Bacteriophage-based bioconjugates as a flow cytometry probe for fast bacteria detection

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ABSTRACT

Robust detection of bacteria can significantly reduce risks of nosocomial infections, which are a serious problem even in developed countries (4.1 million cases each year in Europe). Here we demonstrate utilization of novel multifunctional bioconjugates as specific probes for bacteria detection. Bifunctional magnetic-fluorescent microparticles are coupled with bacteriophages. The T4 bacteriophage, due to its natural affinity to bacterial receptors, namely OmpC and LPS, enables specific and efficient detection of *Escherichia coli* bacteria. Prepared probes are cheap, accessible (even in non-biological labs) as well as versatile and easily tunable for different bacteria species. The magnetic properties of the bioconjugates facilitate the separation of captured target bacteria from other components of complex samples and other bacteria strains. Fluorescence enables simple analysis. We chose flow cytometry as detection method as it is fast and widely used for bio-tests. The capture efficiency of the prepared bioconjugates is close to 100% in the range of bacteria concentrations from tens to around 10^5 CFU/mL. The limit of detection is restricted by flow cytometry capabilities and in our case was around 10^4 CFU/mL.

INTRODUCTION

The development of rapid methods for bacteria detection is crucial in prevention and treatment of bacterial diseases. This is especially important in the case of nosocomial infections (often connected with antibiotic resistant bacteria) and food products with a short expiry date. The conventional detection method depends on culturing and isolation of the target bacteria followed by biochemical confirmation. The whole procedure is laborious and can take up to a few days. Hence, a lot of studies are focused on the development of sensitive, specific and rapid methods for bacteria detection. These methods can be divided into nucleic acid-based (DNA microarrays,[1] polymerase chain reaction (PCR) and derivatives, e.g. multiplex PCR or real-time PCR[2]), immunological-based (enzyme-linked immunosorbent assay[3] and lateral flow immunoassay[4]) and biosensor-based.[5,6] Most of the biosensors are cheap, easy to operate and do not require expensive reagents. However, it is crucial in terms of specificity and selectivity to choose an appropriate bioreceptor responsible for recognizing the target analyte. The most commonly used bioreceptors are antibodies, enzymes and nucleic

acids. Recently, bacteriophages have become an interesting alternative to antibodies in the field of rapid detection of bacteria.[7]

Bacteriophages (phages for short) are viruses whose host organisms are bacteria. Their natural

affinity to host cells can be used to design highly specific tools for bacteria detection. Additionally, there are types of phages that are robust and retain their activity even after exposure to high temperatures[8] and organic solvents.[9,10] Unlike antibodies, phages can be easily produced in large quantities. By simply infecting a bacteria solution one can obtain a large number of progeny phages. These advantages make phages popular biorecognition elements in biosensors[11,12] and other assays.[13,14]

Goodridge et al. proposed an immunoassay for detection of *Escherichia coli* O157:H7, in which fluorescently-labeled phages were used for the visualization of bacteria.[15] Fluorescent phages attached to the bacterial cells allowed the observation of bacteria by means of epifluorescent microscopy by giving the bacteria a "halolike" appearance. This method was combined with immunomagnetic separation of target bacteria from the sample matrix. The determination of bacterial concentration was not reliable. To overcome the limitation of microscopy based method, flow cytometry was used as an alternative and more accurate detection technique. The limit of detection of this assay was 10^4 cells/mL. In this example antibodies, and not phages, were used as bioreceptors. It was later proved that phages can replace antibodies in some of the applications, e.g. magnetic separation of bacteria. Magnetic separation is a fast and simple method to capture target bacteria from complex samples. This technique does not require filtration or centrifugation. Chen et al. developed magnetic bacteriophage-based nanoprobes for bacteria separation.[16] Biotinylated T7 phages were conjugated to mixed metal oxide (FeCo) magnetic nanoparticles capped with streptavidin. The resulting bioconjugates were able to capture between 90 and 50% of *E. coli* in the concentration range 10^2 – 10^7 CFU/mL. Wang et al. developed this approach by utilization of genetically engineered phages that could be efficiently biotinylated *in vivo*.[17] Such modified phages were immobilized on streptavidin coated magnetic beads. After magnetic separation of *E. coli* cells, PCR was carried out to confirm the ability of the phage-based beads to capture bacteria. Additionally, the utilization of phages allowed distinguishing between viable and non-viable bacterial cells. Only the viable cells could be infected by phages, which results in the release of genetic material that thus became available for PCR. Another interesting combination of the use of phages in separation and detection of bacteria was proposed by

