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ORIGINAL PAPER

Development of a biochip using antibody-coated gold nanoparticles to detect specific bioparticles

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Abstract This study developed a method of detecting bioparticles such as *Salmonella* that exist in the biological samples. The method employed a substrate with interlaced comb-like electrodes into which the mixtures of biological samples and antibody-coated gold nanoparticles were added. The alternative signals with appropriate frequency bands were then conducted into the comb-like electrodes to change the dielectrophoresis force. The gold-modified *Salmonella* can be adsorbed on the edges of the electrodes and isolated from various biological samples. The impedance of the adsorbed *Salmonella* on the edges of the electrodes was measured and comparison of the impedance between the electrodes with and without *Salmonella* can quantify the amount of the adsorbed *Salmonella*.

Keywords Biochip · Dielectrophoresis · Gold nanoparticles · Detection · *Salmonella*

Introduction

Bioparticles including viruses, bacteria, and other cells often serve as pathogens or toxic indicators. *Salmonella* is one of the important pathogenic bacteria that can lead to food poisoning and enterocolitis. Salmonella shed from infected animals' intestines can contaminate food and then spread between different hosts like people, dogs, cockroaches, and rodents. Only a small oral inoculum ($<10^5$ cells) of bacteria can induce the manifestations of *Salmonella* infection. Traditional detection methods for *Salmonella* comprise six stages: pre-enrichment, selective enrichment, chromomeric medium, identification of biochemical characteristics, and serum screening test. Through these six stages, to identify pathogens and antibiotic resistance usually takes 3–5 days or even more. In order to shorten the working periods, many detecting methods of *Salmonella* have been rapidly developed and commercialized. Recently, there have been several studies using dielectrophoresis methods to detect biological cells [1–6]. In this study, we primarily introduce a method using antibody-coated nanoparticles to specifically attach the target bioparticles. Furthermore, we change the original dielectric properties of the bioparticles to isolate the pathogens. Finally, we develop a chip to collect the biological particles and measure the changes of impedance to quantify the biological particles. This novel detection method can simplify and accelerate the identification of bacteria or virus with little limitation of personnel and facilities.

First, it is necessary to discover the property of particles. The three most influential factors in dielectrophoresis (DEP) force are the size of particle, permittivity, and frequency. Permittivity and the size of particle generally remain consistent and DEP force varies with frequency of voltage. Secondly, we use the simulated results of Computational Fluid Dynamics software, CFD ACE + (product of CFD Research Corporation) to design the pattern of the electrodes. Finally, the DEP model of bioparticles modified

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by gold nanoparticles is established. And the biochip is designed to conduct several experiments for quantizing the concentration of the sample containing *Salmonella* or *E. Coli*.

Theory

A dielectrically polarized particle moves with the force of non-uniform electric fields, which is called DEP. Equation (1) defines the relationship between the amount of DEP and particles with radius r suspended in a medium with permittivity ε_m [7, 8].

$$F_{\mathrm{DEP}} = 2\pi\varepsilon_m r^3 \mathrm{Re}(K)\nabla E^2 \tag{1}$$

∇E^2 denotes the gradient of the square of the electric field. K listed in Eq. (2) denotes Clausius–Mossotti factor.

$$K = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \tag{2}$$

$$\varepsilon_x^* = \varepsilon_x + \frac{\sigma_x}{j\omega} \tag{3}$$

Here, ε_x^* denotes the complex permittivity which can be rewritten as Eq. (3). σ_x denotes conductivity, j denotes $\sqrt{-1}$ and ω denotes the angular field frequency.

In Eq. (1), if Re(K) is positive, particles will be attracted by the electrodes. On the contrary, particles will be forced to leave the electrodes if Re(K) presents negative. Therefore, K is a main factor to dominate the behavior of particles and can also be defined as Eq. (4)

$$K = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} = \left(\frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m}\right)\left[\frac{j\omega\tau_0 + 1}{j\omega\tau_{\mathrm{MW}} + 1}\right] \tag{4}$$

$$\tau_0 = \frac{\varepsilon_p - \varepsilon_m}{\sigma_p - \sigma_m} \tag{5}$$

$$\tau_{\mathrm{MW}} = \frac{\varepsilon_p + 2\varepsilon_m}{\sigma_p + 2\sigma_m} \tag{6}$$

Equation (4) can be simplified as Eqs. (7) and (8) at high and low frequency, respectively. In our research, we maintained the property of particle at a low frequency and added gold nanoparticles to change the characteristics of the particle.

$$\lim_{\omega\tau_{\mathrm{MW}}\to\infty}[K] = \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m} \tag{7}$$

$$\lim_{\omega\tau_{\mathrm{MW}}\to 0}[K] = \frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m} \tag{8}$$

If we consider the particle as a uniform sphere, the internal permittivity can influence the time and degree of polarization of particle. Therefore, when a multi-layered particle is considered as a layered sphere, the effective permittivity of particle varies and may be substituted by single layered sphere.

