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Proof of principle of a novel impedance microbiology method based on bacteriophages functionalized paramagnetic nanobeads

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Abstract

An unconventional approach to impedance microbiology for *Escherichia coli* detection is under investigation. The detection principle of solution conductivity variation is based on intracellular content escape resulting from bacteriophage cell lysis. Bare electrodes on-chip included in a PDMS chamber were applied to the impedance measurements. Proofs of principle experiments were performed. In parallel, paramagnetic nano-beads were functionalised with selective phages for sample magnetic concentration and future methods integration. The system potential detection limit is about 10 CFU/chamber and provides the means for selective detection of viable cells. The methods integration could provide cost–effective results in less than 1 hour.

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Keywords: Impedance microbiology; bacteriophage; microsystem; paramagnetic nanobeads; pathogen detection.

1. Introduction

Agro-food industries are subject to a high testing rate [1]. In particular, foodborne pathogens detection is costly and time consuming. Hygiene regulations [2] require sensitive analysis of representative samples [3], up to 1 CFU/mL in 25 g for *Listeria monocytogenes* and *Salmonella*. Systems that integrate preparation of representative samples with sensitive and selective detection of viable pathogens are in high demand [4].

At the current state-of-the-art, sample preparation is essential to achieve a sensitive detection [4]. Immunomagnetic concentration is a proven method prone to system integration but antibodies are expensive and

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unstable. Bacteriophages (phages) are a promising recognition element [5]. Phages T4 are lytic viruses specific to their target host. The whole infection cycle takes place in 30 minutes. Most importantly, bacteriophages require viable cells for phage progeny production, thus provide a platform for selective identification of viable cells only.

Impedance Microbiology (IM) is a certified action method [6] for the detection of viable cells. IM is based on solution conductivity variations generated by bacteria catabolism. Its simple implementation employs two bare electrodes. Classic IM uses conventional electrochemical cells of high volumes where catabolites are diluted, resulting in poor method sensitivity. The method depends on bacteria metabolic time frame thus is time consuming. Also its selectivity is limited to selective broth and remains an unsolved issue.

Based on this introduction, our research has focused on a system for a novel and unconventional approach to IM. The method is based on:

- Microsystem for electrochemical impedance spectroscopy analysis;
- Development of low-conductive growth media;
- Selective phages T4 infection of host cell;
- Paramagnetic beads functionalization with phages and phage-magnetic bacteria capture and concentration.

The detection principle is based on solution conductivity variations that are generated by the viral infection of host cells, their burst for phage progeny release and the concomitant dump of endoplasmic material. As case study, the method targeted *Escherichia coli*, a common hygiene indicator [2]. Proof-of-concept results are here presented.

2. Methods and results

2.1. Beads functionalization and bacteria concentration

Paramagnetic beads with 750 nm diameter (Chemicell, ScreenMAG-Amine) were covalently functionalized with phage T4 (ATCC 11303-B4) by the carbodiimide method and as specified in the manufacturer coupling procedure. The phage:bead ratio was first defined via a theoretical calculation based on bead surface, phage dimension and directional phage capsid immobilization. The experimental phages:bead ratios were 84, 112 and 336.

The beads functionalization was characterized in Raman Spectroscopy (Figure 1). The functionalization was proven by similar absorption spectra that were recorded for a single functionalized bead and mean spectra obtained from various functionalized beads. In contrast, bare beads did not show any absorption bands.

E. coli (ATCC 11303) capture experiments were performed at volumes of 0.250, 0.500 and 1 mL. 10 µL of functionalized beads at various concentrations (10^6, 10^7 and 10^8 beads) were added to 10^6 CFU and left to react on a rotating mixer for 15 minutes, followed by magnetic separation. To allow bacteria quantification, the phages were deactivated via UV treatment [7]. Beads-bacteria complex were counted on tryptic soy agar plate (Figure 2). Good capture was obtained in low volumes (250 and 500 µL), with high phage:beads ratio (112 and 336 phage:bead) and with high beads number (10^7 and 10^8). In 1 mL sample the capture was ineffective (data not shown), where only the 336 phage:bead ratio proved a minimal bacteria capture. Therefore the 336 phage:bead ratio will be used in further experiments. The functionalized beads:bacteria stoichiometric relation will require further optimization.

2.2. Detection principle, microsystem and proof of principle experiments

Gold microelectrodes on chip were used for the impedance analysis. The electrodes were produced with silicon microfabrication technologies and included in a PDMS chamber. The electrodes were spaced 600 µm and the chamber volume was 36 nL (530 µm in width, 850 µm long and 80 µm deep).

Impedance spectra were collected at 50 frequencies logarithmically spaced between 1 KHz and 1 MHz, applying 100 mV excitation potential and 10 seconds pause at 0 mV between scans.

Bare electrodes were used for the analyses. In such experimental condition the conductivity σ is expressed as:

$$\sigma = F \sum_i c_i \mu_i \quad (1)$$