Development and Characterisation of Acoustofluidic Devices Using Detachable Electrodes Made from PCB

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Abstract:

Acoustofluidics has been increasingly applied in biology, medicine and chemistry due to its versatility in manipulating fluids, cells and nano-/micro-particles. In this paper, we develop a novel and simple technology to fabricate a surface acoustic wave (SAW)-based acoustofluidic device by clamping electrodes made using a printed circuit board (PCB) with a piezoelectric substrate. The PCB-based SAW (PCB-SAW) device is systematically characterised and benchmarked with a SAW device made using the conventional photolithography process with the same specifications. Microparticle manipulations such as streaming in droplets and patterning in microchannels were demonstrated in the PCB-SAW device. In addition, the PCB-SAW device was applied as an acoustic tweezer to pattern lung cancer cells to form three or four traces inside the microchannel in a controllable manner. Cell viability of ~97% was achieved after acoustic manipulation using the PCB-SAW device, which proved its ability as a suitable tool for acoustophoretic applications.

Introduction

Acoustophoresis is a technique well-known for actuating and manipulating micro[1]-/nano[2,3]-particles using acoustic waves. Its applications have been demonstrated in a wide-range of biomedical applications such as separating blood cells and platelets[4], separating circulating tumour cells from whole blood[5,6], isolating exosomes[7,8], washing[9] and coating of cells[10], handling liquid [11] and versatile manipulations of micro-objects[12–20], alongside the continuous development of acoustophoretic theories[21–24] and simulations[25–27]. Acoustophoretic devices using either bulk acoustic waves (BAWs)[4,28] or surface acoustic waves (SAWs) produce an acoustic pressure gradient and streaming within a fluid, thus achieving the capability of actuating micro-/nano-particles inside. SAW-based devices have been intensively investigated in manipulating biological cells because of their versatility and being less-dependent on the acoustic properties of the microchannel material when compared to those made using BAWs[28].

SAW devices are generally fabricated by patterning an interdigitated transducer (IDT)[17] onto piezoelectric substrate such as lithium niobate ($LiNbO_3$). IDTs typically have two comb-shaped arrays of metallic electrodes, which are driven by radio frequency (RF) signals to produce SAWs. The fabrication process of SAW devices typically employs photolithography, which includes the following steps: 1. mask design and manufacturing; 2. spin-coating photoresist onto $LiNbO_3$; 3. mask aligning for patterning with UV light; 4. metal layer deposition; 5. lift-off process to form the IDT[17]. The brittle[29] bulk $LiNbO_3$ substrate is vulnerable during manufacturing and operation. Furthermore, the SAW device made by photolithography is a one-off component, any modification will require to go through the entire aforementioned manufacturing processes again. It is also difficult to repair a damaged substrate (i.e., scratches). The facilities and skills required for making IDTs limit the use of SAW devices. To address these issues and simplify the process, the creation of shear-SAWs has been demonstrated on the surface of $LiNbO_3$ by stacking aluminum foil strips onto the substrate[30]. IDTs have also been created by pouring low-melting point metal into an IDT mold made by PDMS[31]. Superstrates have also been implemented on the conventional SAW devices to allow their reuse for different applications[32].

Interdigital electrodes (IDEs), with a similar pattern as that in SAW devices, have also been fabricated on printed circuit boards (PCBs). They have been applied in various applications such as moisture sensing[33], water level measurements[34], electro wetting[35], biosensing[36] and even cell manipulation[37]. A standard PCB laminate consists of a layer of thin copper foil and an insulating

layer typically laminated together with glass reinforced epoxy resin (FR4). Further choices for core materials are commercially available such as PET (Polyethylene terephthalate), flexible polyimide or Teflon. This allows versatility based on different applications. The fabrication of IDEs on the PCB by metallisation of the copper layer is routinely employed within the industry with a wide variety of gold or silver electroplating processes commercially available.

