Silicon Compatible Process To Integrate Impedance Cytometry With Mechanical Characterization

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SILICON COMPATIBLE PROCESS TO INTEGRATE IMPEDANCE CYTOMETRY WITH MECHANICAL CHARACTERIZATION

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ABSTRACT

We introduced 3D silicon electrodes to perform impedance cytometry on single cells without compromising practical integration with sensors measuring complementary properties, *e.g.,* mechanical properties. Microfabricated from a highly-doped SOI wafer, some design modifications were made to improve their sensing performance. According to simulations, a trajectory-free measurement can be obtained by replacing the silicon backside under the sensing area with an insulating material. In addition, enlarging the distance between the electrodes and the surrounding silicon structure and filling them with some insulating material result experimentally in better signal quality and reduced parasitics. Combining these two device modifications improves the system frequency response and the signal quality at higher frequencies. The proposed process aims at creating silicon-based electrodes for impedance spectroscopy applications while providing opportunities to integrate them with MEMS sensors and actuators.

KEYWORDS

3D electrodes, impedance cytometry, single-cell analysis, microfabrication.

INTRODUCTION

Cellular systems exhibit a significant degree of heterogeneity. Among all the methods for quantifying cell heterogeneity**,** microfluidic impedance cytometry (MIC) provide biophysical analysis on single-cells in a label-free and high-throughput way [1]. MIC devices detect changes in the impedance signal due to a cell passing through an electric field zone between electrodes. Information regarding dielectric properties, cell size, membrane capacity, cytoplasm resistivity can be extracted [2]. Due to several reasons, *e.g.,* supreme electrical properties and bandwidth, 2D planar gold electrodes are widely used on a glass substrate. However, their inhomogeneous field line inside the channel results in a need for trajectory-specific measurements or more complex fabrication/assembly steps to achieve reliable measurements at the single-cell level. Then, 3D electrodes were created with an electric field distribution more homogeneous and suitable for highly sensitive impedance detection [3]. Nevertheless, these methods show difficulties in combining the sensitive electrical MIC analysis with other biophysical properties, *e.g.*, mechanical properties, to benefit more from MEMS sensors and actuators in a high-throughput format. For example, highly-doped silicon has tremendous fabrication possibilities to integrate with various MEMS sensors/ actuators providing multi-parameter analysis, *e.g.*, electrical and mechanical [4]. However, a silicon wafer requires each electrode to be adequately isolated. Also, the effect of nearby structures and parasitics to be minimized to ensure the most reliable measurement and broader bandwidth.

Here, we target improving silicon-based electrodes for impedance cytometry applications by providing trajectory-free measurements, better signal quality, and reduced parasitics without compromising opportunities to integrate them with MEMS sensors and actuators.

METHOD

Device description

The proposed device is composed of two layers: (i) a PDMS layer assembled on (ii) a MEMS layer. The PDMS layer has an inlet to inject a cell suspension and an outlet to connect to a vacuum pump for controlling the flow along the microfluidic channel embedded in the MEMS layer.

We use the MEMS layer, *i.e.,* the functional layer, by designing in three parts:

(i): A *microfluidic channel* is formed between silicon walls on the sides, the backside silicon layer or some insulating material as the bottom, and the PDMS slab as the top. The microfluidic channel handles the cells with an inlet on one side and an outlet on the other side of the PDMS layer.

(ii) *Electrical characterization area* is fabricated on the front side of an SOI wafer. It includes 3D electrodes facing each other at two sides of a microfluidic channel.

(iii) *Backside silicon layer* is critical for the designs we tested in this study, although it is mainly used for structural integrity and handling. We replaced silicon in some areas, *e.g.*, under the electrical characterization area, with a dielectric material.

Microfabrication

We used three different types of devices. The first type is an all-silicon device with two electrode pairs on the front side of an SOI wafer. The second type uses an insulating material on the sides of the electrode pairs to extend the surrounding silicon structures on the front side. The third type uses the same front side as the second type but has a modified backside by replacing different parts of the backside silicon with an insulating material.

