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PDMS-film coated on PCB for AC impedance sensing of biological cells

Jinhong Guo · Chang Ming Li · Yuejun Kang

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Abstract Microfluidic impedance sensor has been introduced as a cost effective platform in biological cell sensing and counting since several decades ago. Conventional microfluidic impedance sensor usually requires the patterned gold electrodes directly in contact with the carrying buffer to measure the electrical current change due to the blockage of cells. However, patterning metal electrode probes on the silicon or glass substrate is a non-trivial task, which increases the fabrication cost of the impedance sensor. In this paper, we demonstrate an alternating current (AC) impedance based microfluidic cytometer built on a printed circuit board (PCB) coated with polydimethylsiloxane (PDMS) thin film. In addition, circulating tumor cells (*Hela cells*) are used to successfully demonstrate the feasibility of the microfluidic AC impedance sensor in tumor cell detection. The electrodes pre-deposited PCB costs less than US$2.00 and is widely available in the market. This device has a good potential for point-of-care diagnosis in resource-poor settings.

Keywords Microfluidics · AC impedance cytometer · Printed circuit board · Circulating tumor cell · Point-of-care

Electronic supplementary material The online version of this article (doi:10.1007/s10544-014-9872-2) contains supplementary material, which is available to authorized users.

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1 Introduction

The microfluidic cytometer could have a great potential to address the current demands for point-of-care diagnosis, such as for circulating tumor cells (CTCs) due to their advantages of compactness and portability, small sampling size, ultrahigh sensitivity, and flexible choice of detection modalities (Zheng et al. 2013; Arya et al. 2013; Piyasena and Graves 2014). Direct current (DC) resistance based microfluidic cytometry and micro-Coulter counters have been widely applied for the detection of pollen (Jagtiani et al. 2006), biological cells (Wang et al. 2008; Choi et al. 2013; Asghar et al. 2012; Wu et al. 2012; Guo et al. 2014), viruses (Damhorst et al. 2012), DNA and other biomolecules (Lorenz et al. 2010; Bayley and Martin 2000; Dekker 2007). The working mechanism of the resistance pulse sensor is based on the Coulter principle. When a particle carried by a polar liquid passes a micro/nano-scale pore, the electrical impedance of this pore will significantly increase due to the physical blockage induced by the particle, leading to the notable change of the electrical current (a current pulse) across the pore. Similar working mechanism also applies to the AC impedance based sensing. Both resistance or impedance microcytometry is cost effective due to the significant reduction of manufacturing cost as compared to the commercial optical flow cytometer such as Beckman-Coulter FC500, which has a very high price tag of about US$100,000.

However, many available microfluidic cytometers that implement electric impedance or resistance detection modality require patterning the metal electrode probes on a silicon or glass substrate, followed by bonding the microchannel with this functionalized substrate (Song et al. 2013; Piyasena and Graves 2014). Obviously it is a non-trivial or cheap task to pattern microelectrodes at desired locations on a silicon or glass substrate, which requires tedious and expensive surface processing to achieve strong bonding between the metal

probes and the substrate material (Song et al. 2013). Therefore this challenge significantly increases the fabrication cost of the microfluidic cytometer and limits its wide application for point-of-care diagnosis. In a recent study on a microfluidic AC impedance cytometer, a glass coverslip was used to separate the microfluidic channel and the sensing electrodes (Emaminejad et al. 2012). This innovative approach avoided direct contact between the conducting buffer and the electrodes, thereby allowing the electrode substrate to be easily recycled and reducing the cost per device. However, the sensitivity (signal-to-noise ratio) could be limited due to the high impedance loss in glass material as well as the poor affinity between the glass slip and the electrode substrate. To address this problem, in this paper, we propose a microfluidic AC impedance cytometer that is directly built upon a printed circuit board (PCB) coated with a polydimethylsiloxane (PDMS) thin film as an insulator between the electrode and conducting liquid solution. This PDMS thin film protects the metal electrodes from erosion and electrochemical reactions that are always major concerns for DC resistance based sensing, while allowing alternating current to pass and form an electric circuit. This PDMS film is extremely thin with much lower impedance compared to glass material and it provides seamless contact both at the electrode side and the electrolyte buffer side, thereby further improving the sensitivity of the impedance sensing. The PCB substrates are commercially available and can be easily customized to include the pre-deposited metal electrodes that are ready to use. The microfluidic channel can be integrated on this PDMS film coated PCB directly through a simple and fast procedure, enabling a highly cost effective platform for detection of biological samples.