Equation (9) [9] defines that the effective permittivity $\varepsilon_{\mathrm{effective}}^*$ reflecting the attributes of the original particle's structure of single shell particle and the permittivity of Eq. (9) is complex. R_{out} denotes the outer radius, R_{in} denotes the radius of core, $a = R_{\mathrm{out}}/R_{\mathrm{in}}$ and $\varepsilon_{\mathrm{core}}$ and $\varepsilon_{\mathrm{layer}}$ are the permittivity of core and layer (Fig. 1).

$$\varepsilon_{\mathrm{effective}}^* = \varepsilon_{\mathrm{layer}}^* \left\{ \frac{a^3 + 2\left(\frac{\varepsilon_{\mathrm{core}}^* - \varepsilon_{\mathrm{layer}}^*}{\varepsilon_{\mathrm{core}}^* + 2\varepsilon_{\mathrm{layer}}^*}\right)}{a^3 - \left(\frac{\varepsilon_{\mathrm{core}}^* - \varepsilon_{\mathrm{layer}}^*}{\varepsilon_{\mathrm{core}}^* + 2\varepsilon_{\mathrm{layer}}^*}\right)} \right\} \tag{9}$$

Then, based on Eq. (9), ε_p of the Clausius–Mossotti function should be replaced with $\varepsilon_{\mathrm{effective}}^*$ from the single shell particle, and then Eq. (4) can be presented as below.

$$K = \frac{\varepsilon_{\mathrm{effective}}^* - \varepsilon_m^*}{\varepsilon_{\mathrm{effective}}^* + 2\varepsilon_m^*} = \left(\frac{\sigma_{\mathrm{effective}} - \sigma_m}{\sigma_{\mathrm{effective}} + 2\sigma_m}\right)\left[\frac{j\omega\tau_0 + 1}{j\omega\tau_{\mathrm{MW}} + 1}\right] \tag{10}$$

Equation (10) is applied to cells modified by antibody-coated gold nanoparticles with ε_3. First, we calculate the $\varepsilon_{\mathrm{effective}}^*$ of the inner two-layered cell, then estimate the $\varepsilon_{\mathrm{effective}}^{**}$ of whole nano-gold modified cell following the procedure as shown in Fig. 2.

Based on Eq. (9), the main factor affecting permittivity $\varepsilon_{\mathrm{effective}}^*$ is $\varepsilon_{\mathrm{layer}}$, if $\varepsilon_{\mathrm{layer}}$ is much larger than $\varepsilon_{\mathrm{core}}^*$. Therefore, if gold nanoparticles are added to modify the cells and fill their outside membrane, their material behavior will be changed more closely to metal particles. Since the conductivity of metals is greater than that of dielectric materials, the effective conductivity is getting larger and τ_{MV} is getting smaller. Therefore, the operating condition is controlled closely to the status of low frequency. Based on Eq. (8), we realize that the behavior of bio-cells can rely

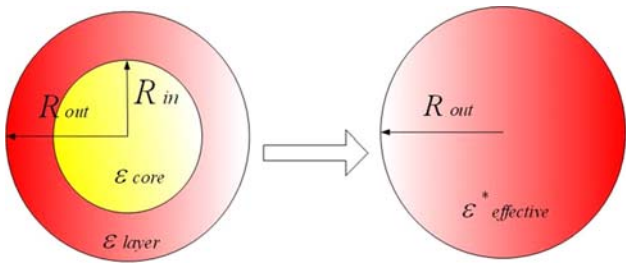


Fig. 1 The single shell model

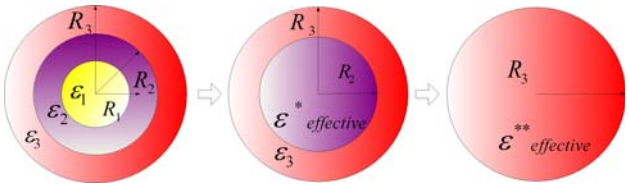


Fig. 2 The simplification procedure by applying single shell model to nano-gold modified cell

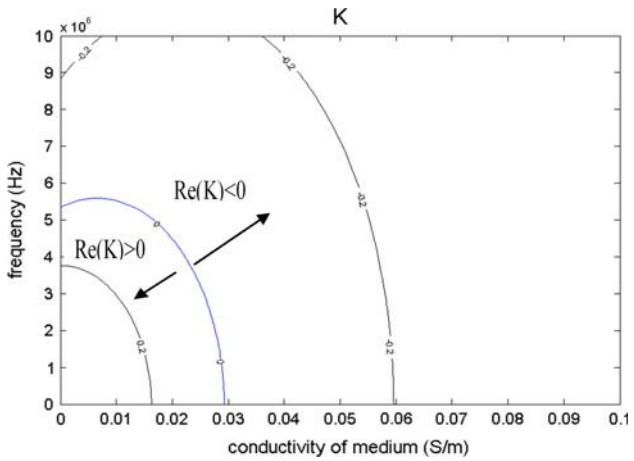


Fig. 3 The distribution of Re(K) versus frequency and medium conductivity for the L929 cell without nano-gold modification. The cell has the properties of $\varepsilon_p = 15 \times 8.84 \times 10^{-12}$ F/m, $\sigma_p = 0.03$ S/m, $\varepsilon_m = 76.6 \times 8.84 \times 10^{-12}$ F/m

on the conductivity of low frequency. Consequently, Re(K) is positive and the cells carrying positive DEP are adsorbed to electrodes. For example, the analysis of Re(K) for the L929 cells without modification of nano-gold is shown in Fig. 3, where Re(K) can be positive or negative depending on the medium conductivity and the applied frequency by the calculation of Eq. (4). However, if the cells are fully modified with nano-gold, then based on the assumption of Eq. (8), Re(K) becomes positive or approaches to 1 (Fig. 4), for most of the medium conductivity and the applied frequency, indicating nano-gold can enhance positive DEP force.

