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

Supplementary information

Cell culture:

The AC16 Human Cardiomyocite cells (EMD Millipore Corporation, USA) were cultured in Cellstar 6-wells plates of 3.5 cm of diameter (Greiner Bio-One, Austria) at 37ºC and 5% of CO_2 in a MCO-50AIC-PE IncuSafe CO2 Incubator. Culture media was obtained by a mixture of DMEM / F12 (Gibco, USA) with 12% of Fetal Bovine Serum (Gibco, USA) and 1x of Penicillin Streptomycin Glutamine (Gibco, USA).

Cell detachment from incubation well, extraction and counting:

First, the 6-wells plate is taken from the incubator. The culture media (3 mL) is removed using by pipetting. Then, the cells are rinsed with PBS (VWR International, USA) for removing the supernatant. 2 mL of PBS are poured in the well, the plate is gently stirred and then the PBS is withdrawn. This process is repeated 4 times. Then, 2 mL of trypsin 0.25% (Cytiva, USA) are poured in the well and incubated for 5 min at 37ºC in the incubator for cell detachment. After this time, 2 mL of culture media are added for stopping trypsin action. The total 4 mL are collected in a 15 mL tube (VWR International, USA) and centrifuged at 300G for 5 minutes using a Compact Star CS8 centrifuge (VWR International, USA) for cell separation from the media. The fluid is then removed and 1 mL of culture media is added. The cells are then concentrated in 1 mL of culture media in a 1.5 mL Eppendorf tube (Eppendorf, Germany). This tube is centrifuged again at 300G for 5 minutes using a Micro Star 12 centrifuge (VWR International, USA) for further washing of the cells from the supernatant. The culture media is withdrawn and 1 mL of culture media is then added. Finally, we obtain the cells in cultured in a well concentrated in 1 mL.
For cell counting, an Invitrogen Countess 3 Automated Cell Counter (Invitrogen, USA) is used. Following the device User Guide, 20 µL of trypan blue and 20 µL of cells are added to an Eppendorf and mixed by pipetting it up and down. Then, 10 µL are pipetted into the Cell counting Chamber Slide by Invitrogen. The sample is let to settle for 30 seconds and then the slide is inserted in the device. The device will provide a counting of cells/mL. This process is done 4 times in total. The cell concentration is then calculated as a mean of the four measurements.

Fluorescent imaging:

For fluorescent imaging of alive/dead cells we use Sytox Orange (ThermoFisher, USA) and Calcein AM (Biotium) dyes. For sample preparation, we follow the User Guides provided by the company.
A solution of 1/1000 of Sytox and 1/250 of Calcein are done. In a different tube, a solution 1/10 of cells is done with a mixture 1/1 of both dyes. Then, the sample is incubated for 30 minutes at 37ºC (15 minutes for the Calcein and 30 minutes for the Sytox are required).
Then, the sample is centrifuged at 300 rpm for 5 minutes for removing the supernatant. Then, the same volumen of PBS as the withdrawn supernatant is added.

Chemical lysis

User-developed protocol *"Purification of genomic DNA from cultured cells using the QIAamp® DNA Micro Kit"* is followed. The chemicals for the chemical lysis were purchased from QIAGEN (Germany).
First, 20 µL of cells suspension were added to a 1.5 mL Eppendorf. Then, 80 µL of ATL buffer were added. After that, 10 µL of Proteinase K were added. Finally, 100 µL of AL buffer were added. Then, the mixture is pulse-vortexed for 15 seconds and finally incubated for 10 minutes at 56 ºC
The extracted DNA, used as a 100% lysis reference is measured using a Qubit 3 (ThermoFisher, USA).

DNA content measurement using Qubit

Following *"Qubit 1X dsDNA HS Assay Kit"* user guide:
Qubit working solution (ThermoFisher, USA) and cells sample are added to a Qubit tube to a final volume of 200 µL.
The volume of the sample can be 1 – 20 µL.
Sample and working solution are mixed in the tube by pipetting.
The final mixture needs an incubation time of 2 minutes before being measured.