Shabani et al.[18] Phages were immobilized on the surface of magnetic beads and carbon screen-printed electrodes by carbodiimide coupling.[19] Phage coated beads were attracted to the electrode surface after bacteria capture and the amount of bead-bacteria complexes were measured with electrochemical impedance spectroscopy. The utilization of magnetic

manipulation enabled the improvement of the limit of detection from 10^4 to 10^3 CFU/mL in comparison with the method utilizing only impedimetric detection.[20] Phage-based separation was also coupled with electrochemical magneto-genosensing for *Salmonella* detection.[21] Utilization of the combination of this two methods allowed obtaining a very low limit of detection (3 CFU/mL). However, it was very complicated and required laborious procedure, including magnetic separation of bacteria, double-tagging PCR, magnetic separation and enzymatic labeling of amplified DNA and finally electrochemical detection. To the best of our knowledge multifunctional phage-based probes, which enable magnetic separation and detection of bacteria, have so far not been described in the literature.

The aim of this work was to develop a simple and cheap tool for both separation and fast detection of bacteria. Multifunctional bioconjugates consisting of magnetic-fluorescent microparticles and specific bacteriophages were chosen for the task. T4 phages were covalently bound to the surface of the microparticles enabling specific capture of *E. coli* bacteria. The magnetic core of the particles was used for separation of target bacteria from the sample matrix and the fluorescent shell enabled the use of flow cytometry as a detection method. Flow cytometry is widely available and routinely used in medicine, molecular biology, pathology, marine and plant biology.[22,23] We aimed to prepare probes, which could be introduced without almost any cost in such bioanalytical laboratories, and which could broaden the spectrum of tests possible to execute. Our goal that the probes should be cheap, easily available (even in non-biological labs), and at the same time versatile and easily tunable for different bacteria species. Therefore we used non-modified, native phages (the strain of choice depend on the target bacteria) and commercially available particles. Bacteriophages are very low cost in comparison to antibodies. Moreover, it is relatively easy to obtain a suitable probe for a desired bacteria strain using phage libraries. The proposed design of the phage-based probe is advantageous as it combines easily accessible components, robust preparation procedure and a simple, yet efficient, bacteria detection protocol, which utilizes commonly available equipment.

RESULTS AND DISCUSSION

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The majority of phages belong to the *Myovirdae*, *Siphovirdae* or *Podovirdae* families.[24] These consist of an icosahedral head containing genetic information and a tail, which is responsible for the recognition and capture of target bacteria. Various phages attack different bacteria, and it so happens that there are phages corresponding to almost all bacterial strains. We decided to

utilize a well-studied pair – the T4 bacteriophage and the *Escherichia coli* bacteria – as a model system. The T4 phage has a structure typical for most phages. Therefore, the method presented here can be easily adjusted to prepare probes for detection of a variety of bacteria strains.

Preparation and characterization of phage-based magnetic-fluorescent bioconjugates

T4 phages were covalently bound to magnetic-fluorescent particles (~0.5 μm in diameter) to obtain multifunctional probes for bacteria separation and detection as schematically shown in Figure 1. Particles with maghemite core and silica matrix terminated with carboxylic groups were used in this study. The size of the magnetic-fluorescent particles was carefully chosen. It should be relatively small to detect the significant change of size upon connection between bacterium and bioconjugates. This facilitated the flow cytometry analysis, as it allowed for easy distinction between target bacteria, other bacteria and bioconjugates themselves. However, too small size of the magnetic core would not provide proper velocity for the magnetic separation.

The whole procedure of preparation of the phage-based bioconjugates was carried out at room temperature and it took only 2.5 hours. Carboxylic groups on the surface of the microparticles were activated with EDC (1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride) to further react with amine groups present on the phage surface. We analyzed different conditions of the preparation process. We considered such aspects as mixing, time of reaction, ratio between a number of beads and phages and also type of used beads. Detailed information is provided in the SI. It was crucial to use beads with carboxylic and not amine groups. In case of EDC activation of carboxylic groups on the surface of the virions they immediately reacted with available amine groups from neighboring phages and not with the beads. This resulted in clustering and sedimentations of the phages.