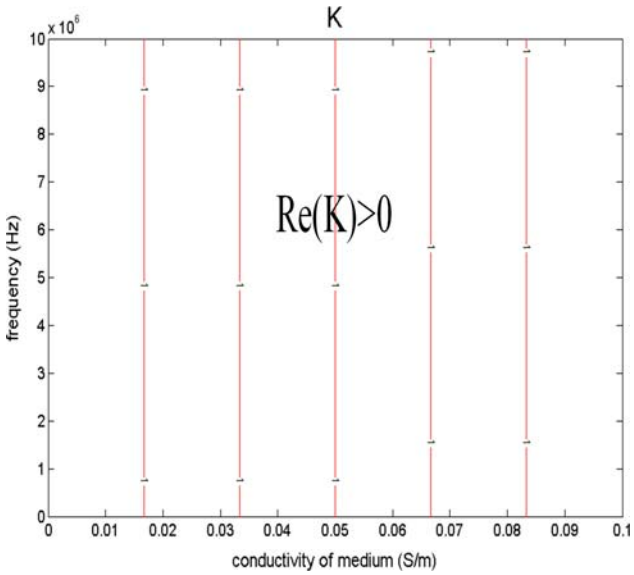


Fig. 4 The distribution of Re(K) versus frequency and medium conductivity for the cells fully modified by gold nanoparticles

Structure of biochip

In our experiment, glass was selected as the substrate to sustain electrodes. Considering the experimental stability and accuracy, the AC electric field should be precisely transmitted to the electrodes. Therefore, we adopted ISA (Industry Standard Architecture) Bus slot to connect the chips.

In this study, electrodes were utilized to isolate and measure all particles. Figure 5 shows the structure of the electrodes. This device consists of two components: the glass substrate with comb-like electrodes and the well mounted on the electrodes.

Simulation

A design of serrated parallel-plate electrodes is used to increase the intensity of the electric field and bioparticles can be easily adsorbed around the DEP electrodes because of sufficient electricity. Figure 6 shows the electrical simulation produced by the parallel saw-tooth electrodes with a gap of 5 μm. The voltage applied to the electrodes is 10 V, and the frequency is 1 MHz. The electric field varies with the applied sinusoidal signal. The highest field intensity occurs at the tip of saw-tooth electrodes as expected.

Materials and methods

Preparation of antibody-coated gold nanoparticles

Gold nanoparticles (Nano Gold-40) were purchased from Taiwan Advanced Nanotech Inc. (Taoyuan, Taiwan). The

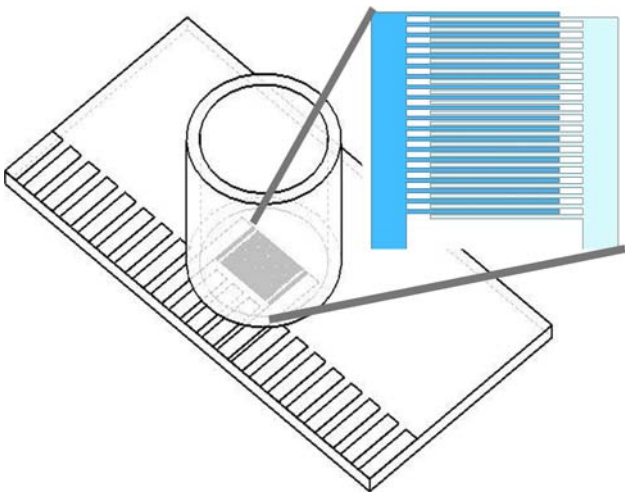


Fig. 5 Structure of the proposed biochip composed of electrodes and an open chamber