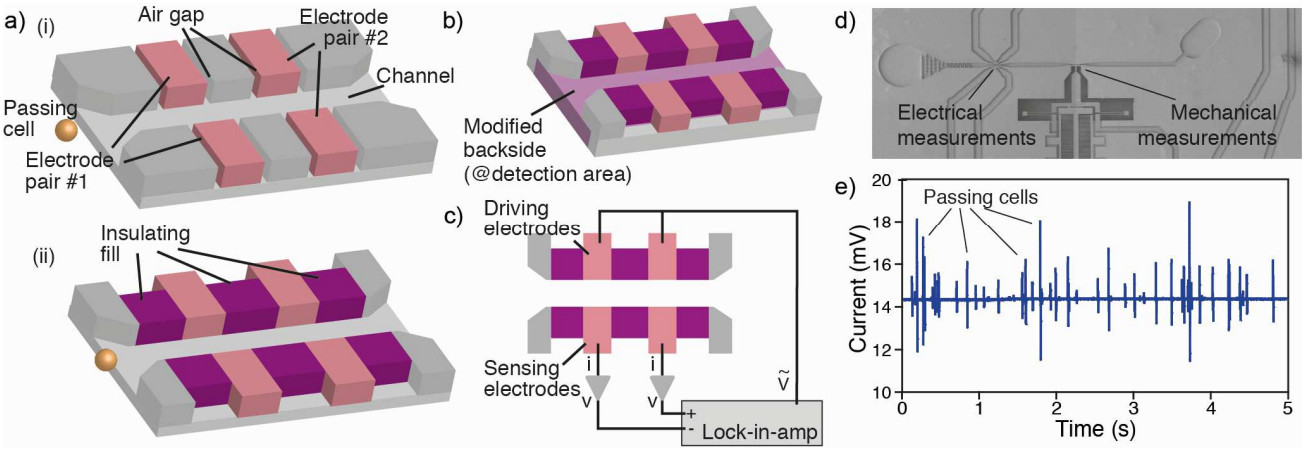

Figure 1: a) Two frontside designs are fabricated and tested: (i) narrow air gaps, (ii) insulating areas. b) Silicon and insulating backside devices are also compared. c) Measurements are performed using a lock-in-amplifier in differential mode connected to silicon 3D electrodes. d) 3D electrodes could be integrated with mechanical MEMS sensors. e) An example of detected cells flowing in the channel.

All types of devices are fabricated on SOI wafers (30/2/300 µm). Electrodes and the microfluidic channel are fabricated on the front side by a photolithography process followed by a 30-µm DRIE etching (Figure 1a-i). Devices with insulating front side regions have an additional step of insulating material filling and patterning (SU8 or CYTOP; Figure 1a-ii). One key point of filling those cavities is to keep the insulating material at the same level as the front side wall to have a proper PDMS adherence on all the devices, especially on the detection area, and avoid any liquid leakage during the experiment. Similarly, devices with modified backside geometry received 350µm DRIE etching of backside and insulating material filling steps (Figure1b).

Working principle

The proposed device targets single-cell impedance spectroscopy in a continuous flow to achieve high throughput. We use either air-liquid interfaces or insulated walls at the electrical characterization area to keep the electrodes at minimal immersion in liquid for better performance. Cells are inserted via the inlet and flow through the 100-µm-width channel to reach the electrical characterization area where the channel width drops to 25 µm. Electrical characterization is performed using two pairs of 3D electrodes working in the differential mode. Being less conductive than the media, cells decrease the current passing between the electrodes while passing through. This change in the current can be detected in real-time.

