

# A SIMPLE AND RAPID METHOD FOR INFECTIOUS WATERBORNE DISEASE MONITORING USING DISPOSABLE PDMS MICROFLUIDIC CHIP BY DIELECTROPHORESIS

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## ABSTRACT

A simple and rapid method to differentiate between the viable and nonviable *Cryptosporidium parvum* (*C. parvum* oocysts) in drinking water using dielectrophoresis on disposable PDMS (polydimethylsiloxane) microfluidic chips was presented here. Additionally, an efficient way to modify *C. parvum* oocysts by silver nanoparticles (AgNPs) was developed, and the varied standard treatment methods were studied for comparison as well. This method is capable to distinguish the viability of *C. parvum* oocysts at concentration levels lower than  $10^5$  oocysts per milliliter.

## KEYWORDS

*Cryptosporidium parvum* oocysts (*C. parvum* oocysts), Dielectrophoresis, PDMS, Microchip, Disposable

## INTRODUCTION

*Cryptosporidium parvum* (*C. parvum*) is an infectious waterborne protozoan parasite, which is a significant cause of diarrheal disease, dehydration, and death in humans, especially among children and immunocompromised patients, has been identified in 78 other species of mammals.[1] The current diagnostic techniques using animal models are time-consuming, require skilled technicians, and are unable to quantify oocysts in fecal and environmental samples.[2-3]

Dielectrophoresis, causes electrokinetic migration of particles, under non-uniform electric fields and is dependent upon the dielectric nature of the cells and their suspending medium. Electrodeless dielectrophoresis (EDEP) methods apply dielectric constrictions to enhance electric fields during dielectrophoresis, while avoiding the dissipation of trapping due to electrochemical reactions at electrodes, as per our previous works.[4-5] In principle, dielectrophoretic force, acting on a polarizable particle in a non-uniform field, is given by:  $F_{DEP} = 2\pi r^3 \epsilon_m Re[K(\omega)] \nabla E^2$ , where  $r$  is the radius of the particle,  $\epsilon_m$  the absolute permittivity of the suspending medium,  $E$  the amplitude of the applied field (i.e., root-mean-squared  $E$  in the case for an ac field), and  $Re[K(\omega)]$ , the real part of the Clausius-Mossotti (CM) factor, representing the frequency-dependent dielectric contrast between the particle and the suspending medium in an external driving field.

Herein, we used a simple PDMS disposable microchip to perform rapid monitoring of the viability of *C. parvum* oocysts. The varied treatments of heat, hypochlorous acid (HClO), and silver nanoparticles (AgNPs)[6] were utilized for the investigation of the capability of AgNPs, and tried to demonstrate the power of AgNPs treatment and its efficiency relied on the DEP behavior. The current disposable PDMS microchip is adequate for the waterborne protozoan parasite monitoring, like *C. parvum* oocysts, to prevent the further outbreak of infectious diseases.

## EXPERIMENT

The layout of our chip design is depicted in Figure 1. A common and biocompatible material, PDMS was selected for the microstructures imprinted from the silicon wafer, and then proceeded to bond coverslip which treated by general oxygen plasma on surface, to be a disposable microfluidic chip. After the adaptable sample loading and platinum electrodes integrating process, the electric field would be applied by function generator through AC power amplifier and then the cell (*C. parvum* oocysts) behavior would be monitored by CCD (charge coupled device) within AC field applied.