2 Theory and method

Figure 1 illustrates the equivalent circuit model of the proposed AC impedance cytometry. The carrying electrolyte solution can be modelled as a resistance. The parasitic capacitance C_e due to the direct coupling between two electrodes is also considered in this model (Emaminejad et al. 2012). The PDMS film between the carrying fluids and the electrodes acts as an insulator and can be modelled as a capacitance C_s. Therefore, the input impedance without the microparticle can be written as

$$Z_{in} = \frac{1}{j\omega C_e} \cdot \frac{j\omega + \frac{2}{RC_s}}{j\omega + \frac{1}{R}\left(\frac{1}{C_e} + \frac{2}{C_s}\right)} \tag{1}$$

where R is the microfluidic channel resistance, ω is the angular frequency of the applied AC field. When a microparticle blocks the constricted aperture temporarily, the impedance variation,

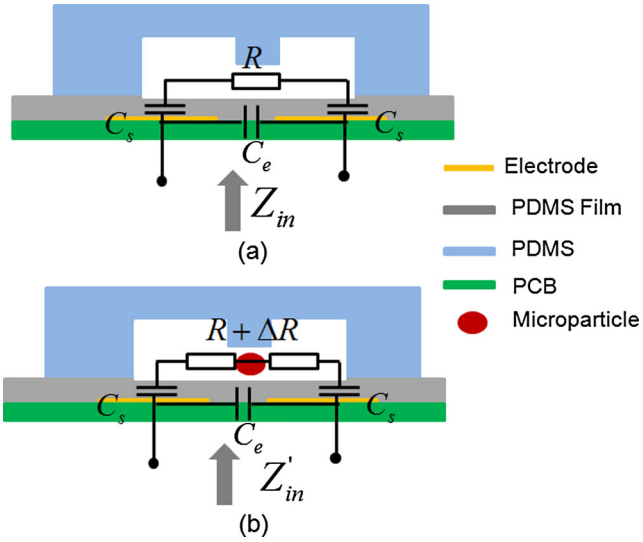

Fig. 1 **a** Equivalent circuit model of the proposed AC impedance cytometer without microparticle in the aperture. R is the impedance of carrying fluids, C_e is the parasitic capacitance between two electrodes. C_s is the equivalent capacitance between electrode and carrying fluids due to the PDMS thin film. **b** The impedance modulation due to the temporary blockage by the microparticle

ΔR, of the microchannel results in a modulation of the alternating electric current, ΔI, which can be expressed as

$$\frac{\Delta I}{\Delta R} \approx \frac{dI}{dR} = \frac{dI}{dZ} \cdot \frac{dZ}{dR} = -\frac{1}{R^2} \cdot \left(\frac{j\omega}{j\omega + \frac{2}{RC_s}}\right)^2 \cdot V_{in} \tag{2}$$

$$|\Delta I| \approx \frac{\Delta R}{R^2} \cdot \left[\frac{\omega^2}{\omega^2 + \left(\frac{2}{RC_s}\right)^2}\right] \cdot V_{in} \tag{3}$$

The PCB with a pair of pre-deposited copper electrodes (2 mm apart) was obtained from a commercial supplier (HQPCB, Shen Zhen, China) for a unit price less than US$2.00. PDMS pre-polymer and the curing agent were mixed at a ratio of 10:1, degassed in a vacuum chamber. The uncured PDMS were then spin-coated on the PCB at 1,000 rpm for 1 min. Subsequently, the PDMS coated PCB was cured in an oven for 2 h at 95 °C. The PDMS film was measured as 30 μm in thickness, which is much thinner than the glass cover slide (150 μm) as used in a previous report (Emaminejad et al. 2012). Since the capacitance C_s is inversely proportional to the PDMS thickness, thinner coating can lead to greater current modulation and hence the sensitivity according to Eq. (3). A 3D microfluidic structure, as shown in Fig. 2 was created by patterning two layers of photoresist (SU-8 25, Microchem, MA, USA) molds (Chen et al. 2011; Zheng et al. 2012). Glass slides were cleaned in acetone, methanol, and DI-water and baked on a hot plate at 250 °C for 30 min.