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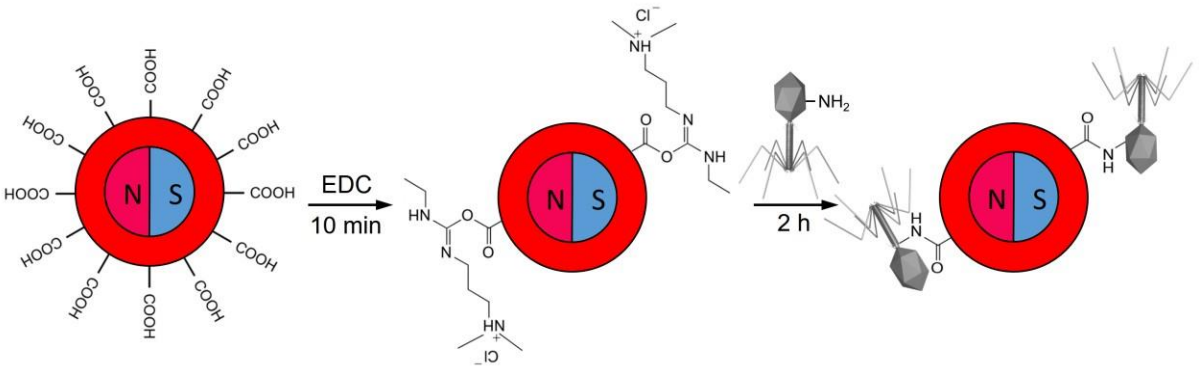

Figure 1. Schematic representation of the preparation of phage-based magnetic-fluorescent bioconjugates. In first step carboxylic groups present on the beads surface were activated with EDC and then phages were added to covalently bind to the beads via amide bonds.

In order to determine the efficiency of the proposed method and the quality of the resulting bioconjugates we prepared the sample, which consisted of magnetic-fluorescent beads modified with fluorescently labeled phages. Images obtained with confocal microscopy showed that the vast majority of particles became modified with at least one virion. Phages are represented by single green dots on the red particles indicating successful conjugation (Figure 2B). Images showing more phage-based bioconjugates in low magnification and fluorescent phages and microparticles alone are shown in Figure S2 in Supporting Information.

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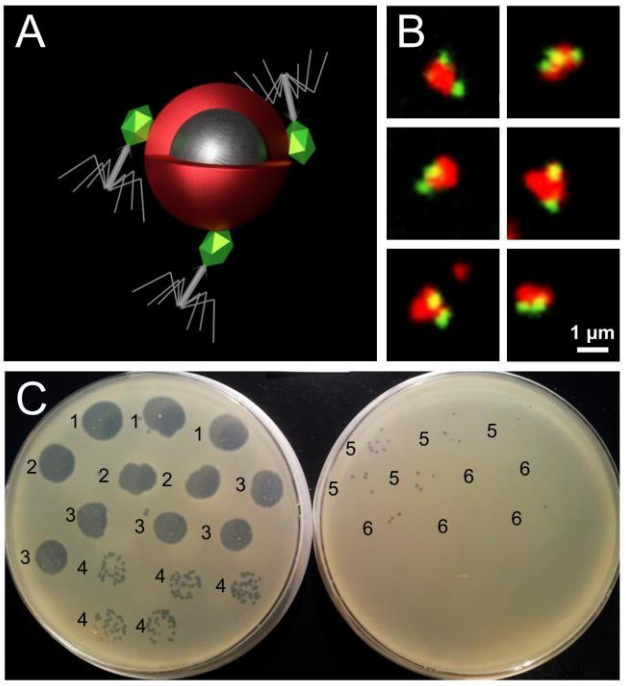

Figure 2. (A) Graphical presentation of prepared bioconjugates. T4 bacteriophages were immobilized

on the surface of magnetic-fluorescent beads. To check the effectiveness of binding procedure, phages stained with SYBR Green were used. **(B)** Confocal microscope images of bioconjugates composed of beads (red fluorescence) and attached phages (green fluorescence). Procedure allowed to obtain designed particles. **(C)** Images of double layer agar plates after titration of the prepared bioconjugates by the plaque count method. The numbers correspond to consecutive tenfold dilutions of the sample. Every plaque corresponds to a single bioconjugate. Thus we proved that bacteriophages coupled with beads are active and infective. The estimated concentration of bioconjugates was 10^8 PFU/mL.

