Electronic Supplementary Material (ESI) for Lab on a Chip.

Electronic Supplementary Information

An Integrated Hybrid Microfluidic Device for Oviposition-Based Chemical Screening of Adult Drosophila melanogaster

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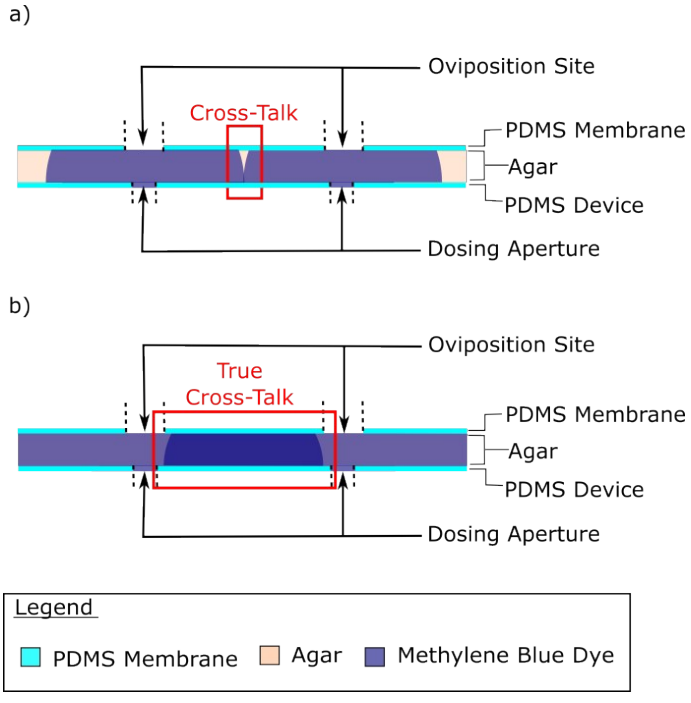
1. Chemical Cross-Talk

Cross-talk (Supp. Fig. 1a) was defined as the instance in which the methylene blue dye infused through two adjacent dosing apertures touched each other in the center. True cross-talk (Supp. Fig. 1b) occurred beyond cross-talk when the adjacent dye from the dosing aperture reached the boundary of the oviposition site defined by the 4mm diameter membrane.



Supplementary Figure 1. Visual demonstration of chemical (a) cross-talk and (b) true cross-talk.

2. Device Fabrication and Testing Protocols

2.1. AGAR-PDMS DEVICE FABRICATION

PDMS Top Layer

1. Silicone tubes (2cm-long Masterflex L/S 1/16" tubing) were erected 70mm apart inside a petri-dish lid (100mm diameter × 7mm height).[1]
2. 5ml of PDMS pre-polymer, Sylgard 184 elastomer and curing agent in a 10:1 ratio (Dow Corning Co., Midland, MI, USA), was spread over a petri dish lid with the erected tubes and was cured.
3. A 40mm diameter dish was laid on top of the PDMS between the tubes.
4. 15ml of PDMS was poured and spread around the dish and cured.
5. The structure was removed from the petri dish lid, and the 40mm diameter dish was removed from the top of the PDMS layer.
6. Dosing apertures (2mm or 3mm diameter through-hole) were incised with a Harris Uni-Core™ hole-puncher (Ted Pella Inc., Redding, CA, USA) and a 3D printed guide.[2]
7. A placement ring for the stock bottle was made via hot-melt adhesive around the perimeter of the voided area on the surface.

PDMS Base Channel Layer

8. 15ml of PDMS was casted into a 3D printed mold that contained the chemical infusion channels (400×200 μm^2 cross-section with a length of 70mm)[3].
9. PDMS was cured and removed from the 3D printed mold.

Chip Preparation via the Sacrificial Ice Technique

10. The top and bottom PDMS layers were plasma bonded together.
11. Chemical barrels (10ml BD syringe) were attached for loading individual chemicals to the inlets of the fabricated microfluidic devices.
12. A piece of tape was used to cover the dosing apertures to enclose the micro-channels underneath.
13. Water was injected into the device via the channels.
14. The device was placed into a freezer (-18° C) until water was completely frozen.
15. The tape over the apertures was removed.
16. 2ml of agar juice solution was coated and cured in the 40mm diameter dish void of the top layer.
17. Water from the melted ice was syringed out.

Oviposition Site Patterning

18. 0.5ml of PDMS pre-polymer was spun on a 6cm×6cm transparency film using a spin coater (MODEL P6700 Series, Specialty Coating Systems Inc., Indianapolis, IN, USA) for 20 seconds at 600rpm.
19. After curing, a 40mm diameter PDMS membrane with patterned 4mm diameter through-holes was incised.[4]
20. The patterned membrane was then overlaid on top of the cured agar of the device.

2.2. DEVICE OPERATION5

1. Chemicals were syringed into the loading barrels and capped at the outlet tubes using caps.[6]

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[1] Toxicity assay chip has a pair of silicon tubes whereas the multi-choice assay chip has three spaced 4.7mm apart.
[2] Toxicity assay chip has one concentric dosing aperture incised while the multi-choice assay chip has three dosing apertures equilaterally spaced by 17mm from the midpoint.
[3] Toxicity assay chip has one channel whereas the multi-choice assay chip has three evenly spaced 4.7mm apart from the middle.
[4] Toxicity assay chip has one concentric through-hole incised while the multi-choice assay chip has three that are equilaterally spaced by 17mm from the midpoint.
[5] Device operations were done at the same time (start and finish) to enhance reliability of results and remove the possibility and effects of clock gene dependency.
[6] Single chemicals (e.g. 2mM, 20mM, and 70mM zinc and 1%, 5%, and 15% acetic acid solutions) were assayed

2. Chemicals were continually added until a 5cm column height was reached
3. 45 adult flies (25 female and 20 male) were anesthetized and counted into a stock bottle.
4. The stock bottle was then capped onto the placement ring of the top PDMS layer.
5. The set-up was placed in an enclosed box and in the dark to allow the flies to oviposit for 24 hours.

2.3. OVIPOSITION AND VIABILITY RATE QUANTIFICATION

Viability Rate

1. Chemicals were syringed out of the device.
2. The device was turned over to count the number of adult fly deaths and survivals.
3. The number of adult flies that survived out of the 45 was converted into a percentage value.

Oviposition Rate

4. The stock bottle with the adult flies was removed exposing the oviposition platform.
5. The number of eggs deposited inside and outside of each oviposition sites were quantified via a dissection probe and a microscope.

independently with the toxicity assay chip. The multi-choice assay chip was done via delivering 2mM and 70mM of zinc or 5% and 15% of acetic acid simultaneously with water as the control. For the chemical delivery characterizations, methylene blue was used in both cases.