Sensors & Actuators: B. Chemical 273 (2018) 689–695

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Capturing fluorescing viruses with silver nanowires

Justyna Grzelak[a], Karolina Sulowska[a], Adam Leśniewski[b], Ewa Roźniecka[b], Marta Janczuk-Richter[b], Łukasz Richter[b], Marcin Łoś[c,d], Martin Jönsson-Niedziółka[b], Sebastian Maćkowski[a,e,*], Joanna Niedziółka-Jönsson[b,**]

[a] Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, Grudziadzka 5, 87-100 Torun, Poland
[b] Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland
[c] Department of Molecular Genetics of Bacteria, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland
[d] Phage Consultants, Partyzantow 10/18, 80-254 Gdansk, Poland
[e] Baltic Institute of Technology, Al. Zwyciestwa 96/98, Gdynia, Poland

ARTICLE INFO

Keywords:
Silver nanowires
Immunoassay complex
Fluorescence imaging
Dynamic sensing

ABSTRACT

We demonstrate fast and simple route to selective virus detection using antibody-functionalized silver nanowires. While the immunocomplex formation takes place in solution, the presence of viruses is determined by correlating transmission and fluorescence images obtained after depositing nanowire solution on a substrate. Importantly, by mixing nanowires in solution we expect to substantially increase the probability for immunocomplex formation, as evidenced by almost complete capturing of viruses after overnight incubation. This approach, based on dynamic search for viruses in solution, gives the possibility to reach medically relevant detection level of 10^3 pfu/ml, which translates to just 5 bacteriophages in a test volume of 5 µl.

1. Introduction

Fast, selective, and sensitive detection of viruses is presently one of the most urgent and attractive fields of research located at the interface between chemistry, physics, and biology. The primary reason for these efforts is rather obvious; there is a strong worldwide need to prevent spreading of diseases among humans, animals, and plants. In addition, virus monitoring is also vital for various biotechnological processes associated with food production or preparation of pharmaceuticals, not to mention virus screening in preventing the threat of bioterrorism.

Most biosensor designs are based on antibodies, which specifically bind to targeted antigens in the analyzed sample [1,2]. So far, among most commonly used techniques in diagnostics are enzyme-like immunosorbent assay (ELISA), polymerase chain reaction (PCR), and serologic tests. Alternatively, many electrochemical immunosensors have been developed; the principle of their function relies on monitoring changes in electrical parameters (current, potential or impedance) of a conducting, specifically-modified substrate, induced by immunocomplex formation [3,4]. Another important class of immunosensors employs photophysical effects [1], where either fluorescence detection [5,6] or surface plasmon resonance effect [7] is exploited, frequently in optical-fiber architectures [8].

Despite significant advancement, each of these methods is limited either by complex fabrication procedures that require complicated modifications of the substrates, or requirements for sophisticated equipment or highly skilled personnel, as well as relatively high costs of reagents and amplification of genetic material. Moreover, sometimes false-positive and/or false-negative results can be obtained by too weak or even non-specific interactions between the sample and the substrate. Last but not least, in most cases, electrochemical or optical biosensor designs are static as far as the active medium is concerned [9], which requires that, in order to facilitate immunocomplex formation and the resulting detection, the viruses must diffuse to the vicinity of appropriately functionalized surfaces.

Nanostructures, in particular made of noble metals [6], have been considered very attractive for novel designs of sensing platforms. Not only their optical properties depend on the metal used, the shape and size, as well as on the distances between them [10], but most importantly, any change in local dielectric environment of such a nanoparticle can cause measurable changes in the absorption or scattering, thus enabling detection of viruses or other biomolecules [6,9]. The potential for using metallic nanostructures as building blocks for

* Corresponding author at: Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, Grudziadzka 5, 87-100 Torun, Poland.
** Corresponding author.
E-mail addresses: justynag@fizyka.umk.pl (J. Grzelak), sulowska@fizyka.umk.pl (K. Sulowska), alesniewski@ichf.edu.pl (A. Leśniewski), erozniecka@ichf.edu.pl (E. Roźniecka), mjanczuk@ichf.edu.pl (M. Janczuk-Richter), lrichter@ichf.edu.pl (Ł. Richter), mlos@biotech.ug.gda.pl (M. Łoś), martinj@ichf.edu.pl (M. Jönsson-Niedziółka), mackowski@fizyka.umk.pl (S. Maćkowski), jniedziolka@ichf.edu.pl (J. Niedziółka-Jönsson).

https://doi.org/10.1016/j.snb.2018.06.119
Received 9 February 2018; Received in revised form 31 May 2018; Accepted 26 June 2018
0925-4005/ © 2018 Published by Elsevier B.V.

sensors (e.g. as transducers) is further enhanced by a variety of developed surface modification protocols, in particular for gold and silver. Consequently, nanostructured metallic systems based on recording changes in the optical properties of such a transducer upon the antibody-antigen interaction can be considered as the alternative to current approaches. Furthermore, when applied in solution, they can provide an additional functionality of dynamic and selective "searching" of specific viruses, by which we mean that both the nanowires with specific receptors and target molecules are diffusing freely in the solution. This approach is different from a more common geometry, where the receptor is immobilized on a transducer surface.

