INVESTIGATION OF OVIPOSITIONAL RESPONSES OF DROSOPHILA MELANOGASTER TO SURFACE MODIFICATION USING PDMS THROUGH-HOLE MEMBRANES

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ABSTRACT

Drosophila melanogaster is a widely-studied model organism in developmental biology. Oviposition is an important assay in fly studies which also should be controlled for experiments that study biological pathways through synchronized embryos. It has been used as an assessment tool to investigate biological responses to environmental cues, such as substrate textures, but in relatively uncontrolled manual manners. Microfluidics have recently been introduced to enhance accuracy and control in many Drosophila assays. Here, we have used microtechnology to study oviposition quantitatively in response to accurate and repeatable exposures to various desirable agar surfaces using a polydimethylsiloxane (PDMS)-based through-hole membrane patterning technique.

KEYWORDS: Drosophila melanogaster, Fruit Fly, Oviposition, Egg-laying, PDMS Membrane, Agar Plate, Surface Modification

INTRODUCTION

Drosophila melanogaster (fruit fly) has been used as a model organism in biology due to its shared biological pathways with humans, short generation times, and the existence of full genomic sequences [1-3]. Behavioral studies such as investigation of oviposition have played an important role in understanding biological processes in fruit flies. Oviposition studies the egg-laying site choices of Drosophila in response to genetic and environmental cues such as physical substrate textures and their chemical compositions [4-8]. Current texture studies are not highly controllable since most of the experiments create their textured substrates by using pre-existing objects (e.g., seeded grapes) or manually incising standard agar medium (conventional laboratory platform for oviposition assays) using sharp objects (e.g., a needle) [7-8]. These techniques have caused a lot of inconsistencies in results because the texture patterns and their intensities were produced differently in each trial. Recently, microfluidic devices have been integrated into Drosophila studies to provide very precise and accurate results [9-11]. They are capable of performing high-throughput transgenic micro-injections in embryos [9] and immobilization of live embryos and larvae for imaging of their developmental and cellular responses to external stimuli (ex. auditory signals) [10-11]. Here, we have applied simple microtechnology techniques such as soft-lithographic surface modification to controllably texture and pattern agar-juice medium, using polydimethylsiloxane (PDMS) membranes, to provide precise exposure to surfaces for quantitative oviposition assays.

EXPERIMENTAL

Our first experimental setup consisted of a grape-juice agar-plate (Fig. 1a, used conventionally for oviposition assays), a through-hole PDMS membrane (hole diameters $d=0.5, 2, 4$ and $8\,\text{mm}$) laid on the agar surface to control exposure area (Fig. 1a), and a stock-bottle to contain adult fruit flies that was inversely attached to the agar-membrane assembly (Fig. 1d). After making the agar-substrate-PDMS membrane assembly with a desired exposure area (controlled by the through-holes), fruit flies (~5 day-old, 25 female and 20 males) were transferred into the stock-bottle, attached to the substrate assembly and left for 24hr in the dark at 21°C for oviposition.

Similarly, in the second experiment, we designed substrates patterned hexagonally with 7 through-holes using diameters and spacing of $0.5, 2$ and $4\,\text{mm}$ (Fig. 1c) and conducted the oviposition assay (Fig. 1b).
1d). In both assays, the survival of adult flies was examined and the number of embryos deposited inside and outside of the through-holes were counted after the 24hr oviposition duration.

![Figure 1: A PDMS membrane-based surface modification technique for control of fruit flies exposure to grape-juice agar in Petri dishes. (a) single-hole substrate platform with PDMS through-hole diameter \(d=8\)mm (b) magnified 8mm-hole substrate after oviposition assay (embryos deposited along edges). (c) magnified hexagonally patterned 7-hole \(d=4\)mm, with equal spacing \(s=2\)mm substrate after oviposition (d) experimental set-up](image)

**RESULTS AND DISCUSSION**

The single-hole oviposition assay was conducted as discussed above. Animals’ survival rate (Fig. 2) and total number of embryos laid inside and outside PDMS through-holes (Fig. 3) were assessed. Survival rate was \(\approx 100\%\) for pure agar while only \(50.8 \pm 5.3\%\) standard error (SE) for pure PDMS surfaces. When the agar exposure was increased (by increasing the diameter of the PDMS through-holes), the survival rapidly increased to \(100\%\) (Fig. 2). This showed that pure PDMS alone was incapable of sustaining most of the 45 flies due to the lack of food and fluids; however, a hole of 0.5mm in diameter or larger could achieve more than \(93.3 \pm 9.4\%\) SE survival rate \((p=4.4 \times 10^{-10}\), Student t-Test).

![Figure 2: Animal survival percentages \(n=25\) females and \(20\) males) on different substrate conditions (single-holes membranes of various diameters) corresponding to exposure-to-agar percentages of \(0\%\) (PDMS), \(0.016\%\) (0.5mm hole), \(0.25\%\) (2mm hole), \(1\%\) (4mm hole), \(4\%\) (8mm hole), and \(100\%\) (Pure Agar). Increasing exposure area by just \(0.016\%\) (0.5mm hole) increased survival significantly \((p=4.42908 \times 10^{-10}\) (t-test)).](image)

Flies avoided laying eggs on pure and 0.5-2mm single-hole PDMS surfaces (Fig. 3), but the number of eggs increased significantly at exposure diameters of \(\geq 4\)mm, which were similar to pure agar. This showed that the flies residing on pure PDMS and 0.5-2mm hole diameter substrates were under physiological stress, potentially since fluids and nutrients were scarce. Thus, less energy was expended on oviposition unlike when diameters of more than 4mm were used. It was also observed that most (73\%) of the eggs were laid inside holes along the edges on the exposed agar (Figs. 1b and 3), which shows that in addition to access to desirable agar surfaces, surface texture is also an important factor in egg-laying.

Likewise, the survival rate in the second 7-hole array assay was 100\% (even for \(d=0.5\)mm 7-hole array) for all platforms except for pure PDMS. However, flies still avoided ovipositing inside 0.5mm-diameter holes although provided with a 7-fold higher surface exposure (Fig. 4), demonstrating the importance of hole-diameter in oviposition. Oviposition on arrayed holes larger than \(d=0.5\)mm showed a statistically-significant increase and similarity to agar control. Exposures on these arrayed platforms \((d \geq 2\)mm) were abundant enough for energy to be expended on reproduction. When comparing the single and the 7-hole platforms with \(d=2\)mm (Figs. 3 and 4), a single-hole access to agar was insufficient for flies to exhibit natural (i.e. similar to agar) oviposition while the increase in the number of holes (7-hole array) did not inhibit this behavior. Therefore, we hypothesize that the threshold for agar-like oviposition

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is between one and seven 2mm-diameter holes. Lastly, spacing between holes had no considerable effect on survival or oviposition for this assay.

Figure 3: Mean number of eggs laid on pure PDMS and agar substrates (no hole) as well as inside (white columns) the single holes (d=0.5, 2, 4, 8mm) and on top of the entire substrate (black columns) with standard errors of mean.

Figure 4: Mean number of eggs laid on pure PDMS and agar substrates (no hole) as well as inside (white columns) the 7-hole array platforms and on top of the entire substrate (black column) with standard errors of mean. Agar exposures higher than 1.75% (2mm) yielded to be statistically significant; p=6.96097x10^{-16} (t-test).

CONCLUSION
This is the first behavioral investigation of Drosophila’s oviposition on surface-modified agar platforms using geometrically-accurate PDMS membranes. Our work can be applied to designing microfluidic chips for embryo collection and to behavioral investigations in D. melanogaster.

REFERENCES

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