Although the successful immobilization was confirmed by means of confocal microscopy, it was necessary to check whether the phages retained activity and were able to bind target bacteria. Chemical immobilization of phages carries the risk that the virions are bound in the wrong orientation and the specific receptors are blocked. To check the activity of phage-based bioconjugates the plaque count method was used similarly to in a regular phage viability test. However, in this case each plaque corresponded to one active phage-based particle no matter how many bacteriophages were attached. As shown in Figure 2C, the bioconjugates were capable of binding and infecting bacteria and produced plaques typical for T4 phages. The number of active bioconjugates was found to be 10^8 PFU/mL. We compared obtained value with measurement of the number of bioconjugates with flow cytometry. The obtained values were similar, which indicated that every created bioconjugate had at least one active bacteriophage. Details are provided in SI.

Determination of capture efficiency of phage-based bioconjugates

In order to determine the efficiency of magnetic separation, bioconjugates with non-stained phages were incubated with different concentrations of bacteria (from 3.3×10^1 to 3.3×10^5 CFU/mL). An incubation time of 15 minutes was chosen as an optimal time for bacteria capture by the immobilized bacteriophages.[12] Longer time can result in lysis of the bacterial cells. After incubation, the bacteria-bioconjugate complexes were separated with a magnet and the number of bacteria remaining in the supernatant was determined using the plate count method as shown schematically in Figure 3. The capture efficiency (CE) was calculated using the equation:

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$$C??(\%) = \left(1 - \frac{N_1}{N_0}\right) \times 100\%$$

where N_0 is the number of bacterial cells present in initial sample (CFU/mL) and N_1 is the number of cells that remain unbound to bioconjugates after the capturing procedure

(CFU/mL). Phage-based bioconjugates were able to bind more than 90% of *E. coli* from the samples in the concentration range 3.3×10^1 to 3.3×10^4 CFU/mL reaching almost 100% for the most dilute samples (Figure 4A). For higher bacteria concentration (3.3×10^5 CFU/mL) a decrease in capture efficiency was observed, which is typical for phage-based magnetic separation method due to the lower number of bioconjugates per bacteria.[16,17] Compared to previous reports we obtained very high capture efficiencies, especially for the lowest bacteria concentrations.[16,18]

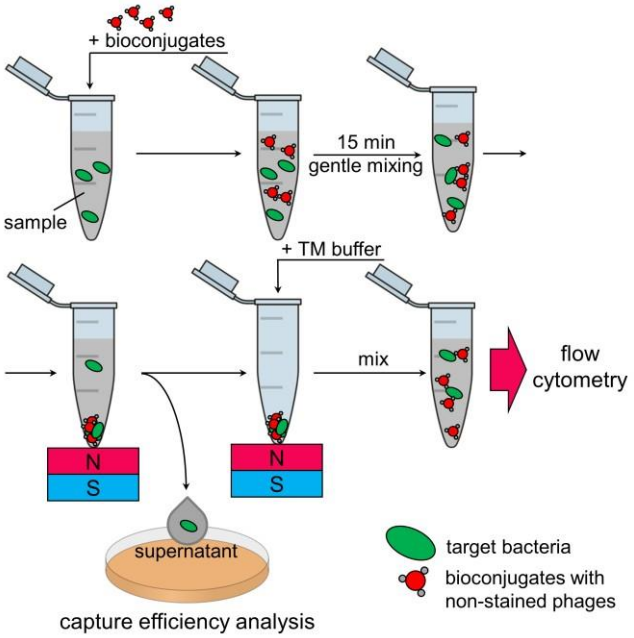

Figure 3. The schematic diagram of the sample preparation for the capture efficiency procedure and flow cytometry measurements. Bioconjugates were mixed with bacteria sample and incubated for 15 minutes at room temperature. Then, the bacteria-bioconjugate complexes were separated with magnet and re-suspended in fresh buffer. The obtained solution was analyzed with flow cytometry to evaluate the number of target bacteria in the sample. For the capture efficiency procedure, the number of bacteria in the supernatant was determined using the plate count method.

Additionally, we performed analogous experiment with bioconjugates containing SYBR Green labeled phages and *E. coli* bacteria modified with blue fluorescent dye. The separated bacterium-bioconjugate complexes were re-suspended in TM buffer and analyzed with confocal microscopy. We observed the complexes of blue bacteria (stained with SYTO Blue

dye) and green-red bioconjugates (phages stained with SYBR Green dye) (Figure 4B) by using three lasers of different wavelengths and proper sets of filters. This experiment additionally confirmed the adequacy of using phage-based bioconjugates for bacteria

separation and detection.