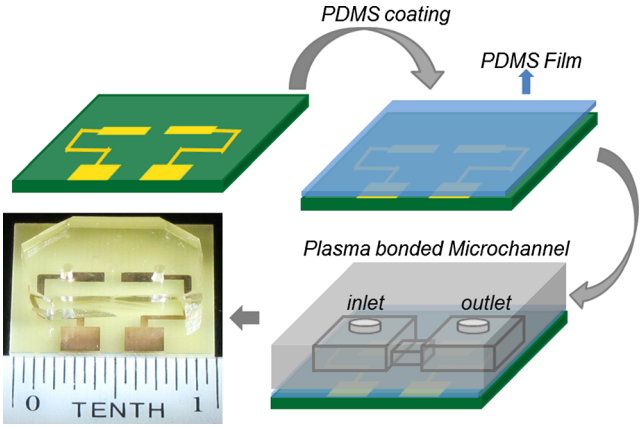

Fig. 2 The fabrication procedure and the final chip device

To create the detection aperture, the first photoresist layer (20 μm in thickness) was spin-coated on a cleaned glass slide at 2,250 rpm, soft-baked for 3 min at 65 °C and 7 min at 95 °C. Then the coated slide was exposed under the UV light using the first photomask with the pattern of sensing aperture, and baked on a hot plate for 1 min at 65 °C and 2 min at 95 °C. To create the main channel for cell loading and draining, a second photoresist layer (SU-8 25, 40 μm in thickness) was spin-coated on top of the first layer (as prepared above) at 1,200 rpm, soft-baked for 5 min at 65 °C and 15 min at 95 °C. The second photomask with the pattern of main channel was aligned carefully to expose the second layer of photoresist under UV light. The coated wafer was then post-baked for 1 min at 65 °C and 5 min at 95 °C, followed by developing in the SU-8 developer for 4 min, and baked for 2 h at 250 °C. Finally a 3D positive relief of SU-8 mold was created. PDMS pre-polymer and curing agents were mixed at ratio of 10:1. The mixture was cast on the as-prepared SU-8 mold, degassed in a vacuum chamber, and cured in an oven for 2 h at 75 °C. PDMS channels were sliced and peeled off from the SU-8 mold and reservoir holes were punched at appropriate locations. The PDMS channel and the PDMS coated PCB were plasma treated for 10 s before they were bonded to form the final chip. During the bonding process, the main channel was aligned carefully and located on top of the PCB sensing electrodes. The chip fabrication procedure is illustrated in Fig. 2. The main channel has dimension of 300 μm in width and 60 μm in height. The sensing aperture has dimension of 20 μm in width and 20 μm in height.

A syringe pump (PHD 22/2000, Harvard Apparatus, MA, USA) was used to provide continuous sample flow in the device. The two electrodes were connected to a function generator (8657A, Agilent Technologies, CA, USA) and a lock-in amplifier (SR850, Stanford Research Systems, CA, USA). The electric current signal was acquired with a data acquisition card and transferred to a PC for post processing.

Polystyrene particles of 15 μm in diameter were used to evaluate the sensitivity of the device under different AC frequencies. The microparticles were stripped from the original buffer through multiple dilution steps, and re-suspended in 1× PBS solution. Bovine serum albumin (BSA) is added in the solution to prevent the agglomeration of the particles.

Hela cell is a type of circulating tumor cell derived from cervical cancer and is a commonly used cell line in cancer research. We used Hela cells as a model to demonstrate the capability of the proposed AC impedance microcytometer for detecting real biological cells. Hela cells (American Type Culture Collection, MD, USA) were cultured in the Dulbecco's Modified Eagle Medium (DMEM) supplement with 10 % fetal bovine serum (FBS), 1 mM sodium pyruvae, and 0.1 mM DMEM nonessential amino acids. The Hela cells were grown at 37 °C under 5 % CO_2 in a T75 flask. The cells were re-suspended in specific concentrations by dilution with 1× PBS solution.