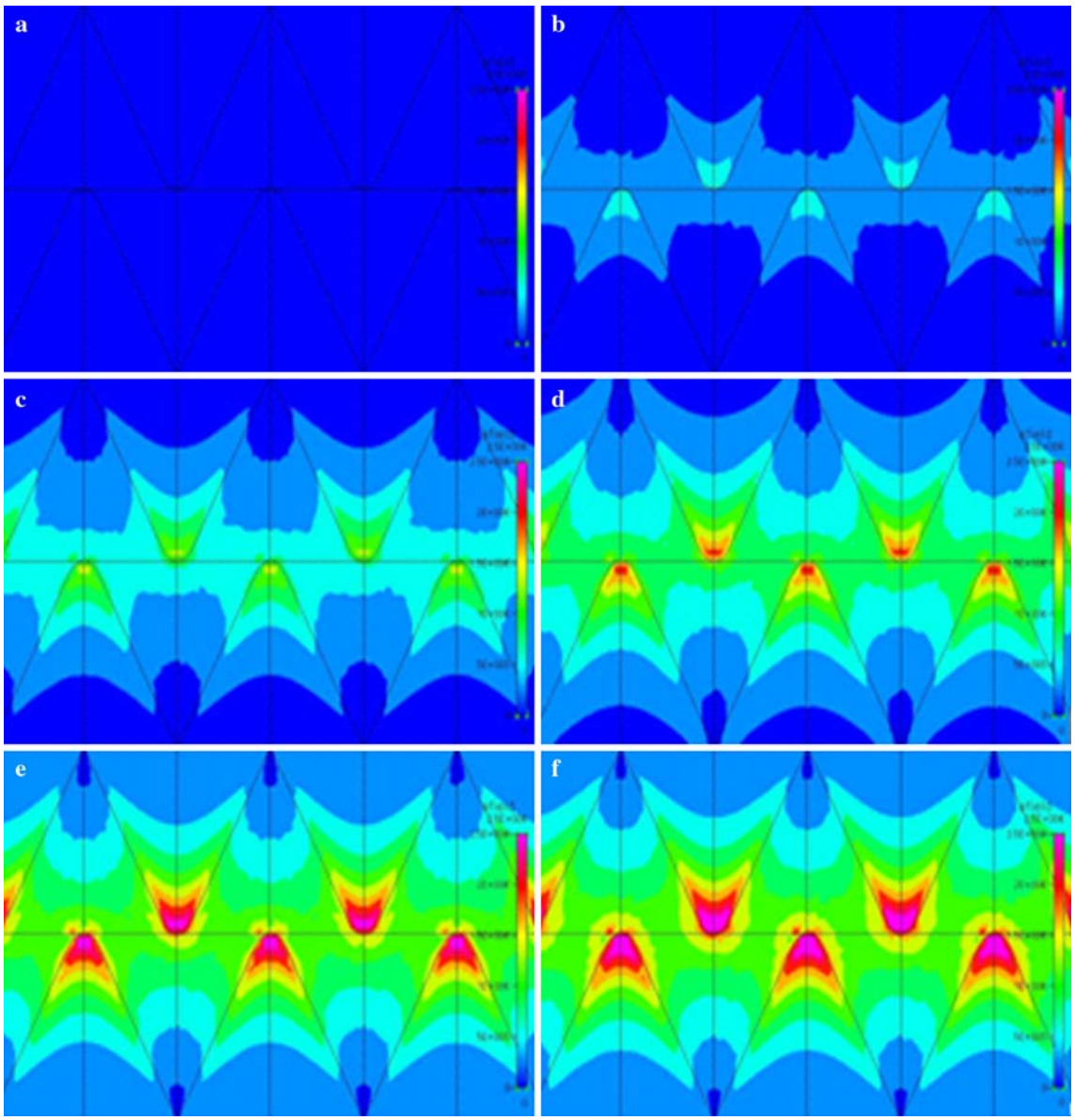


Fig. 6 The distribution of electric fields on the saw-tooth electrodes: the variation of the electric field with the applied sinusoidal signal at phase angle **a** 0°, **b** 18°, **c** 36°, **d** 54°, **e** 72°, **f** 90°

sizes for nanogold used were between 25 and 35 nm. The polyclonal antibody against *Salmonella* spp. (cat. no. B65701R) was purchased from Biodesign (Portland, ME). The antibody reacted with serogroups A, B, C, D, and E of *Salmonella*. Bovine serum albumin (BSA, cat. no. A9647) was purchased from Sigma, Saint Louis. Gold nanoparticles were coated with immunoglobulin G against *Salmonella* [10]. At room temperature, 1 µl of antibody was added in 600 µl of gold nanoparticles solution containing 0.04% trisodium citrate and 0.26 mM potassium carbonate. The mixture was gently mixed for 16 h, blocked by 100 µl of 10% BSA solution for 30 min at room temperature and centrifuged at 6,000*g* for 20 min at 4 °C. After centrifugation, the oiled drop was washed by washing buffer and resuspended in 600 µl of PBS (pH 7.4) as assay solution.

Salmonella from a patient in the hospital were employed to develop new *Salmonella* samples, which were further mixed in KCL solution for detection. A small part of *Salmonella* colonies were taken from the agar plates, immersed in the KCL deionized water (1 mg/3 ml) with conductivity of the 2 µS/cm, and then kept standstill for 3 h. In addition, a group of *Salmonella* samples with concentration from log CFU/ml = 9.3, 8.3, 7.3, 6.3, and 5.3, were, respectively, redeployed and 10 µl of assay solution with antibody-coated gold nanoparticles were

added for 3 h until the complete bonding between antibodies and Salmonella was completed.

Fabrication of DEP electrodes

By use of a thermal evaporator, a layer of aluminum was deposited, photolithographically defined, and etched on the glass to build the electrode patterns. Each electrode array was surrounded by a ring of PR or silicone measuring 4 mm in width and 10 mm in length to form an open chamber, which could accommodate 4–10 μl of solution according to different thickness in layers of PR or silicone. Figures 7 and 8 show the completed biochip and the pattern of saw-tooth electrodes.

Equipment and test setup

The fluid samples were added into the open chamber on the chip using a micropipette. The kinetic movement of nanoparticles and nanoaggregates in the system was observed and recorded using a microscope (Discovery) equipped with a digital camera (Nikon DXM1200). The waveforms of the applied AC signal and voltage on electrodes were monitored using a digital oscilloscope (HP54610B). A function generator (TTi, TGA1244) generated the AC signal, which had a frequency ranging from 100 kHz to 5 MHz and a magnitude ranging from 2.5 to 20 V. An equivalent circuit for the dielectrophoretic impedance measurement method (DEPIM) experiment (Fig. 9) [3–6].

We model the impedance of the electrodes on the chip as a capacitor C_s serially connected with a resistor R_s. Let a resistor $R = 100$ ohm share the supply voltage V_{s1}. The voltage across the electrodes is then defined as V_{s2}. We can define a normalized impedance change, NIC (%), as $(Z_s - Z_c)/Z_c \times 100\%$. Z_s is the impedance magnitude of the electrodes where the samples are collected, while Z_c is that when only gold nanoparticles exist in the medium. Z_s can be calculated by $(1/(\omega^2 C_s^2) + R_s^2)^{1/2}$, where ω is the angular frequency, and Z_c can similarly be acquired.

