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Electrical impedance sensor for quantitative monitoring of infection processes on HCT-8 cells by the waterborne parasite *Cryptosporidium*

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ABSTRACT

Cryptosporidium is the main origin of worldwide waterborne epidemic outbreaks caused by protozoan parasites. Its resilience to water chemical treatments and the absence of therapy led to consider it as a reference pathogen to assess water quality and as a possible bioterrorism agent. We here show that an electrical impedance-based device is able to get insights on *Cryptosporidium* development on a cell culture and to quantify sample infectivity. HCT-8 cells were grown to confluency on Interdigitated Microelectrode Arrays (IMA's) during 76 h and then infected by *Cryptosporidium parvum* during 60 h. The impedimetric response was measured at frequencies ranging from 100 Hz to 1 MHz and a 7 min sampling period. As the infection progresses the impedance signal shows a reproducible distinct succession of peaks at 12 h post infection (PI), 23 h PI and 31 h PI and local minima at 9 h PI, 19 h PI and 28 h PI. An equivalent circuit modeling-based approach indicates that these features are mostly originated from paracellular pathway modifications due to host–parasite interactions. Furthermore, our data present for the first time a real-time monitoring of early parasitic stage development with alternating zoite and meront predominances, observed respectively at peaks and local minima in the impedimetric signal. Finally, by quantifying the magnitude of the impedimetric response, we demonstrate this device can also be used as an infectivity sensor as early as 12 h PI thus being at least 6 times faster than other state of the art techniques.

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1. Introduction

Cryptosporidium is a protozoan parasite which infects a wide range of vertebrates and more particularly human beings (Fayer, 2010). Between 2004 and 2010, 60% of worldwide reported outbreaks of diseases due to waterborne parasitic protozoa were *Cryptosporidium* induced (Baldursson and Karanis, 2011). Cryptosporidiosis outbreaks are present in both developing and industrialized countries (Chalmers, 2012) and can be lethal for immunocompromised or weak patients (Snelling et al., 2007). The largest outbreak took place in 1993 in Milwaukee (US), where 403,000 persons were affected and more than 100 died (Mac Kenzie et al., 1994). Furthermore, it has recently been shown that this pathogenic agent can induce gastrointestinal cancer in mice (Certad et al., 2007).

As no effective therapeutic treatment against the infection currently exists, tools allowing non-invasive and real-time analysis are needed to get a better understanding of the parasite infection processes.

Besides, the high resilience of the parasite to water chemical treatments such as chlorination (Cacciò and Pozio, 2006), the maintenance of its capacity to produce infectious forms, i.e. the infectivity, for more than 13 months (Chen et al., 2007) and the requirement of low inoculum to produce pathogenicity (less than 10 parasites) (Chappell et al., 2006; Okhuysen et al., 1999) lead also to consider this pathogen as a potential waterborne bioterrorism agent. As a consequence, it becomes essential to assess the risk with a rapid evaluation of sample infectivity. A common non-invasive electrical analysis on cell cultures is the so-called "TransEpithelial Electrical Resistance" (TEER). These systems deliver a burst of DC current through cells cultivated on a porous membrane at defined moments to determine cell layer permeability, mostly influenced by intercellular junctions (Ussing and Zerahn, 1951).

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Some developments have been made to make similar measurements continuously, such as the CellZscope ® device (Wegener et al., 2004).

Impedance Spectroscopy (IS) is another common approach which displays several advantages: real-time monitoring, label-free analysis, high-throughput screening and ease of integration (Spegel et al., 2008). The combined use of IS and microelectrodes has demonstrated its usefulness in the field of cell biology. Pioneered by Giaever and Keese (1984) and adapted to Interdigitated Microelectrode Arrays (IMA) by Ehret et al. (1997), it has emerged as a powerful tool and been used to study a wide range of cellular phenomena such as cell adhesion and proliferation (Atienza et al., 2005; Wang et al., 2008; Wegener et al., 2000), cell confluency (De Blasio et al., 2004), cell apoptosis after chemical treatments (Arndt et al., 2004; Meissner et al., 2011; Solly et al., 2004) or stem cell differentiation (Bagnaninchi and Drummond, 2011). However, this technique has never been used either to get continuous information on the host cell response during protozoan parasite infection or to assess their infectivity. We here report that IS on IMA can be used to monitor in real-time the response of a cell culture infected by the waterborne parasite *Cryptosporidium parvum* (*C. parvum*). Furthermore, we show that the IS technique can provide insights on parasite development through their action on the cells which opens significant biological analysis opportunities. Finally, for the first time, we demonstrate that the infectivity of a parasite sample, i.e. its ability to produce infectious forms (King et al., 2012), can be measured and plotted as a dose response curve.