In this work, we demonstrated a novel SAW device fabrication technique done by mechanically clamping IDEs on the PCB to a $LiNbO_3$ wafer. This PCB-based SAW (PCB-SAW) device was characterised and benchmarked against an IDT with the same specifications made using the conventional photolithography process. The PCB-SAW device was used as an acoustic tweezer to actuate and pattern both polystyrene microspheres and cancer cells. The proof-of-concept demonstrated that the simple mechanical clamping technique could be applied as an alternative to the conventional photolithography, by transferring the photolithography effort in fabricating SAW devices to the mature PCB manufacturing industry.

Methods and materials

Design and working mechanism

The schematic illustration of the PCB-SAW device shown in Figs. 1a and 1b consists of six components: a base plate, a $LiNbO_3$ wafer, a PCB with a pair of patterned IDEs, a clamp, a pressure ring and a microchannel. The base plate supports the $LiNbO_3$ wafer and is bolted with the clamp to apply clamping force between the PCB and the $LiNbO_3$ wafer via the pressure ring. Once a proper clamping force is applied by fastening the four screws on the clamp, the pair of IDEs make good contacts to the $LiNbO_3$ wafer to couple RF signals that generate counter-propagating SAWs to form standing SAWs (SSAWs) between the two IDEs. The microchannel is bonded to the $LiNbO_3$ wafer at the middle between the two IDEs for handling fluid samples. Depending on the size of microparticles and other application parameters, the SAW wavelength can be customised by producing PCBs with alternative IDEs specifications.

PCB-SAW fabrication and experimental setup

The PCB was designed using the Eagle software (Autodesk, US) and manufactured externally (circuitfly.com). All the design files are accessible as supporting files of this work. The PCB design had a conventional IDT pattern for SAW devices with the wavelength of 200 μm, corresponding

to Rayleigh mode frequency of 19.9 MHz. This is based on that the speed of sound in the $LiNbO_3$ is 3,980 m/s. Each IDT consists of 40 pairs of 10 mm (aperture size) long finger electrodes. The manufactured single-sided PCB is shown in Fig. 2a. The thickness of the PCB laminate is 1.6 mm with the IDE layer thickness of 34.8 μm of copper. The IDEs and the buses are exposed without pasted solder mask. The PCB dimensions are 10 cm (L) × 10 cm (W) with a milled open window of 3.5 cm (L) × 1.5 cm (W) at the centre for accommodating the microchannel. Alignment markers (holes and lines) are present on the PCB to help align the microchannel and the $LiNbO_3$ wafer. A microscope was used to check the IDE manufacturing quality, details given in Fig. S1. Two coaxial cables were soldered to the bus pads at the edges of the PCB for signal transmissions.

Before the assembly process, both the PCB IDEs and a 3-inch, 500-μm thick, 128-deg-rotated Y-cut X-propagation $LiNbO_3$ wafer were thoroughly cleaned using isopropyl alcohol (IPA) and inspected under a microscope. The pressure ring, clamp, and base plate were printed using a 3D printer (Ultimaker 2+ extended, Utrecht). The exterior dimensions of the PCB-SAW device are 120 mm (L)×120 mm (W)×30 mm (H). Additionally, two localised pressers for focusing clamping force onto IDE region and a round holder for supporting the $LiNbO_3$ wafer, were also printed.

The assembly process is shown in Fig. 2b. The $LiNbO_3$ wafer was placed onto the round holder and its reference flat edge was aligned to be parallel with IDEs to ensure that the SAW generation was in the direction of the X direction of the $LiNbO_3$. The PCB was then placed on the $LiNbO_3$ wafer with the IDEs facing down. The clamp was mounted to the PCB and bolted to the base plate by slightly fastening the four screws. The pressure ring was screwed into the clamp to provide localised force to the PCB via the localised pressers and then the four screws were fully tightened. There was an observation window on the base plate for light transmission during microscopic measurement. A PDMS microchannel with the channel dimensions of 15 mm (L) × 200 μm (W) × 60 μm (H) was bonded to the $LiNbO_3$ wafer using plasma treatment before the above assembly. Tubing was connected to the inlet and the outlet of the single channel. Fig. 2c shows the assembled PCB-SAW device.