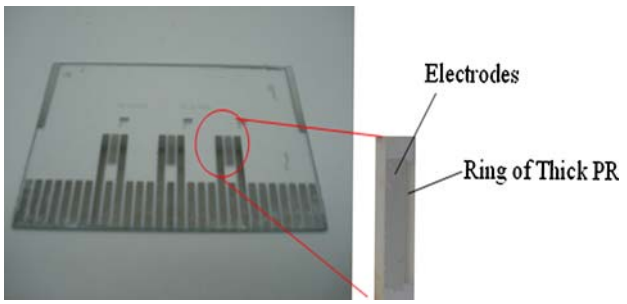


Fig. 7 The proposed biochip with electrodes surrounded by a ring of PR or silicone measuring 4 mm in width and 10 mm in length to form an open chamber accommodating 4–10 μl of solution

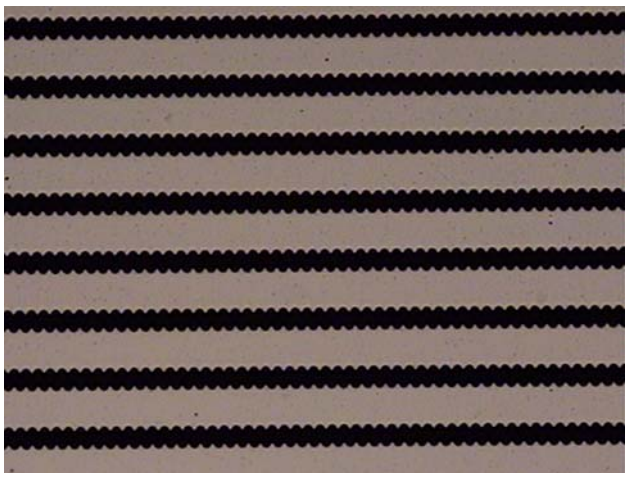


Fig. 8 Magnified picture of saw-tooth shape electrodes with gap of 5 μm

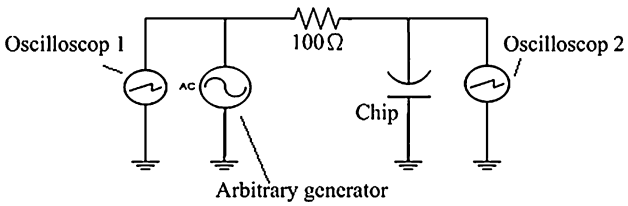


Fig. 9 Equivalent circuit for the dielectrophoretic impedance measurement method (DEPIM) experiment

Results and discussion

Control group: non-modified *Salmonella*

Some of fluid samples were added by micro-titration onto the electrodes of the chip, and covered with a glass coverslip. Figure 10a shows the particle distribution before the electric field was imposed. Following the electric field with amplitude of 10 V at 10 MHz applied, *Salmonella* in solution with conductivity of the 2 μS/cm was adsorbed on the electrode by DEP force (Fig. 10b). The original randomly distributed *Salmonella* were polarized by the effects of electric fields and aligned along the direction of electric field extending several layer surrounding the electrode. When the electric field frequency gradually decreased, the DEP force on *Salmonella* became weaker accordingly and the number of *Salmonella* adsorbed on the electrode was also reduced. When the electric field frequency was turned down to the vicinity of 5 kHz, *Salmonella* were conducted by negative DEP and therefore left the electrode. *Salmonella* originally attached to the electrode were instantly

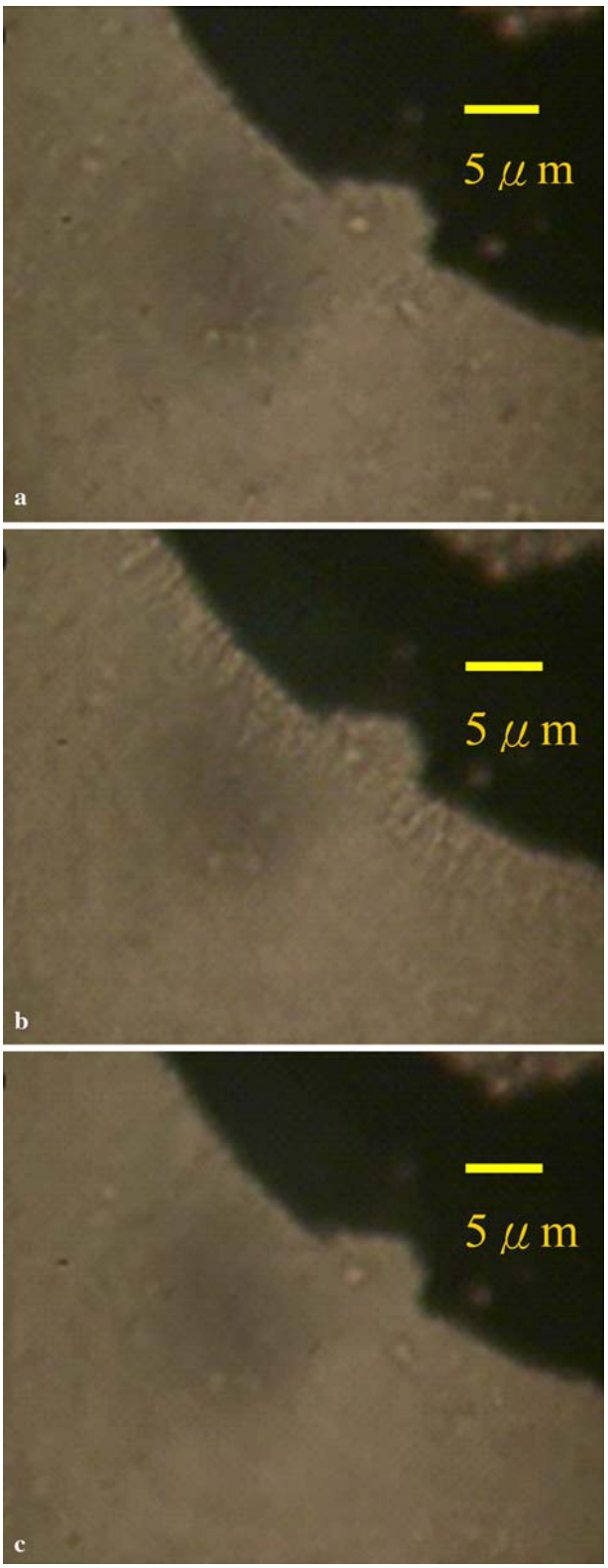


Fig. 10 Under medium conductivity of the 2 μS/cm: **a** *Salmonella* were not adsorbed to electrodes when the electrodes were not energized. **b** *Salmonella* were adsorbed to the electrodes when the voltage of 10 V was applied at 10 MHz. **c** *Salmonella* were expelled by the electrodes when the voltage of 10 V was changed into 5 KHz