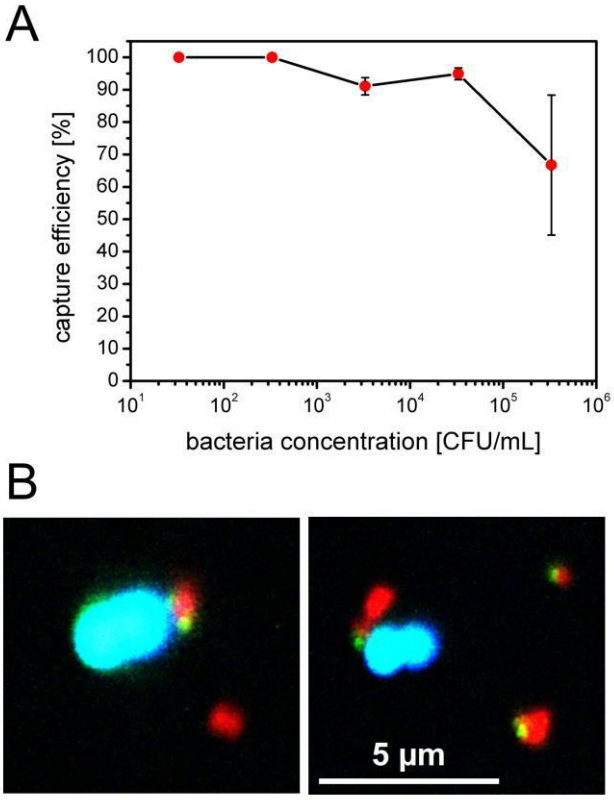

Figure 4. Utilization of phage-based bioconjugates for bacteria separation. **(A)** The capture efficiency of phage-based bioconjugates. The graph presents the percent of bacteria caught by the prepared bioconjugates from samples with different bacteria concentrations. We obtained excellent efficiency (around 90%) even for high concentrations of bacteria (10^4-10^5 CFU/mL). **(B)** A confocal microscopy images of bacterium-bioconjugate complexes. The bioconjugates composed of magnetic beads (red fluorescence) and T4 phages (green fluorescence) are attached to *E. coli* bacteria (blue fluorescence) via the bacteriophage.

Detection of E.coli captured by phage-based bioconjugates

Flow cytometry was chosen as the method for detection of bacteria-bioconjugate complexes as it is routinely used for medical and biological analyses. Bacterial samples were first prepared the same way as for the capture efficiency analysis (magnetic separation). Then, the separated complexes were diluted in fresh buffer solution and immediately after dilution they

were analyzed in triplicate by means of flow cytometry. The procedure is schematically shown in Figure 3.

As a control sample, the bioconjugates were mixed with physiological saline without bacteria

and treated the same way. The obtained results were organized as two-parameter dot plots showing side scatter and red fluorescence. The bioconjugates were small and fluorescent. Therefore they formed one population of fluorescent, weakly scattering objects (Figure 5A). This plot allowed to set the gate in the region with greater fluorescence and side scattering where bacteria-bioconjugate complexes were expected and where the signal from the bioconjugates alone was minimal.

The bacterial samples with concentrations ranging from 10^4 to 10^6 CFU/mL were magnetically separated using phage-based bioconjugates and analyzed with flow cytometer. As shown in Figure 5B a number of events were recorded in the selected gate (red border) indicating the presence of big fluorescent complexes of bacteria and bioconjugates. However, there were still a lot of free bioconjugates available. The results obtained for different bacteria concentrations are shown in Figure 5D. The signal of 10^4 CFU/mL was clearly distinguishable from the background. Moreover, the signal increased for higher concentrations.

A specificity test was also performed with *E. coli* as a target bacteria and *S. epidermidis* as a control bacteria, both in concentration of 10^5 CFU/mL. The two-parameter dot plot for non-specific bacteria is shown in Figure 5C. Only a few events were recorded in the selected gate. The summarized results are shown in Figure 5E. As expected, the signal obtained for *S. epidermidis* was similar to the negative control and significantly lower than the signal recorded for *E. coli*. The specificity of the whole procedure was provided by the magnetic separation step, because T4 phages were specific for *E. coli*.