SAW device fabrication

The IDT's are fabricated by photolithography on 4" 128º Y-X lithium niobite double-side polished wafers of 500um of thickness (University Wafer Inc, USA). The substrates are cleaned using acetone and IPA and with oxygen plasma for 5min plasma cleaner at 50/50 (Diener Electronics, Germany). After this, the sample is heated at 115ºC for 5 min for removing all the remaining humidity on the substrate. The photoresist SPR700 (Micro Resist technology, Germany) is spined at 4000rpm with a ramp of 1000rpm for 45s, obtaining a 900nm thick layer. Then the sample is heated at 105ºC for 1 min. Photolithography is done using a MLA150 (Heidelberg, Germany) using 110 mj/cm^2. Then the simple is heated at 115ºC for 1 minute for Post-exposure baking. The development is done using MF-26A (Micro Resist technology, Germany) for 1 minute, rinsed with water and dried using N2. After the development, a 10nm Ti layer and an 80nm Au layer are deposited on the substrate using e-beam of the AJA Sputter and Evaporator (AJA International Inc, USA). After the metal deposition, the lift-off is performed for removing the photoresist, leaving only the IDT pattern formed by the two metal layers. For this, the sample is placed in a beaker with acetone and subjected to an ultrasonic bath for 1 minute. Then the sample is rinsed with acetone and IPA and dried with N2.

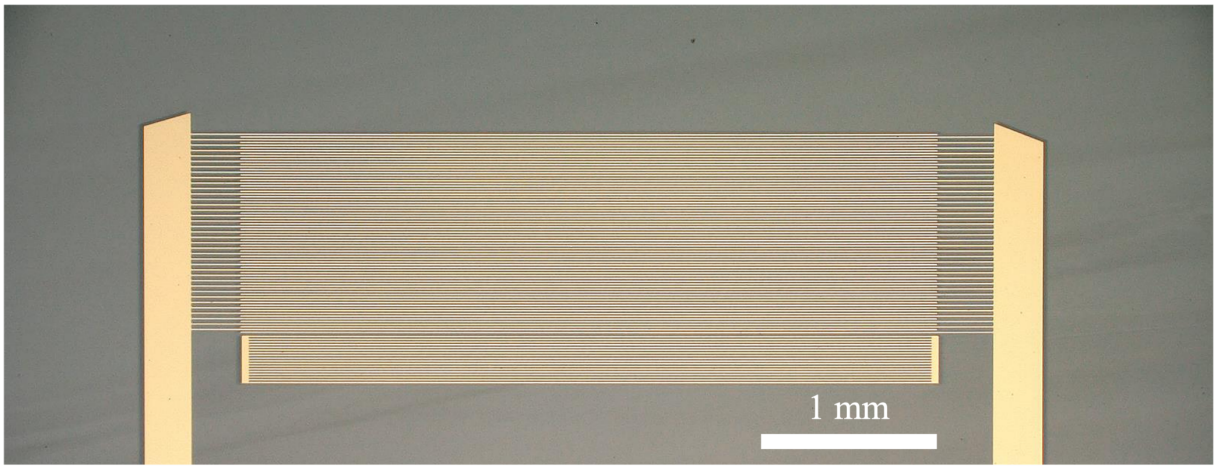

Figure 1: 120MHz Gold IDT

Facility description:

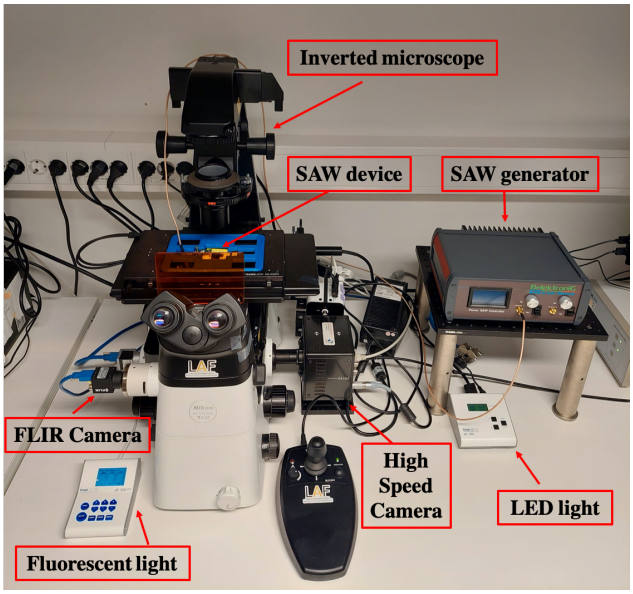

Figure 2: Setup

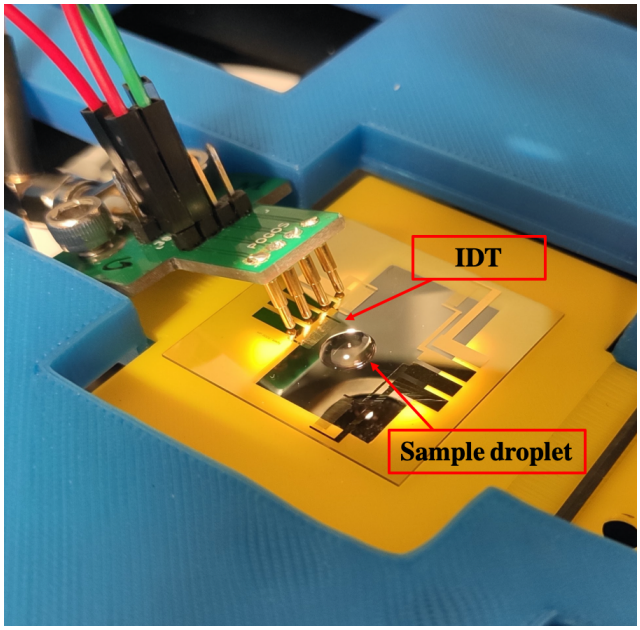

Figure 3: Device experiment setup

The setup is formed by a Nikon ECLIPSE Ti2 (Nikon, Japan) fluorescent inverted microscope. The fabricated SAW device in mounted over the microscope on a 3D printed stage and connected to a SAW generator (Belektronig, Germany). A FLIR Blackfly S camera (FLIR, USA) camera for visualization of the droplet placing and the flow inside the droplet. A pE-100 LED light (CoolLED Limited, UK) is used for visualization. For fluorescent imaging of the alive and dead cells a pE-300 Ultra fluorescent light (CoolLED Limited, UK) is used. A high speed camera Photron FASTCAM UX100 (Serof, Norway) is used for high frame rate acquisition for PIV study.

PIV:

The PIV study was done in order to measure the flow velocity and shear stress created inside the droplet by the actuation of the SAW at for the four different powers used for lysis efficiency assessment (100, 300, 500 and 1000 mW). A solution of culture media and 1µm polystyrene microspheres (Bang Laboratories, USA) was used. In order to reproduce a lysis experiment case, 20 µL droplets of solution were used. Images were recorded 0.5s after applying the SAW on the droplet, using the high speed camera, the fluorescent inverted microscope and LED white light, at frame rates of 1000 fps for the cases of 100 mW and 4000 fps for the rest of the powers. The experiments were repeated at least three times for each case. The frames were processed using DaVis software (LaVision, Germany).

Viscosity Measurements:

Culture media viscosity was measured using a AR-G2 Magnetic Bearing Rheometer (TA Instruments, UK).