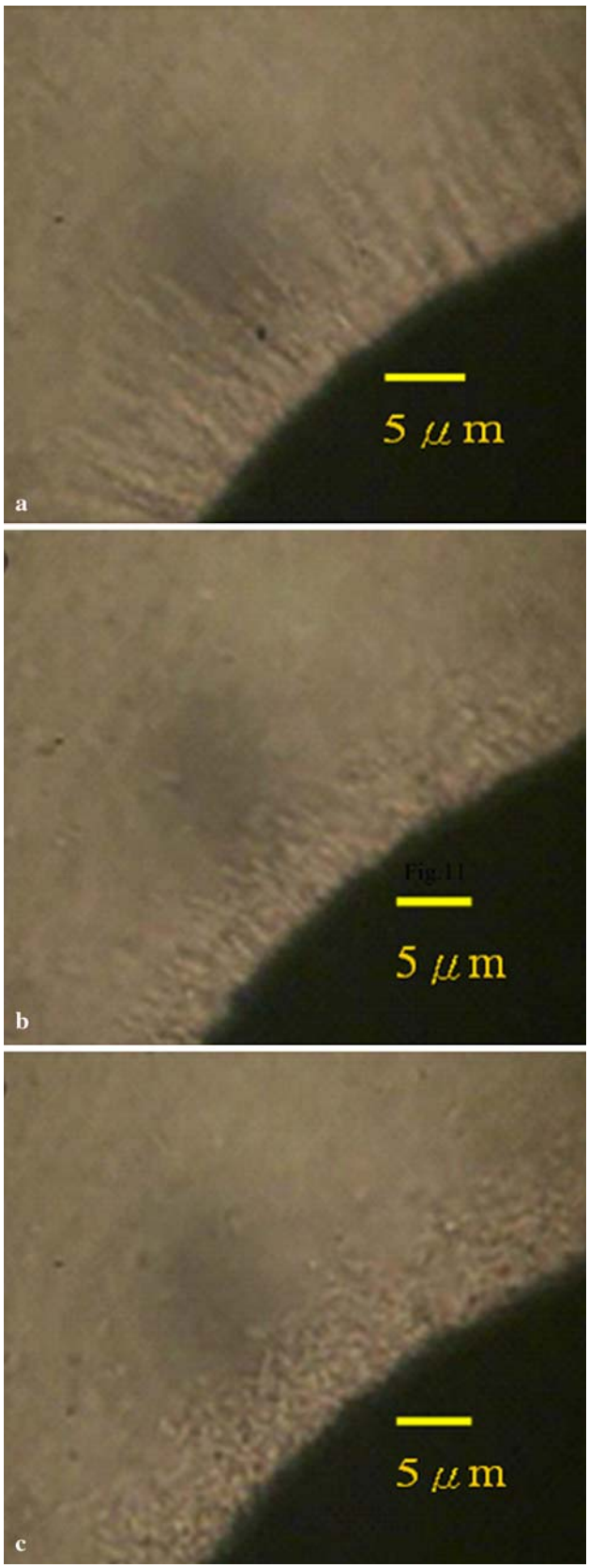
expelled (Fig. 10c). Therefore, if the electric field frequency was restored to more than 5 KHz, several layers of *Salmonella* were adsorbed around the electrode.

Experimental group: modified *Salmonella*

After a sufficient period of time to allow antibody-coated gold nanoparticles bind with *Salmonella* in the sample solution, the mixture was added into the chamber on the chip with comb-like electrodes and then the chamber was covered with a glass coverslip. Before applying an electric field, gold-nanoparticles modified *Salmonella* displayed a similar situation to those non-modified ones. When the electric field of 10 MHz was imposed, *Salmonella* binding with the antibody-coated gold-nanoparticles were strongly conducted by DEP force to adsorb on the electrode (Fig. 11a). Once the frequency was lowered, the force of electrode decreased as well. When the frequency of electric field was instantaneously changed to 5 kHz (Fig. 11b), *Salmonella* connecting the antibody-coated gold-nanoparticles were still exerted by positive DEP and absorbed on the electrode. Supposedly, the decrease of the frequency and reduction of electric field strength avoided adsorption of *Salmonella* on the electrode. However, there were still some but fewer *Salmonella* adsorbed on the electrode. Once the electric field was stopped, *Salmonella* (Fig. 11c) were gradually desorbed away from the electrode and returned to the original display.

