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Ordering of bacteriophages in the electric field: Application for bacteria detection

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ABSTRACT

Only in USA the nosocomial infections cause around 100,000 deaths per year. Majority of them could have been avoided if the pathogens would be identified shortly after infection. However, classical approaches are laborious; can take up to even 72 h. Here we demonstrate a step towards a sensor for fast detection of bacteria. The sensor is based on layer of oriented T4 bacteriophages. The sensitivity of biosensors increases four times for ordered over disordered layer of bacteriophages. This results in the limit of detection of *Escherichia coli* in the range of 10^2–10^3 cfu/mL. Such value is much lower when compared to similar sensors based on physisorbed layer of phages and is of the same order of magnitude as probes with chemically immobilized bacteriophages described in the literature. Proper orientation of bacteriophages prevents receptor binding proteins from being blocked. For the purpose we utilized the electrostatic interactions upon application of electric potential to the surface on which phages are deposited.

1. Introduction

Rapid and reliable detection of pathogens with high sensitivity is important because of environmental and economic reasons. The need for the new methods of detection of pathogens is most urgent in the area of public health. Only in USA, around 100,000 patients die because of the nosocomial infections every year [1,2]. Another source of bacterial infections are pathogens present in food, especially of short expiry date [3]. The issue gains importance due to recent spread of antibiotics resistant strains of bacteria [4]. Routine procedures for bacteria detection and identification are relatively easy and do not require any expensive equipment. However, classical approaches are time-consuming. The whole analysis can take up to 72 h. Such a long time makes classical protocols not applicable in a number of cases. For instance freshly squeezed juices have a shelf life of only 48 h. Therefore the consumers may not be sure whether the pathogenic bacteria are present in the product [5]. Speeding up the analysis will be beneficial for healthcare, food industry and other branches of industry, which are vulnerable to pathogen infections.

The main aim of the presented research is to improve and redesign the idea of biosensors for bacteria detection utilizing the natural viral receptors. Viruses are small pathogens, which can replicate only inside living cells. Viruses hosted in bacteria are called bacteriophages or phages. Most of bacteriophages have high affinity and specificity for species and even strains of host bacteria. The very precise recognition of the bacteria host cells by bacteriophages is assured by receptor binding proteins (RBPs). Phages are cheap and easy to prepare, stable in broad pH and temperature range, and in most cases insensitive for organic solvents [6] and proteases [7].

Utilization of bacteriophages for preparation of biosensors is a known idea. At first, physical adsorption of phages at the surface was studied. Such approach is fast and straightforward, but does not assure precise control over the coverage of the surface and number of phages per unit of area. Olsen et al. [8] prepared biosensor for detection of *Salmonella typhimurium* utilizing physisorbed layer of filamentous phages at the quartz crystal. The detection limit of the device was around 10^2 cells/mL. Magnetoelastic sensor was used to detect *S. typhimurium* in water solution [9] and in milk [3]. Balasubramanian et al. [2] used surface plasmon resonance (SPR) for detection of *Staphylococcus aureus* with detection limit of

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10^4 cfu/mL. Also *Bacillus anthracis* has been a target of phage-based biosensor [10].

In order to increase the amount of deposited phages the physical adsorption was replaced by chemical functionalization of the surface. Several approaches were used for bacteriophages immobilization at the solid substrates. Handa et al. [11] used 3-aminopropyltrimethoxysilane for initial modification of glass. In the next step P22 phages were immobilized *via* classical EDC/NHS reaction. Such sensing element was used to detect *S. typhimurium*. Arya et al. [12] proposed single-step procedure for immobilization of T4 phages at the gold surface. The method utilizes self-assembly monolayer of dithiobis(succinimidyl propionate) (DTSP). DTSP possess thiol group, which formed bond with the solid substrate, and succinimidyl group that bonded with amino groups present at the surface of the phages. To prevent unspecific binding, bovine serum albumin was used to fill the gaps between the virions. SPR was used as the detection method and the detection limit was 7×10^2 cfu/mL. Sugars (dextrose and sucrose) and amino acids (histidine, cysteine) were also used to improve the number of phages deposited at the surface *via* chemical bonding [13]. T4 was used as sensing agent and *Escherichia coli* as target bacterium. Such approach resulted in 7-fold increase of the surface coverage comparing to bare gold surface. When surface was covered with cysteine and activated with glutaraldehyde, the increase of phage coverage was 37-fold comparing to bare gold surface. Such improvement resulted in 4- and 12-fold increase of the sensitivity of the biosensors, respectively.

The efforts to increase the sensitivity of phage-based biosensors are directed towards increasing the number of phages per unit of area. In such case the increase of sensitivity arises from increased number of randomly oriented receptors. Statistically it results in higher number of receptors in proper orientation, which enables contact with target bacterium. This is brute force approach. The majority of receptors of deposited bacteriophages are sterically unavailable for the analyte. RBPs are located at the end of the tail-spike of the virions [14]. Entropy favours the alignment in which the long axis of the phages are parallel to the surface. In such case the receptors are not contributing to the overall sensitivity of studied biosensors. Only orientation in which the long axis of the virions is perpendicular or almost perpendicular to the surface and the tails are facing upwards assures that all of RBPs are involved in the process of the bacteria detection.