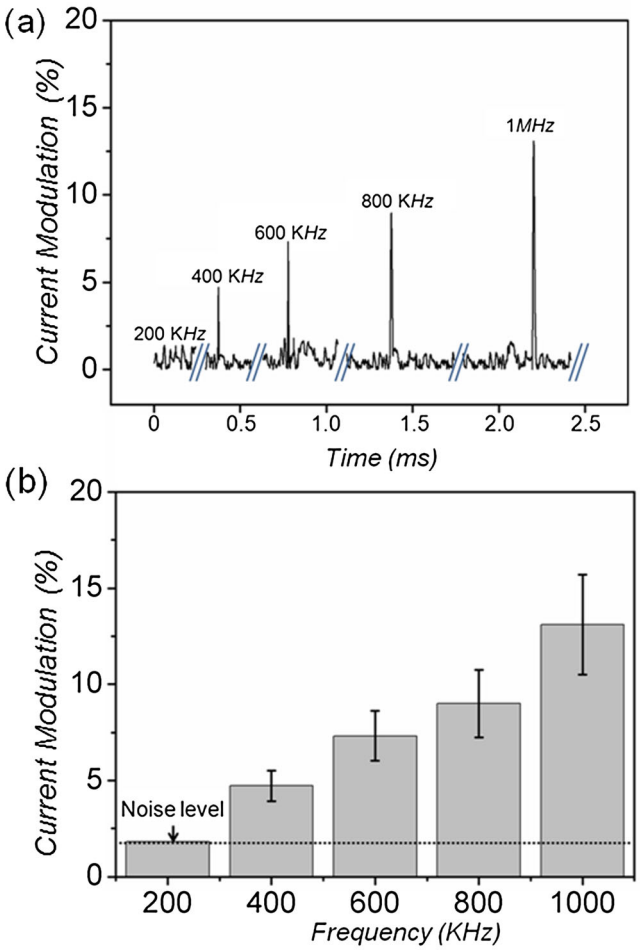

Fig. 3 **a** Typical electric current modulation at 200 KHz, 400 KHz, 600 KHz, 800 KHz and 1 MHz, induced by 15 μm polystyrene particles translocating through the detection aperture of 20 μm in height, 20 μm in width and 40 μm in length. **b** The current modulation by statistical analysis of 50 detected pulses under different frequencies

3 Results and discussion

It can be easily inferred from Eq. (3) that the electric current modulation and hence the sensitivity of this device increases with the excitation frequency. Due to the limit of available equipment setup, we only tested the frequency range from 200 KHz to 1 MHz using the fabricated chip device. Figure 3a illustrates the electric current modulation induced by the passage of 15 μm polystyrene microparticles through the detection aperture under AC excitations at 200 KHz, 400 KHz, 600 KHz, 800 KHz and 1 MHz, respectively. At 200 KHz, no effective signal of current pulse could be detected from the background noise. In contrast, under other four frequencies higher than 400 KHz, each microparticle can be detected with good signal-to-noise (S/N) ratio. The relationship between the magnitude of current modulations and the excitation frequency is shown in Fig. 3b. The S/N ratio can reach 2.61 (8.3 dB), 4.05 (12.1 dB), 4.98 (13.9 dB), and 7.25 (17.2 dB) at 400 KHz, 600 KHz, 800 KHz and 1 M Hz, respectively. Particularly, the S/N ratio at 800 KHz reached about twice as high as that (2.25 or 7.0 dB) reported in a previous work using a cover glass as the insulating barrier between the electrode and the conducting liquid (Emaminejad et al. 2012).

In order to characterize the performance of the fabricated device for practical application in tumor cell detection and counting, Hela cells with different concentrations were