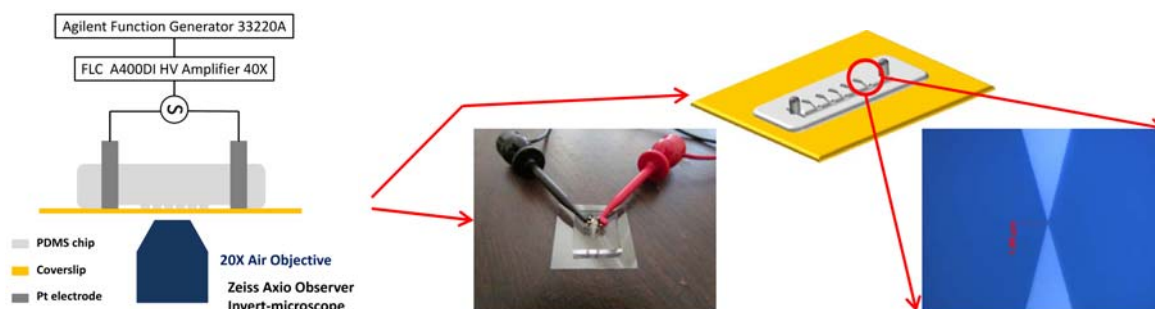


Figure 1: The experiment setup and disposable EDEP chip design

## RESULTS AND DISCUSSION

As stated as above, the DEP behavior of *C. parvum* oocysts is depended on its polarizability, it produced positive, negative and weak DEP behavior by dielectrophoretic force, as shown in the snapshots of Figure 2. Here, we used well substantiated *C. parvum* killing method, heat treatment in boiling water for the comparison to standard.[7] the different doses of treatment by hypochlorous acid (HClO) and AgNPs were investigated. The various DEP behaviors and the results of different treatment and concentration displayed the distinguishable transition curves by frequency in Figure 2. The viable *C. parvum* oocysts (untreated or low dose treatment) expressed positive DEP response in DI water, trapped between two constrictions, in the frequency above 300~400 KHz, and nonviable *C. parvum* oocysts (killed by treatment) expressed negative DEP behavior, repelled away the gaps at all of frequencies. However, the conductivity of viable oocysts is differed from the nonviable one due to the cytoplasm conductivity changed as modeled in the inset of Figure 2, and the distinguishable transition curve demonstrated that it is likely caused by an altered polarizability of oocysts due to changes in conductivity of the cytoplasm.

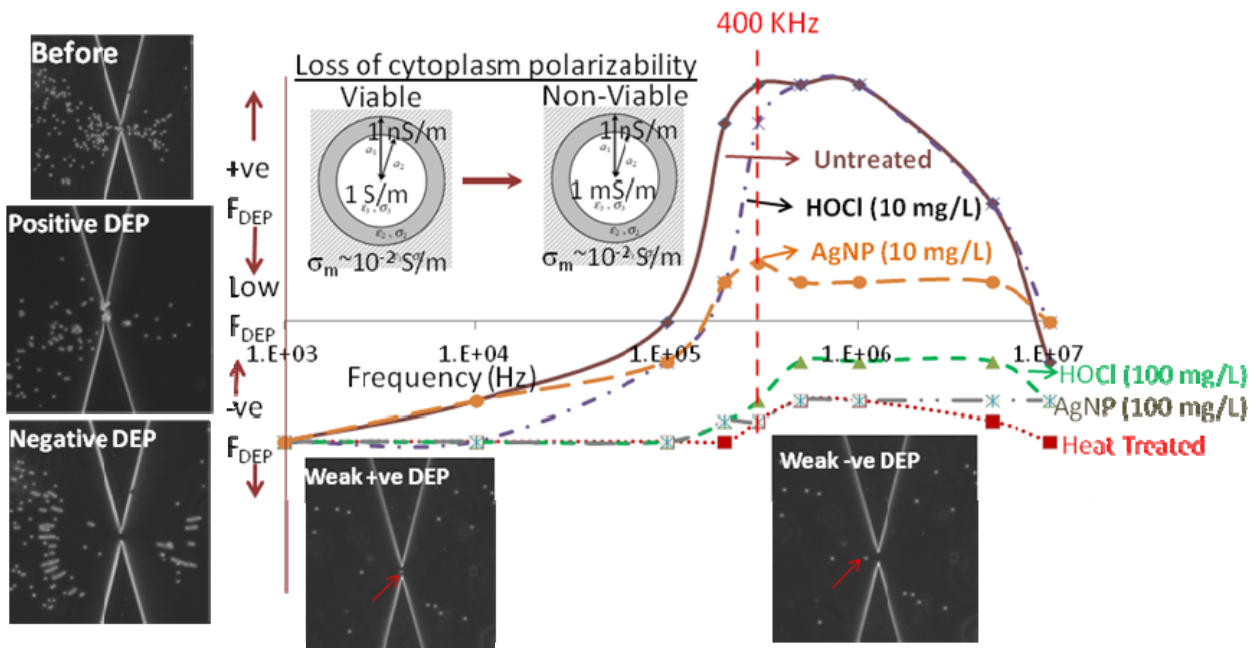


Figure 2. The snapshots of *C. parvum* oocysts behaviors in different DEP condition, and its DEP transition curves in varied treatments and concentration. Inset: The concept of cell models for the polarizability in viable and nonviable *C. parvum* oocysts.

Nevertheless, since the DEP behavior of the *C. parvum* oocysts is dependent on its polarizability, the different conductivity medium will affect the permittivity of *C. parvum* oocysts and medium. The Figure 3 shown our studies when the *C. parvum* oocysts existing in different concentration of medium. It's obvious the DEP transition curve is shifted to the higher frequency when the conductivity of medium is higher to change the permittivity between them.