All electrode designs are 25 µm in width. The 30-µm top layer of the SOI wafer defines the height of electrodes. The device without any insulating material at the front side has a 4-µm gap between the electrodes, forming a stable air-liquid interface due to the surface tension of the liquid preventing any leakage. The other designs have 25 µm space between the electrodes and surrounding silicon structures. This space is filled with an insulating material. A sinusoidal signal (1 Vp-p, 1Mhz) is applied on driving electrodes with a lock-in-amplifier (Zurich Instruments HF2LI). Sensing electrodes are first connected to a trans-impedance amplifier (Zurich Instruments HF2TA), with a gain of 10k, before being fed into the lock-in-amplifier in the differential mode (Figure 1c). This impedance cytometry setup allows us to simultaneously perform measurements at three different frequencies for providing information on cell size, membrane capacitance, and cytoplasm resistivity. The signal demonstrated here is at 1 MHz, related only to cell size measurements.

Setup

Experiments are performed on an upright microscope stage. The outlet of the PDMS slab is connected to a vacuum pump (Fluigent EZ), and the electrodes are connected to the lock-in-amplifier at different channels. Signals obtained from sensing electrodes are first amplified using trans-impedance amplifiers before feeding in the lock-in-amplifier.

Biological materials

SUM159-PT, a triple-negative breast cancer cell line, is tested with the proposed method. Cells are grown in monolayer conditions up to subconfluence. After being trypsinized to obtain a homogenous cell suspension solution, they are put in a PBS 1x solution and passed through a 40-µm filter before being used in the experiment.

Experimental procedure

We first assemble the PDMS slab on the MEMS device. The assembled device is attached to a PCB, and all electrical connections are established. Placing the device on the microscope stage, we complete the fluidic connections. A PBS solution is injected to fill the channel and the tubing until no bubbles remain. Then, 5 µl cell suspension is injected into the channel via the inlet. A constant flow speed of 2 µl/min is sustained inside the channel throughout the experiments by controlling with the vacuum pump. Cells start moving with the flow and go through the electrical characterization area while continuously measuring the passing current. The frequency response measurements are performed by sweeping the frequency from 100kHz to 50MHz (2 Vp-p driving signal) with a gain of 1k.

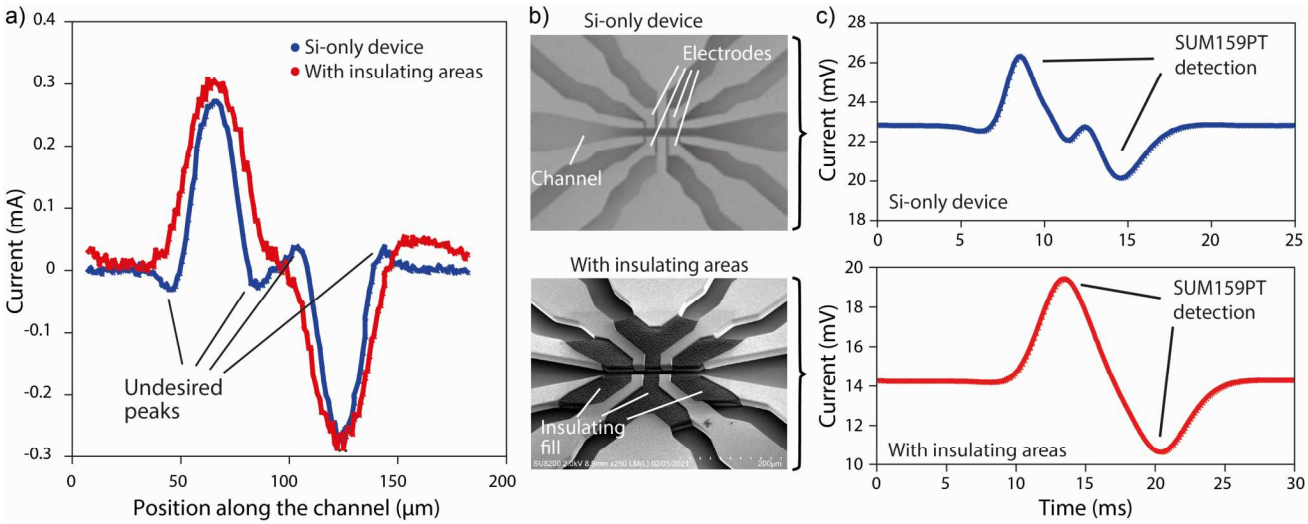

Figure 2: a) Simulations showed undesired peaks when cells are passing nearby silicon structures (blue) while a device with insulating areas showed an improved response (red). b) SEM images of fabricated devices used in the experiments. c) Similar to the simulations, cell experiments showed undesired peaks on Si-only devices but not on devices with insulating areas.