To study the reliability of the assembly, the PCB-SAW device was thoroughly characterised by using *s*-parameters and power transmission test. Details of the electrical characterisation can be found in the Supplementary Information, in which the working frequency is identified and matching networks (MNs) are recommended to couple the power amplifier and the PCB-SAW device to maximise power transmission.

PCB-SAW test with droplet actuation

The device is purely integrated by mechanical clamping and the contact quality between the PCB and the $LiNbO_3$ wafer is associated with the clamping force produced by the bolt torque (Fig. 2b). A droplet actuation test was performed to investigate the relationship between the clamping force and SAW generation indicated by droplet movement. The $LiNbO_3$ substrate was coated with a hydrophobic substance CYTOP™ (AGC Chemicals Europe), which was done by evenly distributing across the $LiNbO_3$ substrate[38].

For the clamping test, the clamping force between the $LiNbO_3$ substrate and the PCB IDEs was increased by adjusting the torque of the M5 screw torque on top of the localised pressers. A digital torque screwdriver (5-50 cNm, Adema, Taiwan) with a digital display was used to apply and read the torque. The torque was converted to clamping force by $F = \frac{T}{cD}$, where F, c, D and T correspond to clamping force (N), coefficient of friction, screw diameter (m) and torque (Nm), respectively. The standard value c for unlubricated steel is equal to 0.2. The readability of the digital torque screwdriver was 0.05 Nm, which allowed a minimum reading of the clamping force of 50 N. The VNA was used to monitor the real-time S_{11} while fastening the bolt. During each assembly, the clamping was adjusted so that the same minimum S_{11} value was achieved. This process facilitated establishing a correlation between the S_{11} and the clamping force, which allowed the use of S_{11} rather than the clamping force to guide the assembly.

Under each measured torque, a 1-µL water droplet was pipetted onto the $LiNbO_3$ substrate 5 mm away from the first finger electrode. Then an input power of 1.26 W was applied to the PCB-SAW device to actuate the droplet. The slight location variance of droplet initial positions in each test is insignificant as the SAW attenuation in the $LiNbO_3$ substrate is negligible[39]. Even though the droplet was placed in nearly identical location before actuation, a variation in speed of droplet transportation can be observed on both the devices. We hypothesise that this could have been caused by slightly uneven CYTOP coating, coating deterioration, slight contact angle variance, droplet volume variation, or a combination of these factors. Therefore, the droplet actuation by SAW was repeated five times before changing to another clamping force. A camera was used to capture the droplet moving and a calibrated software Tracker (www.compadre.org/osp/) was applied off-line to analyse the droplet velocity for indicating SAW amplitude. The captured droplet videos were analysed frame by frame using the leading edge of the droplet, as the reference to

determine the displacement of the same droplet. Any two consecutive frames could produce one velocity using the displacement multiplied by the framerate. Five consecutive frames after the droplet moved were used to get four velocities, which were averaged to get the mean droplet velocity. The pixel size and the frame rate of the camera system were 10 μm and 60 fps, respectively, resulting in a velocity resolution of 0.6 mm/s, which was sufficient for capturing droplet movement.

To benchmark the performance of the PCB-SAW device with the SAW device made by standard photolithography[1], another IDT made by the same $LiNbO_3$ substrate using the conventional photolithography process in cleanroom was prepared using the identical geometry as the PCB-SAW device. The cleanroom-made IDT (CR IDT) was also coated with CYTOP™ for the droplet test.

