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Title: Assembly and Operation of an Acoustofluidic Device for Enhanced Delivery of Molecular Compounds to Cells

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

   If **Yes**, can you record movies/images using your own microscope camera?

   No

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   No

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 19
Number of Shots: 43

Introduction

1. Introductory Interview Statements

Videographer: VO talent will read the introduction and conclusion statements, so you don't need to film those.

1.1. This protocol describes the assembly and operation of an acoustofluidic system that can efficiently deliver biomolecules for a variety of research and cell-based therapeutic applications.

1.1.1. *2.2.1.*

1.2. The main advantage of this system is that it allows rapid delivery of biomolecules to cells while maintaining their viability. It is a platform technology that allows cells to be transformed for a variety of cellular therapeutic applications such as cancer treatment.

1.2.1. *2.6.2.*

Ethics Title Card

1.3. Human blood samples were collected following protocols approved by the Institutional Review Board at the University of Louisville.

Protocol

2. Fabrication of acoustofluidic device

2.1. Begin by combining 54 grams of PDMS base and 6 grams of curing agent in a cup **[1]**. Mix vigorously and thoroughly with a spatula for at least 1 minute **[2]**, then place the cup with the PDMS solution into a desiccator for approximately 30 minutes or until remnant air bubbles are removed **[3]**.

2.1.1. WIDE: Establishing shot of talent combining PDMS and curing agent.

2.1.2. Talent mixing the PDMS solution.

2.1.3. Talent placing the cup into a desiccator.

2.2. Place a photoresist-coated wafer with the patterns facing upward in a 150-millimeter Petri dish **[1]**, then pour the PDMS solution over the mold **[2]**. If needed, place the Petri dish inside a desiccator and apply vacuum until remnant air bubbles disappear **[3]**.

2.2.1. Talent placing the wafer in the Petri dish.

2.2.2. Talent pouring the PDMS solution over the mold.

2.2.3. Talent putting the Petri dish in the desiccator.

2.3. Transfer the Petri dish into a lab oven and bake for 2 hours at 60 degrees Celsius to cure the PDMS **[1]**. After curing, carefully remove the PDMS from the Petri dish by cutting around the edges of the wafer with a razor blade **[2]**.

2.3.1. Talent putting the dish in the oven.

2.3.2. Talent cutting around the wafer and removing the PDMS.

2.4. Cut out each individual device using a knife or razor blade **[1]**, then punch holes through the inlet and outlet ports using a 2.5-millimeter biopsy punch **[2]**.

2.4.1. Talent cutting out individual devices.

2.4.2. Talent punching holes through the inlet and outlet ports.

2.5. After treating the PDMS device with oxygen plasma, immediately place each device onto a clean soda lime glass microscope slide with channels facing the glass surface. Allow the devices to bond overnight at room temperature **[1]**.

2.5.1. Talent placing the device on a slide.

2.6. Gently apply silicone to the surface of the 1-centimeter diameter piezo transducer at a thickness of 1 to 2 millimeters **[1]**, then carefully align the transducer with the concentric spiral and gently press it onto the bottom of the glass microscope slide **[2]**. *Videographer: This step is important!*

2.6.1. Talent applying the silicone.

2.6.2. Talent aligning the transducer and pressing it into the bottom of the microscope slide.

3. Assembly and operation of acoustofluidic system

3.1. Connect a microcontroller to a computer using a USB A to B cable **[1]**. A green power LED indicator should illuminate **[2]**. Use the associated software on the computer to upload a program that generates an 8-megahertz signal **[3]**.

3.1.1. Talent connecting the microcontroller to the computer.

3.1.2. Power indicator lighting up.

3.1.3. SCREEN: 2021-04-20 13-32-00.mkv. 0:05 – 0:21.

3.2. Solder a 1-inch 22-gauge wire to the end of each wire on the PZT transducer **[1]**, then use it to connect the negative terminal wire of the PZT transducer to a GND pin **[2]**.

3.2.1. Talent soldering the wires.

3.2.2. Talent connecting the negative terminal wire of PZT transducer to a GND pin.

3.3. Connect the positive terminal wire of the PZT transducer to the output pin via the soldered wire **[1]**. *Videographer: This step is important!*

3.3.1. Talent connecting the positive terminal wire of PZT transducer to the output pin.

3.4. Cut 3 to 6-inch sections of tygon PVC soft plastic tubing **[1]** and push the tubing into the inlet and outlet ports. It may be necessary to rotate the tubing while applying pressure until it fits in the opening **[2]**. Optionally, glue can be applied at the junction to bond the PDMS and tubing together **[3]**. Optionally, mount the acoustofluidic device and the microcontroller in a 3D-printed case **[4]**. *Videographer: This step is difficult and important!*

3.4.1. Talent cutting the tubing.

3.4.2. Talent pushing the tubing into the inlet and outlet ports.

3.4.3. Talent applying glue at the junction.

3.4.4. Talent mounting the device and microcontroller in a 3D-printed case.

NOTE: The shot number 3.3.2 is moved and placed here after shot number 3.4.3

3.5. Assemble the microfluidic reservoir according to manufacturer's instructions **[1]**.

3.5.1. Talent assembling the reservoir.

3.6. Cut a 3 to 6-inch section of tygon PVC soft plastic tubing and push it over the ID tubing from the microfluidic reservoir output tubing **[1]**. Optionally, wrap the junction with paraffin film to prevent leakage **[2]**. Fill a 60-milliliter syringe with ambient air on the side of the microfluidic reservoir **[3]**.