From the above results, we can clearly realize that the original dielectric properties of *Salmonella* have changed after modification by antibody-coated nanoparticles. When the signal of 5 kHz frequency is imposed, *Salmonella* are conducted by the negative DEP force to be expelled from electrodes. On the contrary, the electrode can exert positive DEP forces to continuously adsorb modified *Salmonella*. Such characteristic can facilitates isolation of *Salmonella* in biological samples to separate from other bacteria. Using the simple modification of cell surface, the dielectric properties of any cells or pathogens can be changed as long as the surface antigens of bioparticles are available for binding with the corresponding antibodies and nanoparticles. Similarly, other metal nanoparticles can be alternatives to isolate *Salmonella* once if their natures and stability allow the binding of *Salmonella* antibody. Moreover, if metal nanoparticles are magnetic beads, the modified cells may be purified and collected in an external magnetic field and electrical field.

If the antibody-coated gold-nanoparticles are more than bioparticles in quantities, modified bioparticles will aggregate to increase the DEP force and become easily attracted by the electrodes (Fig. 12).



◄ **Fig. 11** **a** When the electric field of 10 MHz was imposed, *Salmonella* binding with the antibody-coated gold-nanoparticles were strongly conducted by dielectrophoresis force to absorb on the electrode. **b** When the frequency of electric field was instantaneously changed to 5 kHz, there were still some but fewer *Salmonella* adsorbed on the electrode. **c** Once the electric field was stopped, *Salmonella* were gradually desorbed away from the electrode and returned to the original random display

Quantity measurement of *Salmonella* and *E. coli* without nano-gold

E. coli was selected for our experiment because *E. coli* and *Salmonella* are both gram negative pathogens and share many similar characteristics.

In the experiment we set frequency as 800 KHz and conductivity of medium as 0.001 S/m. By changing the sample concentration of *E. coli* and *Salmonella* on the proposed biochip from 6.5 to 9.5 CFU/ml, we measured the voltage across the chip V_{s2} and supply voltage V_{s1}. As shown in Fig. 13, there is no obvious difference in NIC between most concentrations (6 and 8.5 long CFU/ml) of *E. coli* and *Salmonella*, which indicates the dielectric properties of both pathogens are very similar. The quantity of bacteria on the chip, or the concentration of bacteria in the sample can be easily estimated from the NIC value (Fig. 13). The repeated measurements revealed good reproducibility.

Quantity measurement of *Salmonella* and *E. coli* with nano-gold

After we mixed *Salmonella* and 10 μl of antibody-coated gold-nanoparticles, the mixture was placed standstill for 15–30 min, added into the chip, and the partial voltage was measured on chip using the same experimental parameters as previously described. As shown in Fig. 14, there is significant difference of NIC between most concentrations (5 and 7.5 long CFU/ml) of *E. coli* and *Salmonella,* which indicates the dielectric properties of nano-gold modified *Salmonella* is different from those of *E. coli*. Again, the quantity of *Salmonella* on the chip, or the concentration of *Salmonella* in the sample can be easily estimated from the NIC value since it is defined as comparison of the impedance between the electrodes with and without *Salmonella*.

The microscope images of *Salmonella* corresponding to Figs. 13 and 14 are shown in Figs. 15 and 16, respectively, which prove that antibody-coated nano-gold can alter DEP characteristics of bioparticles and aggregate them.

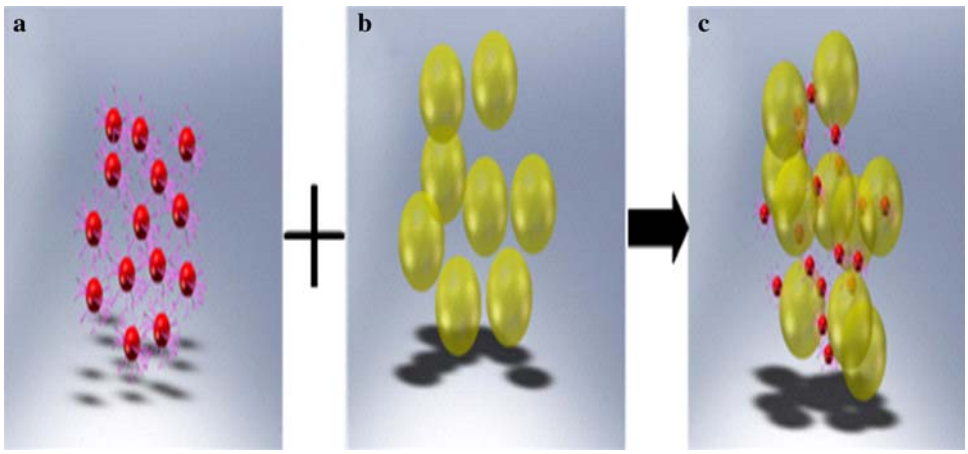


Fig. 12 **a** Gold nanoparticles are coated by antibodies. **b** Bioparticles form a group. **c** Bioparticles and antibody-coated gold nanoparticles aggregate in a group, lead to an increased DEP force, and thus are easily attracted by the electrodes

