

Vibration-Assisted On-Chip Oocyte Denudation

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Primary CNF Tools Used: ABM Contact Aligner, Heidelberg Mask Writer - DWL2000, Dicing Saw - DISCO, P7 Profilometer - Versalaser Engraver/Cutter Tool

Abstract:

We developed an open-surface microfluidic platform for on-chip removal of cumulus cells and oocyte denudation through vibration-induced flow. Unlike traditional methods that depend on repeated manual pipetting—labor-intensive, operator-dependent, and potentially stressful for oocytes—our technique employs precisely controlled fluid movement around microscale structures. This method effectively detaches cumulus cells while keeping the oocytes securely within the chip.

Summary of Research:

Recent advances in micro/nanofabrication and microfluidics have improved the precise manipulation of biological cells. We engineered an open-surface microfluidic platform for on-chip removal of cumulus cells and denudation of mouse oocytes, utilizing vibration-induced flow (VIF). (Favakeh et al.) This system features a microstructured chip positioned on a computer-controlled XY piezoelectric stage. The stage was custom-designed on a PMMA substrate using a VersaLASER tool, with two piezoelectric actuators embedded along the X and Y axes. Applying two sinusoidal signals with a 90° phase difference to these actuators produces circular vibrations and localized streaming around the micropillars, as shown in Figure 1.

Figure 2 illustrates that the open-surface chip can be manufactured through various microfabrication methods, such as direct SU-8 photolithography on glass, PDMS soft lithography, hot embossing with polystyrene, and 3D printing. These techniques facilitate the creation of cost-effective, open-surface devices that simplify the loading, observation, and recovery of oocytes with a pipette.

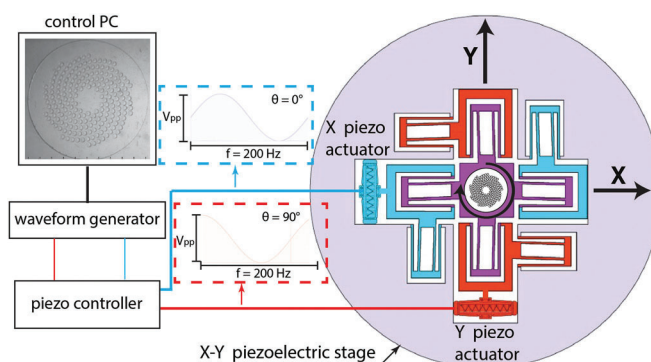


Figure 1: Vibration-induced flow platform. Schematic of the experimental setup, including the control computer, waveform generator, piezoelectric controller, custom XY piezoelectric stage, and open-surface microstructured chip. Two sinusoidal signals with a 90° phase difference are applied to the X- and Y-axis piezoelectric actuators to generate circular vibration.

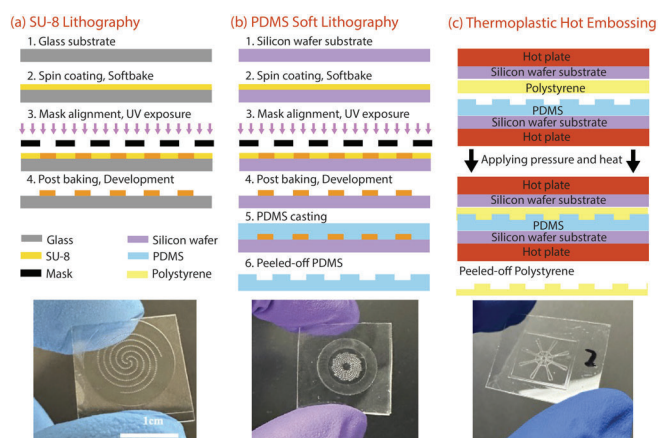


Figure 2: Fabrication methods for the open-surface microstructured chip. Schematic illustration of chip fabrication using (A) direct SU-8 photolithography on glass, (B) PDMS soft lithography from an SU-8 master, and (C) hot embossing of polystyrene using a PDMS mold.

Figure 3 illustrates the flow field and size-based separation mechanism. Each micropillar vibrates circularly, creating a localized whirling flow. The interactions between neighboring pillars' flow fields facilitate fluid movement across the micropillar array. The pillar diameter, spacing, and arrangement were optimized so that larger cells stay confined in the central chamber, while smaller detached cells and debris pass through the gaps and are transported outward.