3.6.1. Talent pushing the PVC soft plastic tubing over the ID tubing.

3.6.2. Talent wrapping the junction with paraffin.

3.6.3. Talent filling the syringe with air.

3.7. Set the syringe pump to a rate of 200 milliliters per hour to push the contrast agent solutions through the acoustofluidic device at a volumetric flow rate of 50 milliliters per hour **[1]** and collect the samples from the output of the device into a 50-milliliter centrifuge tube **[2]**.

3.7.1. Talent setting the syringe pump rate.

3.7.2. Talent positioning the centrifuge tube.

4. Preparation of ultrasound contrast agents

4.1. Prepare a phospholipid solution in a 20-milliliter scintillation vial by adding 25 milligrams of DSPC, 11.6 milligrams of DSEPC, 0.26 milligrams of DSPG, and 0.88 milligrams of polyoxyethylene 40 stearate **[1-TXT]**. Add chloroform until all phospholipids are dissolved **[2]**.

4.1.1. Talent adding DSPC and DSEPC to the vial. **TEXT: DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine; DSEPC: 1,2-distearoyl-sn-glycero-3-ethylphosphocholine; DSPG: 1,2-distearoyl-sn-glycero-3-phosphoglycerol**

4.1.2. Talent adding chloroform and the lipids dissolving.

4.2. Evaporate chloroform in a desiccator for 48 hours to form a dry lipid film **[1]**. Rehydrate the film with 10 milliliters of sterile PBS **[2]**, then sonicate the lipid solution for 3 minutes at 40% amplitude to form a cationic micellar solution **[3]**.

4.2.1. Talent putting the solution in the desiccator.

4.2.2. Talent adding PBS to the lipid film.

4.2.3. Talent sonicating the solution.

4.3. After sonication, the phospholipid solution can be stored at 2 to 6 degrees Celsius for up to 1 month **[1]**.

4.3.1. Talent putting the solution in the refrigerator.

4.4. To prepare ultrasound contrast agents, add 200 microliters of cationic micellar solution and 600 microliters of sterile PBS to a 2-milliliter glass septum vial **[1]**. Seal the vial by crimping the cap **[2]**.

4.4.1. Talent adding cationic micellar solution and PBS to the vial.

4.4.2. Talent sealing the vial.

4.5. Use a 1.5-inch 20-gauge needle to fill the vial head space with decafluorobutane gas for 30 seconds **[1]**. Amalgamate the vial to form perfluorobutane gas-filled ultrasound contrast agents **[2-TXT]**. *Videographer: This step is important!*

4.5.1. Talent filling the vial head space with decafluorobutane gas.

4.5.2. Talent amalgamating the vial. **TEXT: 45 s at 4,350 cpm**

4.6. Add 25 microliters of ultrasound contrast agent solution per 1 milliliter of cell solution **[1]**, then immediately pump the combined contrast agent and cell mixture through the acoustofluidic device **[2]**. *Videographer: This step is important!*

4.6.1. Talent adding contrast agent to cell solution.

4.6.2. Talent pumping the mixture through the device.

Results

5. Results: Acoustofluidic treatment enhances intracellular molecular delivery

5.1. This protocol produces an acoustofluidic system that can be used to enhance intracellular molecular delivery in multiple cell lines. Intracellular delivery of a fluorescent compound, fluorescein, to primary human T cells was improved with acoustofluidic treatment compared to an untreated control group **[1]**.

5.1.1. LAB MEDIA: Figure 2 C.

5.2. The fluorescence intensity of T cells increased by 5-fold after treatment, indicating enhanced delivery of fluorescein **[1]**. Cell viability decreased slightly after acoustofluidic treatment but remained above 80% **[2]**.

5.2.1. LAB MEDIA: Figure 2 A. *Video Editor: Emphasize the treatment bar.*

5.2.2. LAB MEDIA: Figure 2 B.

5.3. Acoustofluidic treatment enhanced intracellular delivery of a preservative compound, trehalose, to human A549 lung carcinoma cells compared to flow alone and to cells in the untreated control group **[1]**.

5.3.1. LAB MEDIA: Figure 3 A.

Conclusion

6. Conclusion Interview Statements

6.1. Adding the cationic microbubbles to the cell solution is critical. Intracellular delivery of biomolecules will be limited without cationic microbubbles in solution.

6.1.1. *4.6.1, 4.6.2.*

6.2. This platform enables intracellular delivery of various biomolecules, which can alter cellular function for research or therapeutic purposes. After completing this procedure, additional cellular characterization can be performed to evaluate the impact of delivering various biomolecules.

6.2.1. *4.1.1, 4.1.2.*