Silver nanostructures have been used previously to detect viruses [6,11,12]. For example, an array of silver nanorods served as a platform for surface-enhanced Raman scattering to distinguish and quantify attachment of respiratory viruses [11]. Also metallic nanowire barcodes with stripes of different metals were applied to determine MS2 bacteriophage in a multiplex sandwich immunoassay system [9]. Antibody replacement by molecular beacon chemistry enabled the detection of DNA of HIV, SARS and HCV [13]. In contrast to previous reports, where metallic nanostructures were employed in a chip-like static geometry, we used a suspension of cysteamine-stabilized silver nanowires modified with a polyclonal antibody for specific virus detection. The functionality of our approach is demonstrated for T7 bacteriophages that – due to their similarity in shapes and sizes – can be considered a model system for adenoviruses, and which are non-contagious for humans. Since viruses are small objects (most of them have diameters from 20 to 300 nm) they cannot be observed directly with an optical microscope. Therefore, in order to facilitate their detection, it is necessary to label them with a florescent dye which intercalate virus DNA. This manipulation step opens ways for fast, efficient, and highly sensitive detection, which takes advantage of the extremely rapid development in fluorescence detection techniques. Such techniques should allow for investigating intermolecular interactions of immunocomplex formation and, in principle, detecting single viruses, if the quantum yield of the dyes used is sufficient [14].

The scheme of virus detection developed in this work (Fig. 1) is not only considerably simpler than any previous approach, but also offers high selectivity and very low detection limits, both of which can be presumably further optimized. The detection limit, equal to 10^3 pfu/ml, was obtained without any additional amplification. This was achieved by applying nanostructures that were suspended directly in the virus-containing solution. This results in unlimited instances of interaction between antibody-modified silver nanowire and viruses and therefore leads to obtain medically relevant detection limit and is competitive with respect to other available biological sensors. Feasibility of this approach is verified by fast and efficient optical detection based on correlating fluorescence images with corresponding transmission images. We consider this concept to be pretty general and thus easily applicable towards other sensing schemes and analytes. In particular, control over the surface properties of the nanowires, as well as the possibility to exploit plasmonic properties thereof, open highly intriguing and promising pathways of adopting this approach in real-life applications.

2. Materials and methods

2.1. Chemicals and materials

Silver nitrate ($AgNO_3$), copper chloride ($CuCl_2$), calcium chloride ($CaCl_2$), calcium sulfate ($CaSO_4$), magnesium chloride ($MgCl_2$), polyvinylpyrrolidone (PVP, M_w 55,000), anhydrous ethylene glycol (EG), cysteamine, deoxyribonuclease I (DNAseI), SYBR Green I nucleic acid stain, phosphate buffered saline (PBS) tablets, and Tris base were purchased from Sigma Aldrich. PBS consists of 10 mM phosphate buffer, 2.7 mM potassium chloride and 137 mM sodium chloride, pH 7.4; TRIS buffer contains 10 mM Tris base (tris(hydroxymethyl)aminomethane), 10 mM $MgSO_4$, 5 μM $CaCl_2$, and pH was adjusted to 7.4 using HCl. SYTO™ 62 Red Fluorescent nucleic acid stain was from ThermoFisher Scientific. LB broth and LB-agar were bought from Carl Roth as 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 sodium chloride. LB-agar additionally contained 15 g/l of agar and top LB-agar contained 5 g/l of agar. Acetone and ethanol, both HPLC grade, were from POCH. Polyclonal anti-T7 antibody was purchased from MBL International. Bacteria *E. coli* MG1655, T7 bacteriophages, and T4 bacteriophages were obtained from University of Gdansk (Poland).

2.2. Bacteriophage preparation and purification

T7 and T4 bacteriophages were prepared and purified according to procedures provided by Sambrook et al. [15].

2.3. Bacteriophage staining

1 ml of $4.8 \cdot 10^{11}$ pfu/ml purified T7 bacteriophages were stained with 1 μl of SYBRGreen in PBS buffer containing 1 mM $MgCl_2$, 1μM $CaCl_2$, and 3μM DNAse I for 24 h in 4 °C in darkness. Stained bacteriophages were purified from free dye by dialysis in PBS over 48 h. Plaque count method was then used to determine viability of phages.

1 ml of $3.9 \cdot 10^{10}$ pfu/ml purified T4 bacteriophages were stained with 10 μM SYTO 62 Red Stain in TRIS buffer with 0.2 μg/ml DNAse I for 24 h in 4 °C in darkness. Stained phages were purified from free dye on column with diameter 7 mm and length 150 mm filled with polyacrylamide Bio-Gel P-30 Fine (Bio-Rad, USA). TM buffer was used as eluent. Plaque count method was then used to determine viability of phages.

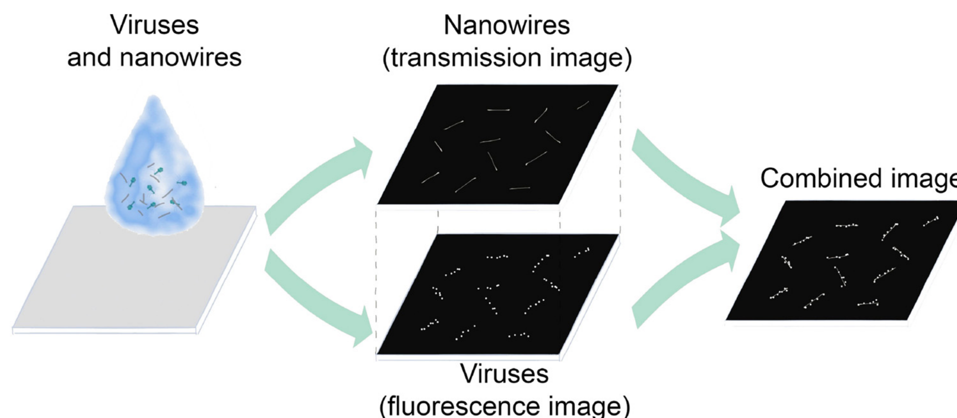

Fig. 1. Schematic representation of virus detection based on correlating fluorescence images with corresponding transmission images.

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