Sample preparations

To demonstrate the PCB-SAW device capability in manipulating microparticles within droplets, a 3-4 μL glycerol droplet (3 mm in diameter) was prepared on the $LiNbO_3$ substrate and 20 μm polystyrene microspheres were pipetted into the glycerol droplet (concentration of ~18,000 / μL). An input power of 0.2 W was used for manipulating the microspheres.

To demonstrate the PCB-SAW device in manipulating microparticles inside the microchannel, 10 μm polystyrene microspheres were mixed with a custom media at a volume ratio of 1:2.7. The custom media consisted of glycerol and phosphate-buffered saline (PBS) with a volume ratio of 1:4.4, which was made to prevent particle deposition. Before sample introduction, the microchannel was flushed with bovine serum albumin (BSA) solution (water:BSA = 100:1, mass ratio) for 20 min at a flow rate of 20 μL/min. The input power in this experiment was 0.5 W.

For the cell manipulation, the human non-small-cell lung carcinoma (NSCLC) cell line- A549 (ATCC® CCL-185™) were from the American Type Culture Collection (Virginia, USA), and were grown in Dulbecco's modified eagle media and supplemented with L-Glutamine (200 mM at 1:100 dilution, Gibco), Penicillin/Streptomycin (10,000 U/mL at 1:100 dilution, Gibco), and 10% foetal bovine serum (FBS) in 75-cm^3 cell culture flasks until their density reached 1×10^7 /mL. The cells were harvested from the plastic surface by trypsinisation, and then concentrated by centrifugation (3500 rpm, 5 min) to 2×10^7 /mL. The input power was set to be ~1 W in the experiment.

Viability test

There were three sample groups for viability test: (1) SAW-on Group, in which the NSCLC cells were continuously run through the PCB-SAW device for 5 minutes under the input power of ~1 W and flow rate of 20 µL/min. (2) SAW-off Group, in which the cells were running through the PCB-SAW device at the same flow rate and duration without applying SAW. (3) Control Group, in which the cells were kept in a steady tube on an ice bath for the same period of time.

Acridine orange (AO, 30 µg/mL) and di-amino-phenyl-indole (DAPI, 100 µg/mL) were mixed at the volume ratio of 3:10 to prepare an AO-DAPI solution for cell staining. For both SAW-on and SAW-off Group, 100-µL sample in total was collected after 5 minutes, of which three 10-µL samples were taken out to mix with the AO-DAPI solution at the volume ratio of 5:1 to stain the cells. The three stained samples were then pipetted into three cell chambers on a cell counter slide for viability analysis using a cell counter (NucleoCounter® NC-3000™). For the Control Group, the same amount of the sample was taken for staining and viability test. All the tests were repeated three times.

Results and discussion

Characterisation of the PCB-SAW device

The average width and spacing of the finger electrodes on the PCB were measured to be 38.7 µm and 61.1 µm, respectively (Fig. S1), which led to a SAW wavelength of 199.6 µm. The MNs were designed for the PCB-SAW device, which managed to reduce the device's reflection coefficients to -18.4 dB and -21.4 dB (Fig. S2c) and improve the transmission coefficients to -11.9 dB (Fig. S2e).

Under the unique clamping bonding of the PCB-SAW device, Fig. 3a shows the $|S_{11}|$ and droplet velocity against the clamping force. Despite large variance of SAW amplitude indicated by the droplet velocity, the optimal clamping force of 50 N produced the minimum S_{11} of -46 dB and the maximum average droplet velocity of 24.4 mm/s. Further increase in the clamping force to the PCB-SAW device decreased the $|S_{11}|$, the SAW amplitude and its variance. The reduction of the droplet velocity at a higher clamping force could be a result of over compressing the piezoelectric material, thus resulting in reduced SAW amplitudes or higher power reflection. The use of the

MNs improved the sensitivity of the S_{11} reading, which allowed to easily achieve an optimal clamping assembly by reading the real-time S_{11} spectrum.