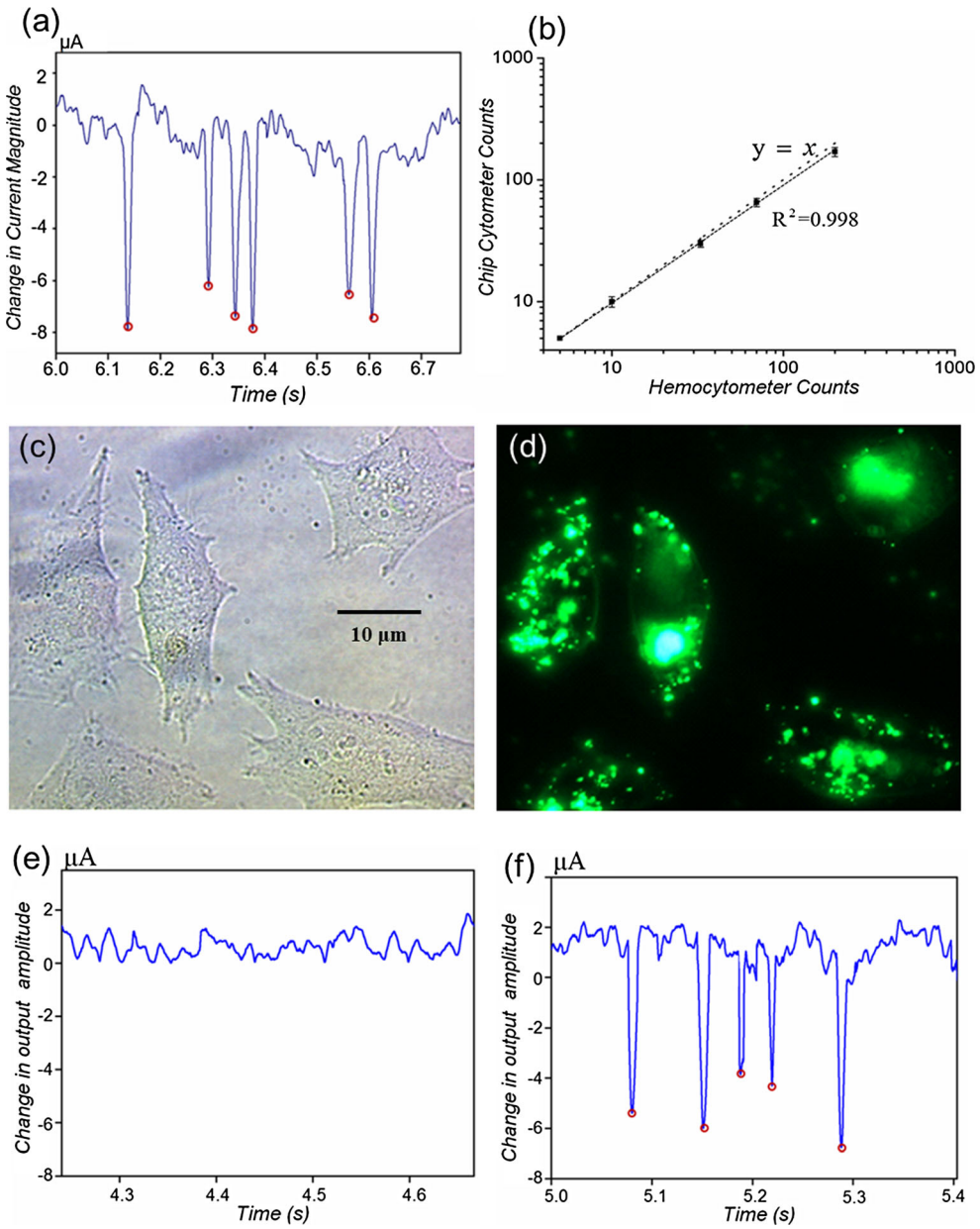

Fig. 4 **a** The measured electric current modulation at frequency of 1 MHz induced by Hela Cells. **b** Comparison of Hela cell enumeration using the proposed chip device and a hemocytometer at different concentrations, based on 50 μL sample size. **c** A contrast image of Hela cells under a light microscope. **d** A fluorescent image of Hela cells stained in green. **e** RBCs could not be detected by the impedance sensing at 1 MHz. **f** Tests on the mixture of RBCs and Hela cells only showed impedance modulation induced by Hela cells at 1 MHz

perfused through the chip and the same aliquots were verified by a commercial hemocytometer in parallel. The Hela cells were cultured following the protocols as described above, and were washed with PBS buffer. Four groups of cells were re-suspended in 1× PBS with specific concentrations (0.2×10^3 mL^{-1}, 0.8×10^3 mL^{-1}, 1.6×10^3 mL^{-1}, and 4×10^3 mL^{-1}). Each group was further split into two equal aliquots for the chip device and the hemocytometer, respectively. Figure 4a shows a series of typical pulses of the electric current modulation induced by several Hela cells under excitation frequency of 1 MHz, which is the highest frequency for the available setup. The data measured using the chip were benchmarked with those obtained from a commercial hemocytometer (UIC-BCC01, UNIWISE, Zhejiang, China) in Fig. 4b, showing comparable accuracy between the chip and the commercial cytometer. We have some preliminary results for using this impedance device for testing the red blood cells (RBCs). However the signals of RBCs can hardly be distinguished from the background noise (Fig. 4e). Also we have tested the mixture of RBCs with the Hela cells while only Hela cells can be detected (Fig. 4f). We speculate that this limited sensitivity for RBCs is due to the large sensing aperture in the current device, for which the smaller RBCs cannot induce enough impedance change to be detected. On the other hand, these results also imply that different types of cells show different impedance signals. Further studies are ongoing to improve the sensitivity of this device for detection of more types of biological cells. In addition, we have studied the effects of the PDMS film thickness and the detection frequency on the magnitude of impedance modulation by numerical modeling (details provided in the supporting information), which is developed using a commercial Finite Element Method package COMSOL Multiphysics 4.3b (COMSOL, CA). The results in Fig. 5a implies that the impedance modulation hence the sensitivity can be significantly increased if the PDMS film thickness decreases to below 20 μm, which is expected because of the reduced impedance loss between the sensing electrodes and the electrolyte buffer. Figure 5b shows that the impedance modulation increases under higher detection frequency, which agrees well with the theoretical prediction in Eq. (3) and also are consistent with the experimental results in Fig. 3.