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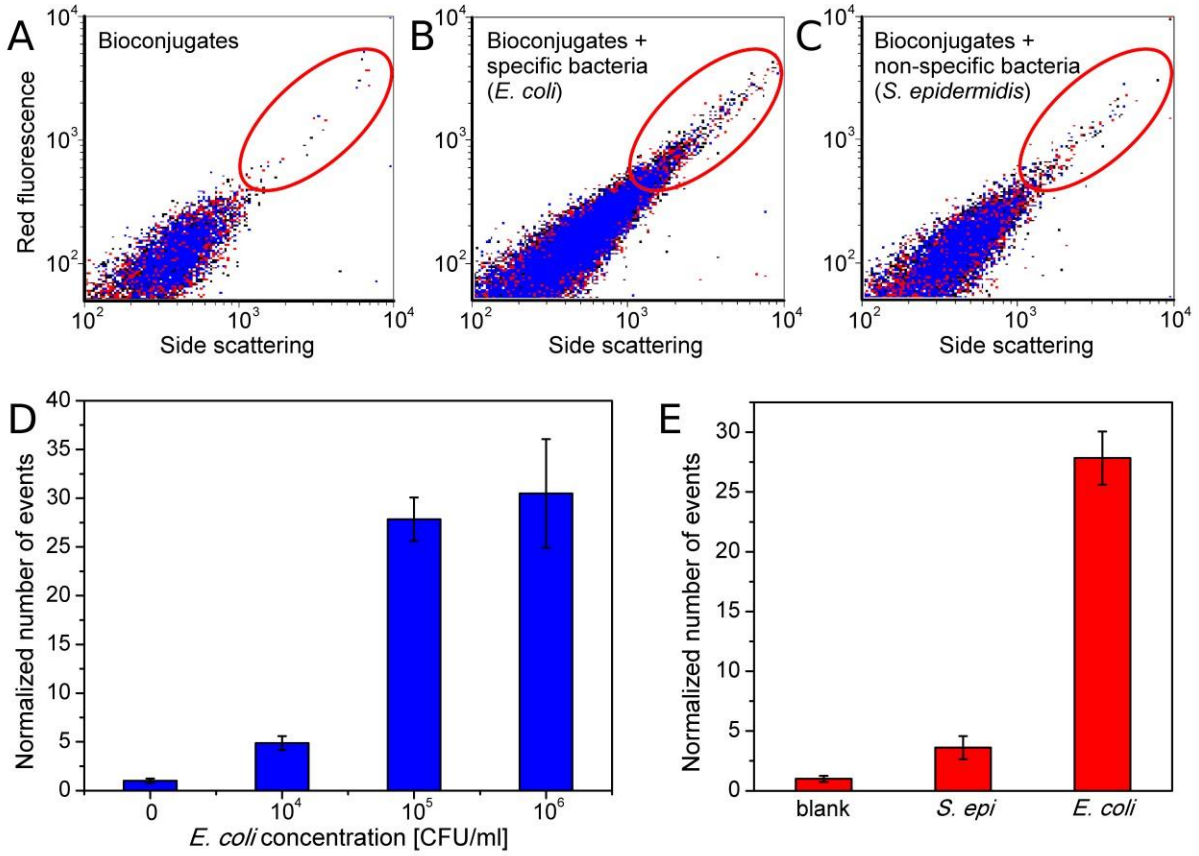

Figure 5. The results of flow cytometry measurements for detection of bacteria. The two-parameter dot plots of side scattering and red fluorescence for phage-based bioconjugates. The results were obtained for samples with **(A)** bioconjugates without any bacteria in solution, **(B)** bioconjugates with target bacteria (*E. coli*) and **(C)** bioconjugates with non-specific bacteria (*S. epidermidis*). The gate (red border) was judiciously chosen to detect signal coming from bacteria-bioconjugate complexes only. Three repetitions were plotted on each graph. **(D)** The results obtained from the chosen gate for different concentrations of target bacteria (*E. coli*). **(E)** Selectivity analysis of the designed method. The graph presents results obtained for specific (*E. coli*) and non-specific (*S. epidermidis*) bacteria in concentration of 10^5 CFU/mL. The presented values prove the selectivity of the designed method.

CONCLUSIONS

There is a number of various preparation techniques of bacteriophage-based probes for bacteria detection described in the literature.[17,21,25] However, there are still issues that should be improved. 1) Avoiding expensive and complicated genetic modifications, 2) a simple

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preparation process and 3) single-step detection are aspects that needs to be properly addressed. Here we demonstrated a method that meets all of these requirements. We designed and optimized the method for preparation of multifunctional bioconjugates for bacteria detection. Bacteriophages were used as highly specific and selective bioreceptors. The phages

are bound with fluorescent-magnetic sub-microparticles by EDC coupling. All the components are easily accessible and relatively cheap. Also, the protocol for bacteria detection using our bioconjugates as flow cytometry probes is straightforward, yet efficient, specific and selective. All these beneficial traits of the technique renders the presented approach suitable for scaling up and utilization as a new method for bacteria detection in all the facilities using flow cytometry as standard method for bio-analyses. We aimed to create a method that could be easily applied, even in non-biological laboratories and without additional equipment, i.e. at low initial costs.

