Mech. Dev.* **124**(4), 318–326 (Apr. 2007).
- ⁶⁴L. LeGoff and T. Lecuit, "Mechanical forces and growth in animal tissues," *Cold Spring Harb. Perspect. Biol.* **8**(3) (2016).

- ⁶⁵Y. Pan, I. Heemskerk, C. Ibar, B. I. Shraiman, and K. D. Irvine, "Differential growth triggers mechanical feedback that elevates Hippo signaling," *Proc. Natl. Acad. Sci.* **113**(45), E6974–E6983 (2016).
- ⁶⁶M. O. Shaikh, L.-Y. Chang, C.-H. Chen, T.-F. Wu, and C.-H. Chuang, "Paper-based immunosensor utilizing dielectrophoretic trapping of microprobes for quantitative and label free detection using electrochemical impedance spectroscopy," *Biomicrofluidics* **12**(6), 064102 (2018).
- ⁶⁷N. Mavrogiannis, M. Ibo, X. Fu, F. Crivellari, and Z. Gagnon, "Microfluidics made easy: A robust low-cost constant pressure flow controller for engineers and cell biologists," *Biomicrofluidics* **10**(3), 034107 (2016).
- ⁶⁸N. Nguyen, P. Thurgood, J. Y. Zhu, E. Pirogova, S. Baratchi, and K. Khoshmanesh, "'Do-it-in-classroom' fabrication of microfluidic systems by replica moulding of pasta structures," *Biomicrofluidics* **12**(4), 044115 (2018).
- ⁶⁹J. Greer, S. O. Sundberg, C. T. Wittwer, and B. K. Gale, "Comparison of glass etching to xurography prototyping of microfluidic channels for DNA melting analysis," *J. Micromech. Microeng.* **17**(12), 2407 (2007).
- ⁷⁰A. Subramaniam and S. Sethuraman, "Biomedical applications of nondegradable polymers," in *Natural and Synthetic Biomedical Polymers*, edited by S. G. Kumbar, C. T. Laurencin, and M. Deng (Elsevier, Oxford, 2014), Chap. 18, pp. 301–308.
- ⁷¹S. N. Bhatia and D. E. Ingber, "Microfluidic organs-on-chips," *Nat. Biotechnol.* **32**(8), 760–772 (2014).