Simulations

A finite element model is used to investigate the sensitivity of a MEMS sensor over the detection and the quantification of cells. In particular, we evaluated the sensitivity of the sensor according to different electrode geometries and the cell positions while flowing through a channel. Several sensor topologies are considered to define proper electrode design guidelines.

The model was built on COMSOL platform, referring to the Electric Current (EC) model, and given as,

$$-\nabla.((\sigma + j\omega\varepsilon_0\varepsilon_r)\nabla V - J_e = 0 \qquad (1)$$

where σ is the conductivity [S/m], ω is the angular frequency, ε_0 is the vacuum permittivity, and ε_r is the relative permittivity of the material, V is the applied potential, J_e is an externally generated density, defining the electric field by the constitutive law,

$$D = \varepsilon_0\varepsilon_r E \qquad (2)$$

where D is the electric displacement field, and E is the electric field.

The cell membrane was represented as contact resistances:

$$n.J_1 = \frac{1}{d_s}(\sigma + j\omega\varepsilon_0\varepsilon_r)(V_1 - V_2) \qquad (3)$$

where, d_s is the surface thickness. Analyses were solved in the frequency domain, setting the parametric solver to evaluate the response of the system at several working cell displacements. The input signal was given using a Voltage Terminal, and the current amplitude was evaluated as a function of cell displacement.

Results and Discussion

Two pairs of 3D-silicon electrodes (Figure 1d) were used in the differential mode to detect passing cells in a high-throughput way (Figure 1e). Air gaps on both sides of an electrode provided electrical insulation while the surface tension of the liquid protected the channel from leaking out. Both simulations and experimental results showed that the silicon structures surrounding electrodes significantly affect the detected signal as multiple additional peaks appear before, after, and in between the peaks corresponding to the electrodes. Those additional peaks altered measurement performance on the intrinsic cell

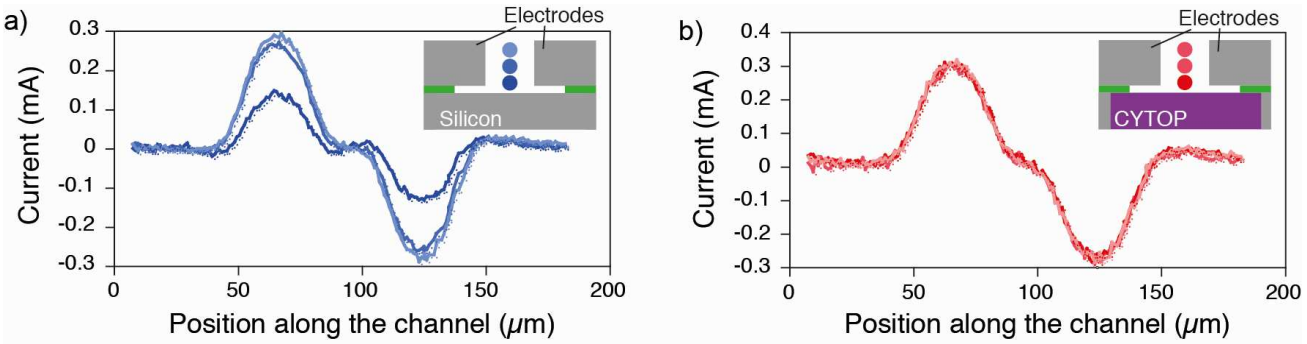

Figure 3: Simulations showed that: a) The vertical position of passing cells caused significant changes in the obtained current amplitude when the backside of the detection area was silicon. b) Removing the silicon and filling the detection area backside with a dielectric material such as CYTOP resulted in a trajectory-free measurement. Insets correspond to cross-sectional views.