The procedure proposed by us is easily applicable to all the bacteriophages or even viruses, depending on specific target, against which the probe should be sensitive. The method does not use any genetic modification or laborious preparation of phages/viruses. Wild type strains or even libraries might be used as bioreceptors. For each bacterial strain there is a corresponding phage. Thus, the presented bioconjugates are universal probes for bacteria detection. Their sensitivity depends on the chosen phage only.

We demonstrate the bioconjugates using the well-studied pair, T4 phage – *E. coli* bacteria, as a model system. The measured capture efficiency is close to 100% in a broad range of *E. coli* concentrations (from 10 to 10^5 CFU/mL). This proves that the sensitivity of the method and limit of detection is restricted by the applied detection method and the quality of the used equipment. We decided to utilize flow cytometry, since it is a common equipment in laboratories. The limit of detection in our case is around 10^4 CFU/mL, which makes our method suitable for fast (15 minutes) screening tests that are intended to quickly and precisely determine bacterial species in samples that are already heavily infected. The method combined with a pre-incubation step could be also applied to detect initial stages of bacterial contamination. The speed of analysis and limit of detection might be balanced depending on the specific needs.

EXPERIMENTAL PROCEDURES

Chemicals and materials

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Carboxyl-terminated superparamagnetic-fluorescent beads were purchased from Chemicell (Berlin, Germany). These particles were 0.5 μm in size (hydrodynamic radius) and they were functionalized with red fluorescent dye (excitation max 633 nm, emission max 672 nm). All chemicals were purchased from Sigma Aldrich (USA). LB medium and LB-agar medium

were purchased from Carl Roth (Germany) as instant mixes. LB medium consisted of 10 g/L of NaCl, 10 g/L of tryptone, and 5 g/L of yeast extract. LB-agar additionally contained 15 g/L of agar. TM buffer consisted of 10 mM Tris base (tris(hydroxymethyl)aminomethane), 10 mM $MgSO_4$, 5 μM $CaCl_2$ and pH was adjusted to 7.4 using HCl.

TM buffer, LB medium and other solutions were autoclaved prior to use.

Phage preparation and bacteria culturing

T4 phage lysate was prepared by infection of early logarithmic culture of *Escherichia coli* MG1655. After lysis the phages were precipitated by polyethylene glycol precipitation. After centrifugation of precipitate and re-suspension in TM buffer with 1M NaCl, the phage was purified by ultracentrifugation in step CsCl gradient in Beckman Optima XL70 ultracentrifuge (Ti50 rotor). The phages formed a band, which was collected by aspiration using syringe. Next, the phages were dialyzed against TM buffer. After completion of the dialysis 0.2 μg/mL of DNase I was added in order to digest DNA released from capsids damaged during dialysis.

The bacteria were cultured according to the standard protocol. *E. coli* MG1655 was used as target (specific) cells and *Staphylococcus epidermidis* WT (obtained from the Faculty of Biology, Warsaw University, Poland) was used as control (non-specific) cells. First, a single colony from agar plate was inoculated into LB medium for overnight culturing (37 °C, 200 rpm). The small amount of overnight culture was inoculated into a new portion of LB medium and cultured to obtain suspensions with desirable OD_{600}.

Phage viability

We used the plaque count method for determination of bacteriophage viability. Plaque forming units (PFU) can be directly correlated with a number of active phages within the sample. In this approach 20 mL of bottom LB-agar medium was poured onto the plastic petri dish and left to solidify. Then 4 mL of top LB-agar was mixed with 200 μL of overnight culture of *E. coli* MG1655 and poured onto the plate. Top LB-agar consisted of LB medium and 0.5% agar. Tenfold dilutions of phage solution were prepared and 5 μL droplets of each

dilution were spotted onto the top agar layer. Plates were incubated at 37 °C for 24 h and plaques were counted.

Preparation of fluorescently labeled bacteriophages and bacteria

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The bacteriophages and bacteria cells were labeled with fluorescent dyes in order to observe and control the processes of bioconjugation and bacteria detection.

The purified T4 phages were stained with SYBR Green dye (ThermoFisher Scientific, USA, excitation max 497 nm, emission max 520 nm) for 24 h at 4 °C. The ratio of volume of dye solution to volume of phage solution (5×10^{10} PFU/mL) was 1:1000. Then, the stained phages were purified from free dye by dialysis against TM buffer for 48 h. The plaque count method was used to determine phage viability.