The study showed that the presented AC impedance microfluidic cytometer built on PDMS coated PCB is capable of providing a cost effective (less than US$2.00 for materials) solution of rapid cell detection for point-of-care diagnosis in resource-poor developing countries. Further studies can be focused on improving the sensitivity and reliability of this impedance sensing device. The particle focusing strategies (Xuan et al. 2010), such as hydrodynamic sheath flow, can be introduced into the microfluidic channel in order to focus the particle samples into a thin stream before they flow through the sensing aperture. The particle pre-focusing could prevent the channel from clogging due to particle clustering, and hence allow for even smaller aperture to be designed to further improve the device reliability and sensitivity.

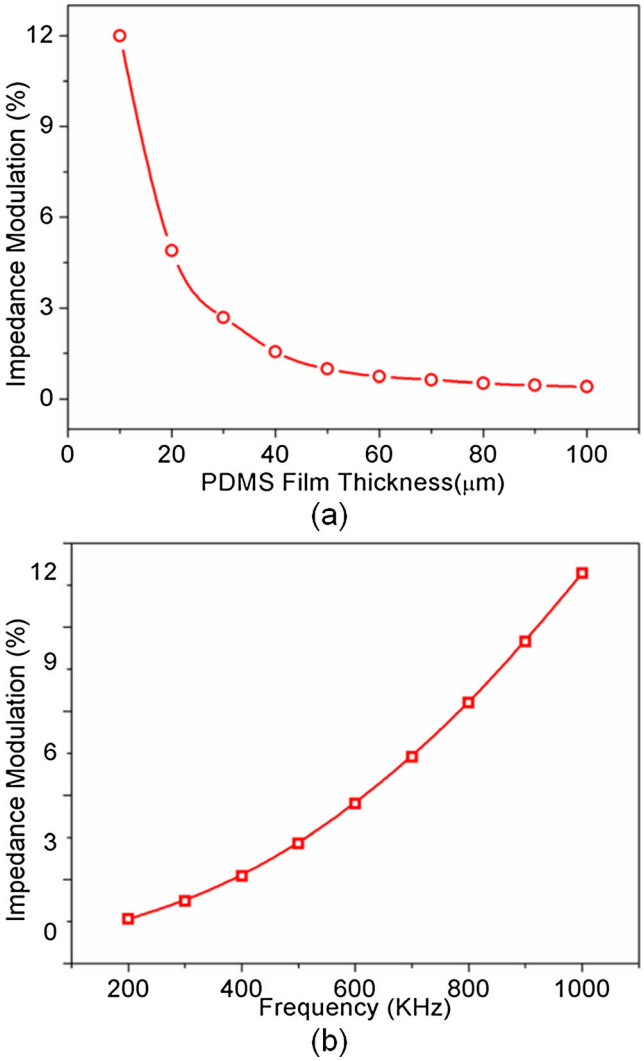

Fig. 5 **a** The simulated impedance modulation induced by a 15 μm particle as a function of the PDMS film thickness under detection frequency of 1 MHz; **b** The simulated impedance modulation induced by a 15 μm particle as a function of detection frequency

4 Conclusion

In summary, we reported a cost effective microfluidic device based on AC impedance sensing on a PCB coated with PDMS thin film. The PCB substrate has pre-deposited metal electrodes and is widely available in the market with unit price less than US$2.00. The major advantage of using PCB lies in that it opens up a new possibility of using the mature technologies in IC (integrated circuit) industry to interface with and further functionalize the microfluidic chip. For instance, the PCB can be easily customized with integrated amplifiers,

filters, and other electronic circuits on board in order to improve the sensitivity and reliability of the device. In addition, the electronic components that can convert the DC input into any AC signal with desired frequency and wave forms can be designed for specific applications of AC impedance sensing, without inducing extra cost for instrumentation under mass production. As a proof-of-concept study, this brief communication has demonstrated that this device has a good potential in detecting and counting microparticles or biological cells, with comparable accuracy as the commercial hemocytometer, for low cost point-of-care diagnosis, especially in the resource-poor developing countries.

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