2. Material and methods

2.1. Biosensor fabrication

Inspired by a previously described protocole (Houssin et al., 2010), a square Pyrex substrate (5 cm × 5 cm × 0.7 mm thick) is first cleaned with a 5 min Piranha bath (1/3 H_2SO_4 in 2/3 H_2O_2), directly followed by Acetone and Isopropyl alcohol baths. Then, after a dehydration step, Dow ST hexamethyldisilazane (HMDS) is spin coated (2000 rpm, 1000 rpm/s, 20 s) to allow adhesion, again by spin coating, of AZ Electronic Materials AZ-nLOF 2020 photoresist (3000 rpm, 1,000 rpm/s, 20 s) afterwards. After a softbake step (110 °C, 1 min), 8 mm circular interdigitated round microelectrode arrays (with finger widths and gaps respectively of 100 μm and 7 μm in average) are patterned by UV-Lithography (I=53.9 mJ/cm²; postbake: 110 °C, 1 min) and developed with AZ Electronic Materials AZ MIF 326 (55 s) so that a titanium adhesive layer (10 nm) and gold (100 nm) can be deposited by evaporation. Lift-off is done using Dow ST SVC-14 solvent (70 °C, 40 min). Then, 6 mm wells are punched in 5 mm thick square-shaped polydimethylsiloxane (PDMS) and electrostatically fixed on the chip, which have been activated beforehand by Plasma O_2 (30 sccm, 150 W, 100 mT, 30 s) to improve the adherence. The well diameter is designed to be smaller than the IMA total area to ensure that the any cell growing at the bottom sits on the electrode and thus contribute to the signal. The microchip and its dimensions are presented in Fig. 1A and B. Finally, the chip is mounted on a home-made Printed Circuit Board (PCB). Previous to cell seeding, biosensor chips were sterilized with a 70% (vol/vol) alcohol treatment for 15 min at room temperature followed by three rinse steps with sterilized culture medium.

2.2. Cell culture

One standard method to evaluate *Cryptosporidium* infectivity is based on its development on cell cultures. HCT-8 cell lines (Human ileocecal adenocarcinoma, ECACC No 90032006) were maintained with regular subculturing in a growth medium consisting in a RPMI 1640 medium (Gibco, Invitrogen Corporation, France) supplemented with 1 mM Sodium Pyruvate, 2 mM L-glutamine, 10% (vol/vol) of heat inactivated fetal calf serum, 170 μM Streptomycin, 210 μM Gentamycin and Penicillin (100 U/L). Cells were grown in an incubator at 37 °C with 5% (vol/vol) CO_2 until monoloayers

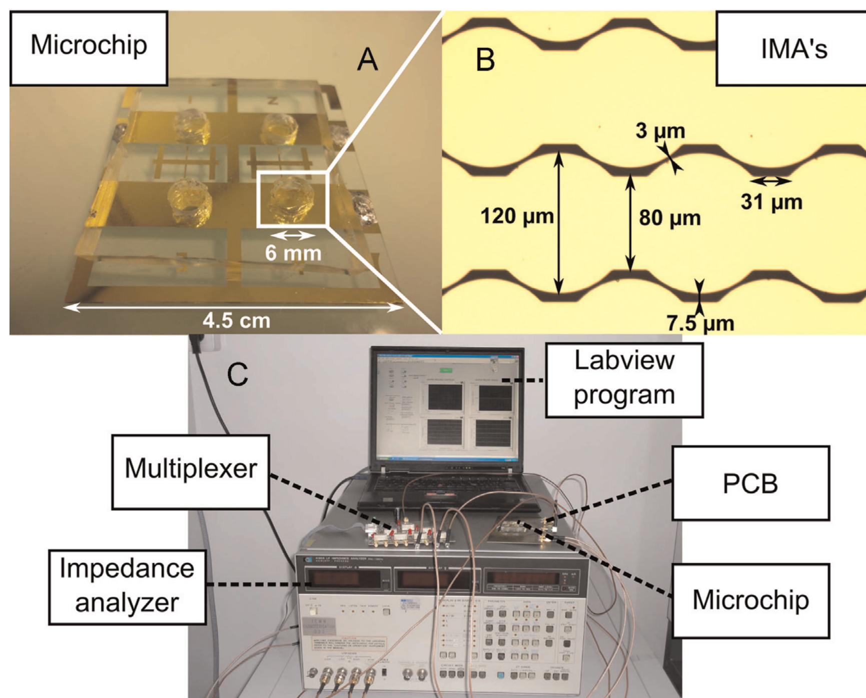

Fig. 1. Experimental set-up pictures. (A) Pictures of the whole setup, with the microchip mounted on a Printed Circuit Board (PCB) plugged with a multiplexer and a HP4192A impedance analyzer, driven by a Labview program. After cells or parasites were inoculated on the chip under a class 2 laminar flow hood, PCB and microchip were put in an incubator at 37 °C with 5% (vol/vol) CO_2. (B) Picture of the microchip, including 4 cell culture PDMS wells at the bottom of which Interdigitated Microelectrode Arrays (IMA's) were designed. (C) Picture of cells cultivated on IMA's. Average electrode width and gap are respectively 100 μm and 7.5 μm.

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