The bacteria *E. coli* MG1655 were stained with SYTO Blue dye (ThermoFisher Scientific, USA, excitation max 420 nm, emission max 441 nm). Overnight culture was diluted to OD=0.40 and bacteria were centrifuged (5000 rpm, 5 min) and re-suspended in physiological saline (0.9% NaCl). Then 500 µL of bacteria suspension was incubated with 5 µL of dye solution for 80 min at 37 °C with rotation (250 rpm). To remove free dye, stained bacteria were centrifuged (5000 rpm, 5 min) and re-suspended in fresh physiological saline.

Preparation of phage-based bioconjugates

T4 phages were covalently immobilized onto carboxyl-terminated magnetic-fluorescent beads by EDC (1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride) coupling[19] as schematically shown in Figure 1. The magnetic beads (66.7 µL, 7.5×10^9 particles/mL) were washed 2 times with MES buffer (0.1 M, pH 4.5) using hand-made separator with neodymium magnet. The beads were re-suspended in 5 mM EDC in MES buffer and incubated for 10 min at room temperature on the orbital shaker (300 rpm) to activate carboxyl groups. The magnetic beads were washed 2 times with MES buffer and once with TM buffer. T4 phage solution (200 µL, 5×10^{10} PFU/mL) was added to the beads and incubated in the dark for 2 h at room temperature with agitation in order to create amide bonds between activated carboxyl groups of magnetic particles and amine groups presented on the phage surface. The phage-based bioconjugates were washed 3 times with TM buffer and re-suspended in 100 µL of blocking/storage buffer (TM supplemented with 0.1% bovine serum albumin). The bioconjugates were stored in the dark at 4 °C for further use.

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The biocojugates were prepared in two variants: with stained and non-stained T4 phages. SYBR Green-labeled phages were used for conjugation to check the effectiveness of proposed method with confocal microscope. The non-stained phages without fluorescent labels were used to prepare proper bioconjugates for bacteria detection with flow cytometry.

Additionally, a number of active bioconjugates was studied by the plaque count method according to phage viability assay described above.

Capture efficiency

Overnight *E. coli* MG1655 culture was centrifuged (5000 rpm, 5 min) and bacteria were re-suspended in physiological saline (0.9% NaCl). Tenfold dilutions were prepared to obtain bacteria solutions from 3.3×10^1 to 3.3×10^5 CFU/mL, as confirmed with the plate counting. The bacteria solution was mixed with phage-based bioconjugates in the ratio 5:1 and incubated at room temperature for 15 minutes. Then the bacteria-bioconjugate complexes were magnetically separated and the supernatant was collected. A number of bacteria remaining in the supernatant was determined by plate count method.

Bacteria detection – flow cytometry analysis

Different concentrations of bacteria were suspended in physiological saline. For sensitivity measurement *E. coli* bacteria in concentrations ranging from 10^4 to 10^6 CFU/mL was prepared. For selectivity analysis concentration of *E. coli* and *S. epidermidis* solutions was 10^5 CFU/mL.

The bioconjugates with non-stained phages were mixed with bacteria solution in volume ratio 2:1 for 15 min at room temperature. Then, bioconjutages with attached bacteria were separated by pulling down in hand-made separator with neodymium magnet and re-suspened in 50 times bigger volume of fresh TM buffer (50 times dilution). Afterwards the solution was three times analyzed with flow cytometer. The bioconjugates were mixed with physiological saline without bacteria and then treated the same way as described above. This solution was a control for all experiments.

Instrumentation

Nikon Ti Eclipse with confocal system A1R was used for evaluation of the quality of prepared phage-based bioconjugates and for controlling of the bacteria catching process. The system included ion laser IMA101040AL5 (Melles Grior, USA) emitting light of wavelength

of 488 nm, laser 405 nm, laser 635 nm, objective CFI Plan Fluor 40× and NIS-Elements AR 4.13 software.

Samples were analyzed by using Becton Dickinson FACS CantoII cytometer (Becton

Dickinson, San Jose, USA) equipped with argon, red and violet laser and Diva software.

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SUPPORTING INFORMATION

Analysis of different conditions of preparing bioconjugates. Confocal microscopy images of phage-based bioconjugates, phages stained with SYBR Green and magnetic-fluorescent beads. Analysis of viability of phages attached to particles.

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