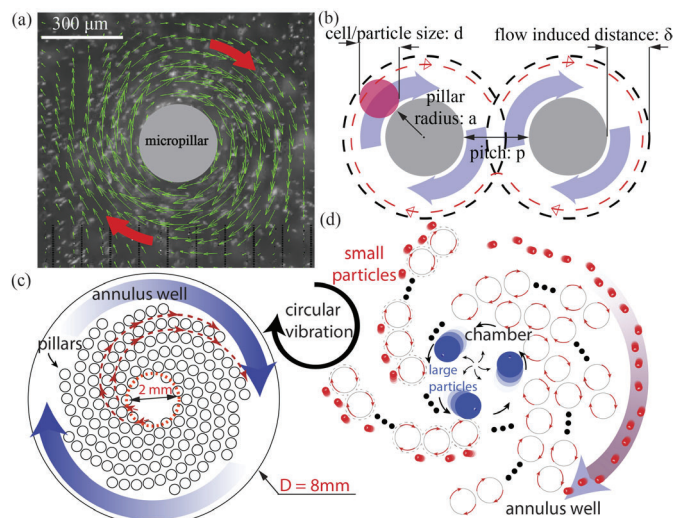


Figure 3: VIF-based flow generation and size-selective separation. (A) Circular vibration generates localized whirling flow around each micropillar. (B) Transportation flow caused by circular vibration through the array of pillars. (C) Topographical feature of induced flow by circular vibration through the spiral pillar array for the proposed chip. (D) Particle size separation using VIF.

Figure 4 depicts the on-chip denudation process. Cumulus–oocyte complexes are initially loaded into the central chamber. Applying circular vibration creates vortical flows around the micropillars, which transport and rotate the complexes within the array. This controlled navigation ensures the oocytes move through the micropillars in the loading chamber area, while detached cumulus cells are effectively separated. The denuded oocytes are confined and easily collected from the open-surface device. These results indicate that VIF provides a controllable platform for automated oocyte processing.

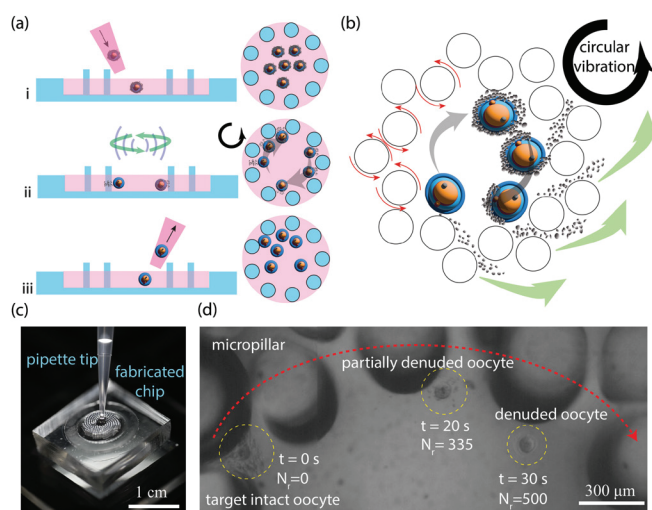


Figure 4: On-chip cumulus removal and oocyte denudation procedure. (A) Cumulus–oocyte complexes are loaded into the central chamber, (B) transported and rotated by vibration-induced flow, and exposed to hydrodynamic forces that detach the surrounding cumulus cells. The denuded oocytes remain confined within the device and (C) can be recovered using a pipette. (D) Stacked images of freeze/thaw oocyte denudation within the loading chamber at different times (t) and different numbers of rotations (N_r).

Conclusions and Future Steps:

We developed an open-surface VIF platform for on-chip cell separation and mouse oocyte denudation. Circular vibration produced localized flow around micropillars, enabling oocyte transport, rotation, and removal of surrounding cumulus cells. The device kept denuded oocytes in the central chamber while moving smaller cells and debris outward. This shows that VIF provides a simple, low-cost, contact-minimized method for oocyte denudation, potentially reducing manual pipetting.

References:

- [1] Favakeh, Amirhossein, et al. "On-Chip Oocyte Cumulus Removal Using Vibration-Induced Flow." *Lab on a Chip*, vol. 25, no. 21, Oct. 2025, pp. 5551–62.