Once the optimal state of the PCB-SAW device was achieved by applying the clamping force of 50 N, the S_{11} spectrum was compared with that of the CR IDT with the same specifications as shown in Fig. 3b. It can be observed that the minimum S_{11} for both the devices had a difference of ~0.21 MHz, which could be caused by the errors in the PCB manufacture and the parasitic capacitance and inductance introduced by the MN circuits.

Benchmarking the PCB-SAW device at the optimal state with the CR IDT in terms of actuating droplets under a range of input powers is shown in Fig. 3c. The CR IDT showed higher efficiency in converting the input power to SAW comparing with the PCB-SAW device. This is reasonable as the electrodes for the PCB-SAW device were mechanically clamped onto the piezoelectric substrate resulting in an imperfect signal coupling. This issue can be easily compensated by doubling input power to the PCB-SAW device. For example, operating the CR IDT at ~0.6 W drives the droplet velocity of 20 mm/s, which can be achieved by the PCB-SAW device working at ~1.2 W.

Manipulation of microparticles

SAW devices have been previously demonstrated in manipulating microparticles within droplets for sample mixing[38,40]. On the PCB-SAW device, a droplet sample containing polystyrene microspheres was placed at the centre between the two IDTs (Fig. 4a). When one of the IDTs was activated, a streaming pattern with two major vortices was observed (Fig. 4b, Video S1), which was in good agreement with the pattern formed on conventional SAW devices[40]. When both the IDTs were activated, a four-vortex streaming pattern was generated (Fig. 4c, Video S2), which again agreed with that produced on conventional SAW devices[38]. Each of the IDT in the tests was driven by an input power of 0.2 W.

Further tests using the PCB-SAW device as an acoustic tweezer were performed by introducing polystyrene microspheres into the PDMS microchannel. The acoustofluidic model of the PCB-SAW device was developed to study acoustic pressure distribution and predict the microparticle trajectories as shown in Fig. S5, which was adopted from conventional SAW device modelling[25,26].

A polystyrene microsphere sample was injected into the microchannel. After an evenly dispersed pattern was formed within the microchannel (Fig. 5a), RF signals with the same phase

($\Delta\varphi$=0°) were applied to both IDTs to produce SSAWs with the PNs located at the centre and near the two walls, which trapped microspheres to form three aggregation traces as shown in Fig. 5b and Video S3. By applying a 180° phase difference ($\Delta\varphi$=180°) to the RF signal driving one of the IDTs, ANs were formed at the centre and near the two walls, resulting in four microsphere traces as shown in Fig. 5c and Video S4. Both these cases show good agreements with the simulation results (Fig. S5).

Manipulation of cancer cells

To validate the manipulation of cells and test the biocompatibility, the PCB-SAW device was filled by the NSCLC cell sample (Fig. 6a) and repeated the same operation for microspheres. Applying RF signals with $\Delta\varphi$=0° and $\Delta\varphi$=180° to the two IDTs resulted in the formation of three-cell column (Fig. 6b, Video S5) and four-cell column (Fig. 6c, Video S6), respectively. The results demonstrated that the PCB-SAW device can be used as an acoustic tweezer to manipulate and re-position cells controlled by changing RF signal phase.

The ability of the PCB-SAW device in preserving cell viability was tested using three sample groups, including Control, SAW-off and SAW-on. The results shown in Fig. 6d denote the viabilities of 98.2±0.8% (average ± SD), 97.6±1.2% and 96.9±0.6%, respectively. The analysis of variance showed no significant differences among these three groups (p = 0.166).

Conclusion

In this paper, we demonstrated that the novel PCB-SAW device is capable of performing all the functions realised using the standard SAW devices. The PCB-SAW has the main advantages of easy fabrication and low-skill entry requirement. The systematic characterisation to the PCB-SAW device and the comparison with the standard SAW device confirm the new technique has similar ability in actuating droplets. The PCB-SAW device can also be used as an acoustic tweezer to pattern microspheres and cells in a controllable manner, while maintaining high cellular viability.

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