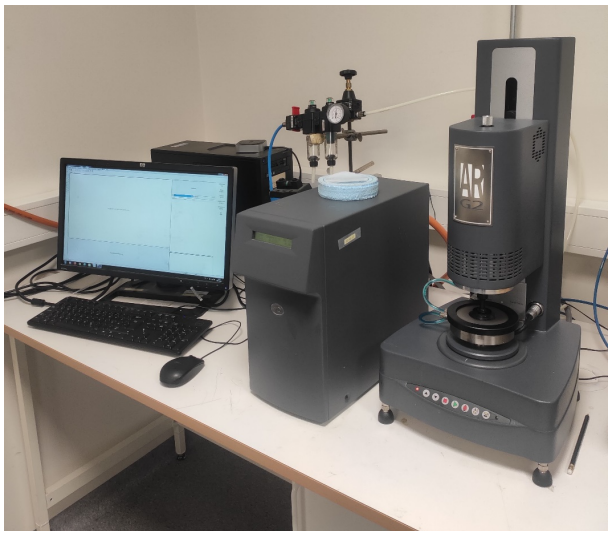

Figure 4: Rheometer

First, a calibration/test experiment was done using DI water. The experiment was performed with a 4 cm plate and 1.3 mL, at shear rates from 40 to 100 1/s.
The measurement of viscosity obtained for DI water was ~ 1*10^-3 Pa·s, being this result consistent with the literature. This experiment confirmed that the tool was properly calibrated and therefore we could proceed to measure trustfully the viscosity of the culture media.
For culture media viscosity measurement, the same plate of 4 cm and 1.3 mL of culture media were used. The experiment was done using a steady state flow.
Viscosity was measure at 5 different shear rates from 40 to 100 1/s. The average measured viscosity for the culture media was 1.3 Pa·s.

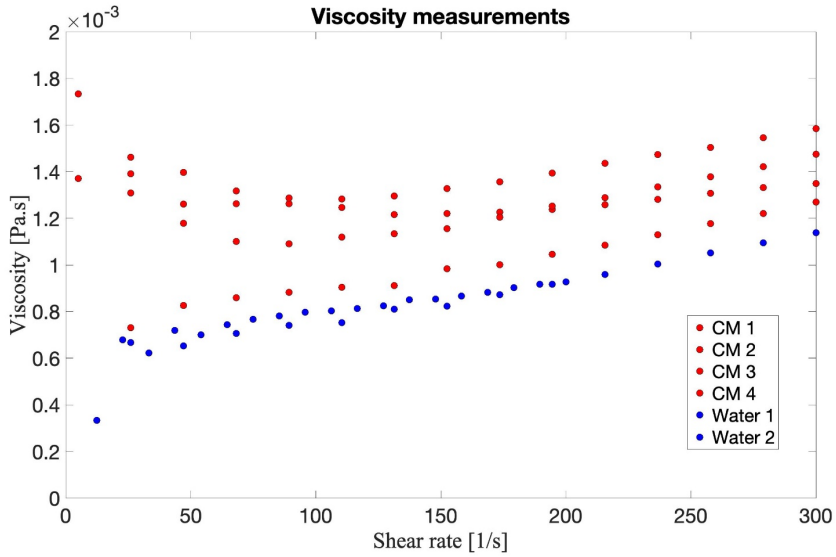

Figure 5: Viscosity measurements of water and culture media using rheometer

Temperature study:

The temperature effect of the acoustofluidic device on the sample droplet was studied using a FLIR X6800sc IR camera (FLIR, USA) and FLIR Research IR Max Version 4.40.11.35 software. The data was processed using MatLab.

The experiments were performed for the study of the temperature effect using the acoustofluidic device at the maximum power used for lysis (1 W) for the longer actuation time (120s using cycles of 20s ON/OFF). First, the setup is calibrated using a black body for inhomogeneity correction on the camera generated image and correction of the possible reflections into the camera. Then, a 20 µL droplet is placed with a pipette, in front of the IDT, at a distance between 1-3 mm. The recording is done at 50 fps and the SAW is activated after 15 seconds of recording. The SAW acts on the droplet for 120s on cycles of 20 s ON/OFF. After the actuation of the last cycled, the recording continues for a total recording time of 5 min and 30 seconds. During this time, the sample cools down since no heat is produced by the device.

Then, a second set of experiments is performed at 1 W and 120 seconds without cycles. The SAW is activated at 15 seconds after the start of the recording. After the 120 seconds of SAW actuation, the sample is let to cool down until a total recording time of 200 seconds.