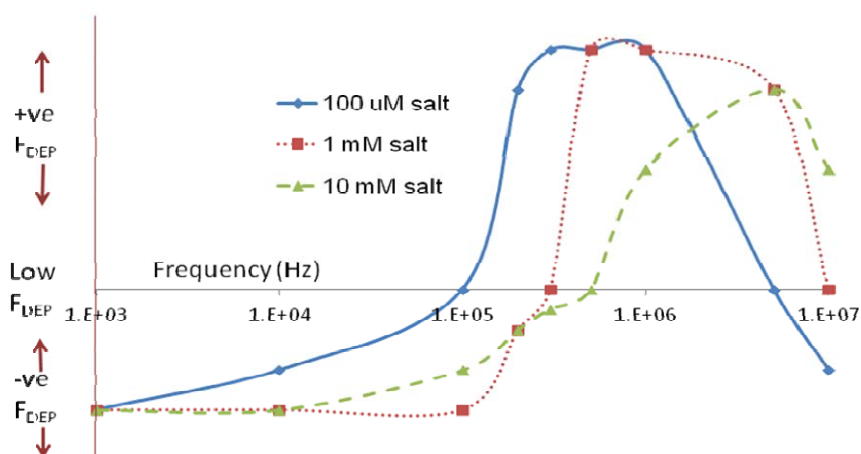


Figure 3. The DEP transition curves of *C. parvum* oocysts in various concentration of medium

Furthermore, we also studied the influence of different doses of varied treatments by time, performed in Table 1, to monitor the DEP behavior changing by treatment time of HClO or AgNPs at particular frequency, 400 KHz, and

the treatment time associated to the different concentration of *C. parvum* oocysts (lower to  $10^5$  oocysts/mL). The results brought this *C. parvum* oocysts monitoring method into a powerful scale due to the AgNPs treatment performed within very short time and its nontoxic agent property. It will appropriate to be a good waterborne protozoan parasite determination method, rapidly diagnose and prevent the infectious diseases outbreak.

Table 1. The time monitoring on different doses of hypochlorous acid (HClO) and silver nanoparticles (AgNPs) treatment.

Treatment time / mins	5	10	15	20	30	35	~ 120	150	180	210	240
HClO (100 mg/L)	+	+	+	+	+	-	-	-	-	-	-
HClO (50 mg/L)	+	+	+	+	+	+	+	-	-	-	-
AgNPs (100 mg/L)	-	-	-	-	-	-	-	-	-	-	-
AgNPs (50 mg/L)	-	-	-	-	-	-	-	-	-	-	-
AgNPs (35 mg/L)	less	less	-	-	-	-	-	-	-	-	-
AgNPs (20 mg/L)	less	less	less	less	less	-	-	-	-	-	-
AgNPs (10 mg/L)	+	+	+	+	+	+	+	+	+	+	+
AgNPs (1 mg/L)	+	+	+	+	+	+	+	+	+	+	+
AgNPs (35 mg/L)**	-	-	-	-	-	-	-	-	-	-	-
AgNPs (20 mg/L)*	-	-	-	-	-	-	-	-	-	-	-
AgNPs (15 mg/L)*	+	+	+	+	+	+	+	+	less	-	-
AgNPs (10 mg/L)*	+	+	+	+	+	+	+	+	+	+	+

DEP conditions: 200  $V_{pp}/cm$ , 400 KHz; +: positive DEP, -: negative DEP, less: less effective

[*C. parvum* oocysts]:  $5 \times 10^7$  oocysts/mL,  $*5 \times 10^6$  oocysts/mL, and  $**5 \times 10^5$  oocysts/mL

## CONCLUSION

In presenting investigation, we demonstrated a simple and rapid method to differentiate the viable and nonviable *C. parvum* oocysts in drinking water using disposable microfluidic chip by dielectrophoresis for waterborne disease diagnostics. We illustrated the viability of *C. parvum* oocysts would be distinguished by DEP behavior obviously in various oocysts treatments, especially in a developed nontoxic treatment by silver nanoparticles, and also studied the dose effect by treatment time, influence of the medium conductivity as well. It's apparent the *C. parvum* oocysts are monitored the varied DEP behaviors at particular frequency by different treatment, summarized in table 2.

Table 2. The summary of DEP responses for *C. parvum* oocysts in varied treatments

	No treatment	Heat treatment*	AgNPs treatment**	HClO treatment**
<b>DEP behavior in D.I. water</b>	<b>+</b>	<b>-</b>	<b>-</b>	<b>+ → -</b>

\*Heat treatment: 15 mins in boiling water

\*\*Concentration used is 100 mg/L for  $5 \times 10^7$  oocysts/mL

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