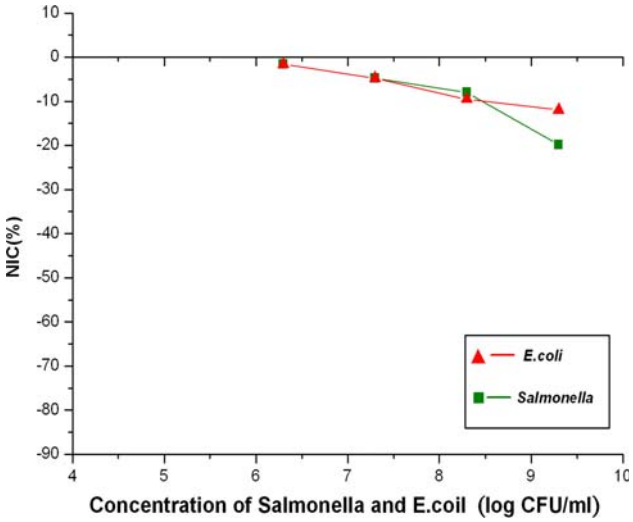


Fig. 13 NIC of *Salmonella* and *E. coli* without nano-gold

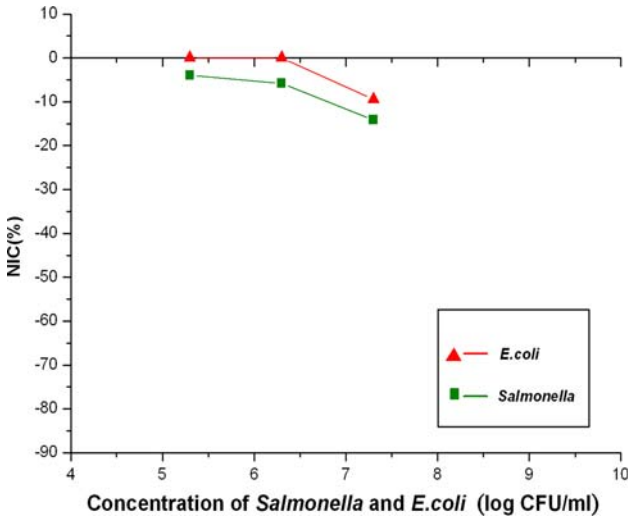


Fig. 14 NIC of *Salmonella* and *E. coli* with nano-gold

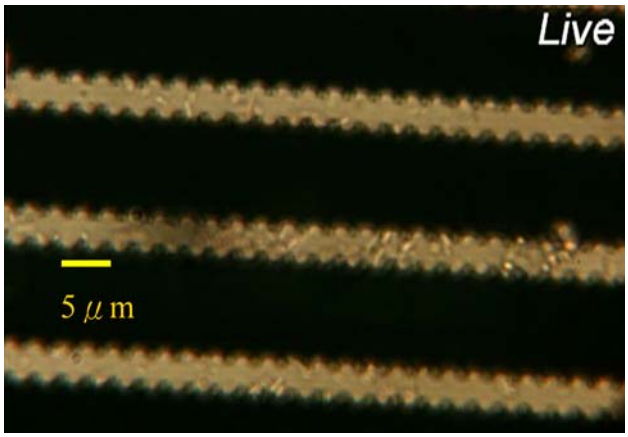


Fig. 15 Non-modified Salmonella (log CFU/ml = 6.3) adsorbed to the edge of the electrodes under DEP force

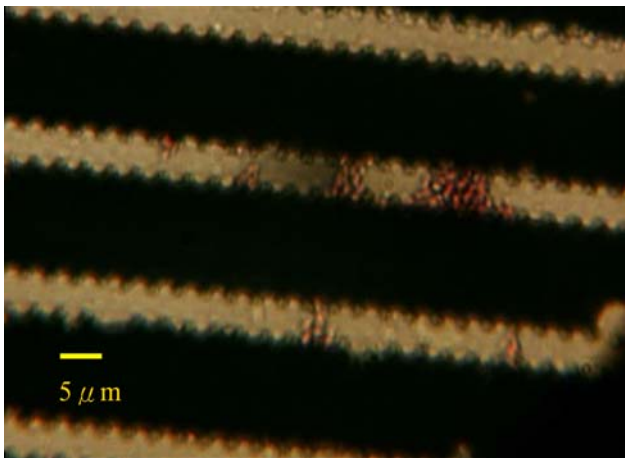


Fig. 16 Nano-gold modified *Salmonella* aggregating along the edge of the electrodes under DEP force (log CFU/ml = 6.3)

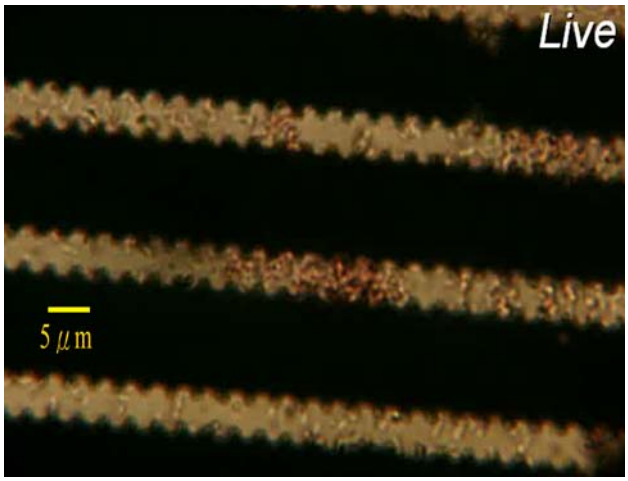


Fig. 17 Aggregating *Salmonella* bonded with gold nanoparticles (in *red color*) and floating *E. Coli*(in *white color*) (log CFU/ml = 6.3)

When we mixed *Salmonella*, *E. Coli* and nano-gold together, the result is shown in Fig. 17. Because modified *Salmonella* (in red color due to nano-gold) aggregated between the electrodes, the floating *E. Coli* (in white color) could be isolated.

We have validated the effect of positive DEP on capture of bioparticles which are modified by gold nanoparticles. Antibody-coated nano-gold can alter DEP characteristics of bioparticles and aggregate them. Simple impedance measurement method can quantify the collected *Salmonella.* Nano-gold can increase the DEP effects of bioparticles and increase the sensitivity up to 10^5 CFU/mL, or even 10^4 CFU/mL if we reduce depth of the chamber and the gap width of the comb-like electrodes. Separation of *Salmonella* from *E. Coli* is feasible by using such DEP.

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