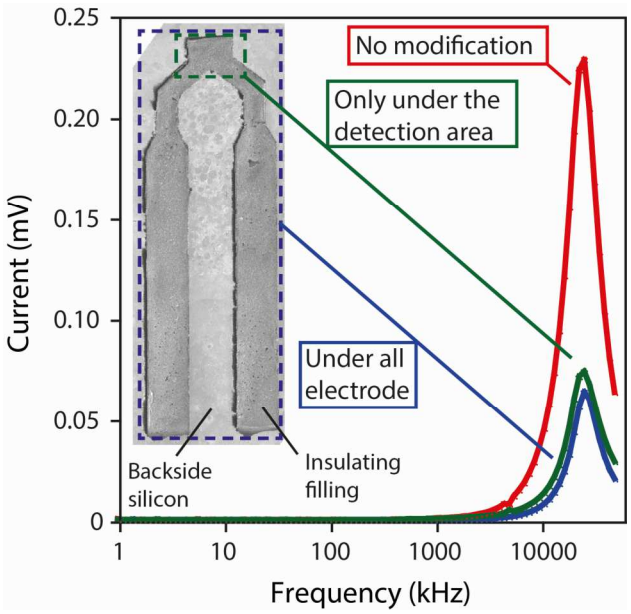

Figure 4: Three different backside designs (Red: Si-only, green: detection area-limited modification, and blue: extended to the electrode area modification) were fabricated and frequency responses of the devices were measured. The y-axis corresponds to the current value after the trans-impedance amplifier. Modified versions performed better at high frequencies by decreasing parasitics. Inset: The SEM image of the modified backside with PDMS filling. The green rectangle corresponds to the detection-area limited modification version.

properties. Those parasitics could be eliminated by extending the distance to the electrode and filling this hole with some insulating material, *e.g.*, SU8 and CYTOP, as demonstrated with simulations (Figure 2a). This measurement improvement was also shown experimentally between the two geometries, the Si-only device and the one with SU8 fillings between electrodes on the front side (Figure 2b,c).

The simulation of the effect of backside material on the measured current concerning the vertical position of cells showed significant differences (Figure 3). To obtain a trajectory-free measurement for single-cell analysis, we had to minimize the effect of cells' vertical position on the measured current. According to the simulations, a cell passing closer to the bottom surface results in a lower current passing between the electrodes (Figure 3a), which compromises reliable measurements. On the other hand, an alternative design with the backside silicon near the measurement area being replaced with an insulating material solves the problem. Simulations show identical measurement results regardless of the position of the passing cell, achieving a trajectory-free measurement (Figure 3b).

Frequency response analyses showed that the backside silicon also causes parasitics at high frequencies. Three different devices with different backside structures were tested for their frequency responses. All of these three designs had the same front side structures, *i.e.*, SU8-filled spaces between electrodes. The first type had an all-silicon backside. The second and third devices had PDMS-filled backside holes under only the electrical characterization area (green rectangle in figure 4 inset) and an area extended along the electrodes (blue rectangle in figure 4 inset), respectively. The frequency response was swept from 100kHz to 50MHz (2 Vp-p) with a gain of 1k. As these measurements were taken in air, we expected complete insulation between the electrodes. However, all devices showed elevated current values at higher frequencies suggesting the parasitic currents. Among the tested devices, the all-silicon device performed poorly compared to the others. As a result, the modified backside devices show improved performance for trajectory-free cell measurements and frequency measurements with extended bandwidth.

CONCLUSION

Demonstrated fabrication steps significantly improved the use of 3D silicon electrodes for continuous flow single-cell impedance spectroscopy applications. Extending the space between electrodes and the surrounding silicon structures and filling those spaces with insulating materials significantly improved the cell signal quality by reducing parasitics and simplifying the detected signal geometry. The device showed improved bandwidth and potential trajectory-free measurements by replacing the backside silicon with an insulating material. Those optimizations suggest a reliable use of 3D silicon electrodes for impedance cytometry without compromising the integration possibilities with other MEMS sensors and actuators.

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