The problem of proper orientation of phages was recognized, yet not addressed. For instance, Tawil et al. [15] suggested that smaller phages are more likely to attach to a surface by its head. No evidence for that was given in the literature. Tolba et al. [16] showed the biosensors based on genetically modified T4 phages. Biotin carboxyl carrier protein gene was fused with small outer capsid protein gene. Therefore, the heads were tagged with ligand, which formed stable complex with streptavidin-coated magnetic beads. This forced the orientation of phage with receptors exposed towards the analyte. Anyhow, such method required preparation of recombinant phages, which is laborious process that can inactivate the phages. Orientation of phages was used also to control the growth of the bacteria in ready-to-eat and raw meat. Anany et al. [17] immobilized phages on cellulose membranes, which surface was modified to possess a positive surface charge. The authors reasoned that as a result the negatively charged heads of the virions were in proximity of the surface, whereas the positively charged tails were pointing upwards. Similar approach was utilized to increase the number of phages deposited on the nanostructured interface [18]. This was achieved by electrophoretic capture upon application of positive potential to indium thin oxide electrode. However, there is no evidence in the literature for orientation of phages and how it improves the characteristics of biosensors for bacteria detection.

We studied well described T4 phage – *E. coli* pair as exemplary system. The T4 phage is a member of the T-even phages, a group including also T2 and T6 [19]. T4 has an icosahedral capsid of size of 110×80 nm and tail of length of 98 nm. The tail is contractile and equipped with fibres at the end. The genetic information is stored in capsid in form of dsDNA of size of 168.9 kb [20–22]. The tail and fibres act twofold: they recognize the host and inject the genetic material inside host cell, as a syringe. The structure of T4 is typical for majority of bacteriophages.

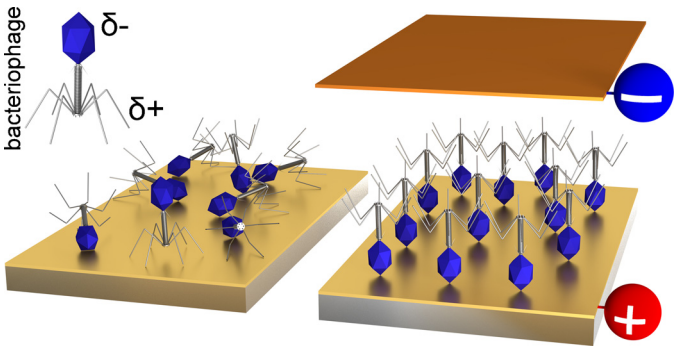

Fig. 1. Proper orientation of the T4 phages was obtained upon application of the electric potential. Tail up–head down arrangement of virions is beneficial for phage-based biosensors as majority of the receptors are available for bacteria detection.

The most extensive electro-optic work characterization of the T-even phages was that of Greve and Blok [23–26]. The authors analyzed electric birefringences to confirm existence of permanent dipole moment of T-even phages. For instance three forms of T4 phage were studied: (1) slow – tail fibres in extended conformation, (2) fast – tail fibres retracted upward along the tail and (3) fiberless phages. The values of permanent dipole moment were around 200,000 D, around 20,000 D and around 95,000 D, respectively. Baran and Bloomfield deduced that tail fibres have a substantial positive charge and the head–tail structure – a large negative charge [27]. The authors connected such charge distribution with process of detection of the bacteria cell by the phage. The tail fibres are attracted by the negatively charged surface of bacteria to facilitate the recognition of the host. It was even possible to state that the products of gene 12 carry a negative charge [28] and to estimate the positive charge of single tail-fibre [29]. The summary on early works on measurements of dipole moment of bacteriophages was given by Allen [30].

Here we demonstrate the method for orientation of phages upon application of electric potential to solid substrate on which phages are deposited (Fig. 1). As virions are in fact permanent dipoles they may align along the field lines or orient due to electrostatic interactions between charged surface and capsids and/or tails. Proper sign of the potential applied to the sensing plate assured head down–tail up orientation. The general design is similar for majority of the bacteriophages, thus the developed method is applicable for wide variety of phage species. As such it might be utilized for preparation of biosensors for detection of variety of bacteria strains.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma Aldrich (USA) and were used without further purification. Lysogeny broth (LB) medium and LB-agar medium were bought from Carl Roth (Germany) as an instant mixes ready to dissolve in deionized water. LB medium consisted of 10 g/L of tryptone, 5 g/L of yeast extract and 10 g/L of NaCl. LB-agar additionally contained 15 g/L of agar. Physiological saline was prepared by dissolving 9 g NaCl in 1 L of deionized water. TM buffer consisted of 10 mM TRIS (tris(hydroxymethyl)aminomethane) and 10 mM $MgSO_4$ dissolved