Investigation of the Mechanical Property of *Drosophila* Mature Oocytes using Microfluidic Devices

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Primary CNF Tools Used: Lithography toolset, PDMS casting station

Abstract:

This project aims to study the mechanical properties of mature oocytes of the fruit fly *Drosophila melanogaster* using a microfluidic device, to better understand a conserved biological process required for embryonic development, egg activation.

Introduction:

At the end of oogenesis, the mature oocyte is developmentally arrested and needs to be "activated" to transition to embryonic development. Egg activation is conserved across species and is accompanied by a rise of intracellular calcium. In vertebrates, this calcium rise is triggered by fertilization. However, in *Drosophila*, despite conserved downstream events, egg activation is uncoupled from fertilization, and is triggered by mechanical pressure during ovulation. This process can be mimicked *in vitro* by letting the mature oocyte swell in a hypotonic solution containing calcium: the oocyte experiences pressure and takes up calcium-containing fluid from the environment, initiating a calcium rise and activating. The calcium rise initiates at the oocyte poles, and traverses the entire oocyte as a wave (analogous to the calcium waves seen in activating vertebrate and echinoderm oocytes). The *Drosophila* calcium wave can be visualized by our genetically-encoded calcium sensors GCaMP3 and GCaMP6.

Summary of Research:

To better understand the trigger for *Drosophila* egg activation we need to elucidate the mechanical requirement that initiates the calcium wave. Although all-round pressure on the membrane in a French Press can accelerate activation, and all-around pressure on the membrane as the oocyte swells in a hypotonic solution can to trigger calcium wave, our preliminary data have shown that point pressure by a microneedle can only induce a local rise of calcium level that does not propagate like a wave. This suggests to us that a larger regional or circumferential force must be needed to trigger the calcium wave.

To test this, we designed a set of microfluidic chambers with a tunnel-like shape and a narrow constriction in the middle. Mature oocytes can be squeezed through the constriction and we can observe whether a calcium wave starts when the oocyte experiences pressure from the narrow constriction. The wafers used as a mold for the chambers were fabricated at CNF. We then used them to cast our microfluidic devices using PDMS.

GCaMP-containing mature oocytes were loaded into a chamber, pushed through the constriction with syringe-driven fluid flow under a fluorescence microscope to check calcium level changes. To date, we have examined 27 oocytes, but only one of them showed a calcium rise when passing through the constriction in the chamber. Currently we are optimizing the design of the device to increase the chance of inducing a calcium rise and to obtain statistically meaningful results. One way would be to reduce the size of the constriction so that oocytes cannot pass through, allowing us to apply greater pressure using a syringe. The other possibility is to increase the length of constricted part of the tunnel so that oocytes are exposed to pressure for a longer period of time.