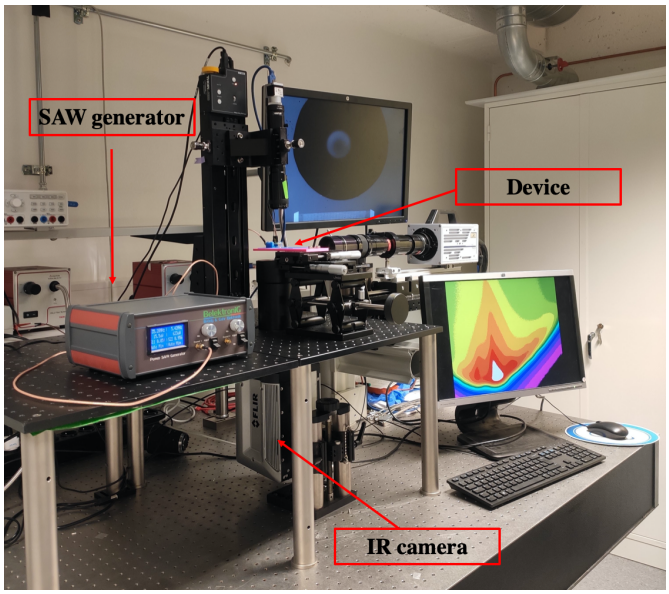

Figure 6: IR setup

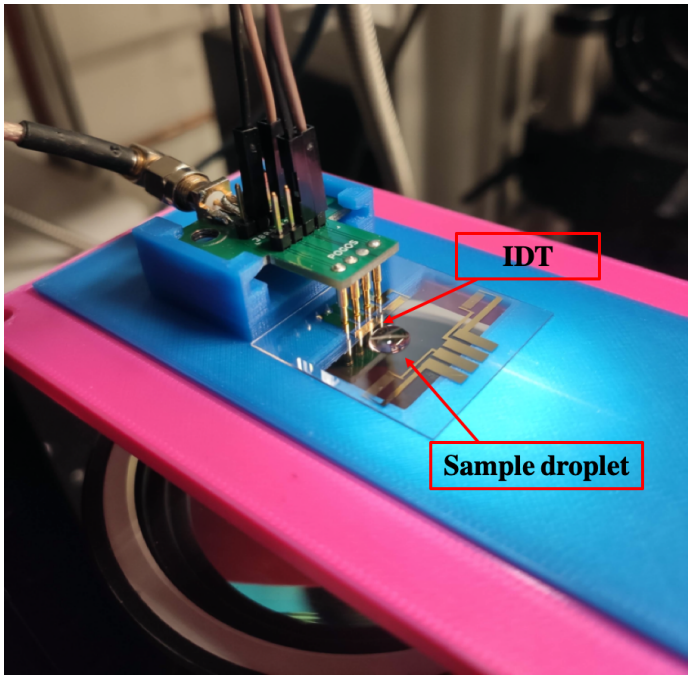

Figure 7: Device on IR setup

RNA Analysis

For this experiment we took 4 independent populations of AC16 cells and carried out 3 replicates of the SAW experiment plus a control sample from each population. The saw experiments were carried out as explained in the materials and method section. Immediately after applying the SAW the samples were placed in 700 ul of QIazol reagent to prevent RNA damage and kept it in a freezer until the study was performed.

For the RNA study, the material was purified and concentrated using a RNAeasy Mini (QIAgen) extraction kit following the protocol specified by the company. Briefly, 140 µl of chloroform was added and kept again at room temperature for 3 minutes. Then the sample was spined for 15 minutes at 12000g at 4C. After this, the upper aqueous phase (containing the RNA) was transfer to a collection tube and mixed with 1.5 volumes of 100% ethanol. This was then transferred to a RNeasy spin column and centrifuged at 8000 g for 15 s where the flowthrough was discarded. The RNA was then eluted using RNase-free water and centrifugation for 1 min at 8000 g.

After purification, the RNA sample purity was measured using by placing 1 µl of the sample in a Nanodrop 2000 and reading the A260/A280 and A260/A230 ratios.

Thermal lysis study:

The effect of the temperature in the DNA content in the sample was studied using a thermal bath at different temperatures. For each temperature, three Eppendorf tubes with a volume of 20 µL were introduced in the thermal bath for 2 minutes. Then, the DNA content of each

sample was measured on the Qubit. The temperatures used in this study were 40, 50, 60 and 70 ºC.

Figure 8: Thermal bath