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Sensors and Actuators B 125 (2007) 615–621
SENSORS AND ACTUATORS B CHEMICAL
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Immobilization of biotinylated bacteriophages on biosensor surfaces

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Received 24 October 2006; received in revised form 26 February 2007; accepted 7 March 2007
Available online 13 March 2007

Abstract

Bacteriophages are viruses that recognize specific receptors on the bacterium surface to which they bind and inject genetic material. The specificity of this recognition opens remarkable possibilities for biosensor development. The chemical attachment of T4 bacteriophages onto gold surfaces is being reported. This attachment leverages the genetic biotinylation of the capsid heads of bacteriophages, and the natural affinity of the biotin/streptavidin system. The development of a streptavidin-immobilization chemistry that minimizes non-specific binding of the target bacterium is first described. The attachment of genetically biotinylated T4 bacteriophages onto these streptavidin-coated surfaces is then reported. Such chemical immobilization results in a 15-fold improvement of attachment when compared to the simple physisorption of the wild-type phage onto bare gold. The attachment procedure was then used to investigate the effect of a biotinylated phage-terminated surface on the growth of the host bacteria. This assessment was conducted in an electric cell-substrate impedance sensing device. The streptavidin-mediated attachment of biotinylated phages significantly delays the growth of the host bacteria by up to 17.2 h. In comparison, non-specific binding of wild-type phages onto the streptavidin surface is found to cause a lesser growth delay of 13 h.

Keywords: Bacteriophage; Biosensor; Bacteria; Impedance sensing; Biotinylation

1. Introduction

Increasing public health concerns related to bacterial diseases, as well as the need to monitor food and water supplies have prompted interest in the development of low-cost and low-footprint pathogen detection systems. The detection and identification of pathogens in food products, drinking water supplies and hospitals continue to mostly rely on conventional microbiological culture techniques. These tests are based on assessing a bacterium's ability to grow in plates or tubes containing a variety of media (solid or liquid) under various conditions. While detection of a small number of bacteria is possible by incubation, growth of bacteria to numbers sufficient for identification can take several days. In addition, further biochemical and serological tests are required to confirm the identity of the agent.

Polymerase chain reaction (PCR) may also be used to amplify a small amount of genetic material from bacteria [1,2]. Alternatively, bacterial identification using enzyme-linked immunosorbent assay (ELISA) is conducted by testing antibody–antigen interaction with the targeted bacterium and can be performed within a working day [3]. Combined PCR-ELISA increases sensitivity of the conventional PCR method [4,5]. However, these techniques still require an enrichment step during which bacteria are grown to the levels required for detection. In addition, problems associated with enzyme inhibition and DNA extraction have made direct detection of low numbers of bacteria in foods by PCR difficult to achieve.

Therefore, there has been sustained interest towards the development of biosensing systems that would circumvent the limitations of conventional techniques. A typical biosensor platform couples a physical transducer (electrochemical, mechanical, thermal, or optical) with a specific recognition probe such as an enzyme, nucleic acid, cell, antibody or artificial receptor. The interaction between the probe and the

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doi:10.1016/j.snb.2007.03.007

target is converted to a quantifiable signal by the transducer. Micromechanical resonators [6,7], quartz crystal microbalances (QCM) [8–10], surface plasmon resonance (SPR) [11–15], and amperometry [16] have been demonstrated as potent platforms for such applications. For example, *Salmonella* has been detected by immobilizing polyclonal antibodies on a quartz crystal acoustic wave device using Langmuir–Blodgett films [17]. Another example includes the use of SPR to effectively detect *Escherichia coli* bacteria [18]. The development of these technologies as viable biosensing systems requires the use of a recognition probe offering high levels of specificity, selectivity, and stability. Antibodies are frequently used as recognition receptor systems for the specific detection of antigens. While antibodies may offer some degree of selectivity and speficity (especially monoclonal antibodies), they suffer from environmental instabilities and require arduous and cost-intensive methods for their production, isolation and purification. In addition, polyclonal antobodies are limited by their heterogenicity towards other species, strains or molecules. Thus, there is a need for alternative probe selection [19,20].

Bacteriophages (or phages) offer such potential as alternative probes for specific biosensing. They are viruses that recognize specific receptors on the bacterial surface to which they bind and inject their genetic material. Such injection allows replication of the phage and release of a new generation while killing the bacteria. These viruses recognize target bacteria through functional receptors located on their tail extremity (Fig. 1(a)) [21]. This recognition is routinely employed in phage typing where a group of phages are used to differentiate between different bacteria. This unique level of specificity also presents remarkable possibilities for biosensor development. For instance, the use of a lytic phage for the SPR detection of *Staphylococcus aureus* was recently reported [22]. However, the attachment of phages was accomplished by simple non-oriented physisorption of the viruses onto the sensor surface.

Alternatively, chemical attachment of phages onto sensor surfaces could significantly improve the stability and performance of the overall platform, and enable their employment in applications where patterning of the probing element is required. The chemical biotinylation of phages has already been shown to significantly increase the efficiency of phage-based biosorbents when compared to simple physical adsorption [23]. In the case of microsensors, bacteriophages bound from the head capsid protein onto the sensor surface would also allow the tail fibers to face the medium, enabling a more efficient capture of the bacteria.

The biotin–avidin complex has been used for binding purposes in previously reported bacterial sensing applications [24,25]. Edgar et al. [26] has recently reported the genetic modification of the T7 bacteriophage to display a small peptide on the major capsid protein that is subsequently biotinylated by the biotin-ligase protein (BLP) present in the host bacterium. The biotin was attached postranslationally by the BLP to a specific lysine residue in the tagged peptide. Such genetic biotinylation opens the possibility of leveraging the affinity of the streptavidin/biotin system for the attachment of the phages onto

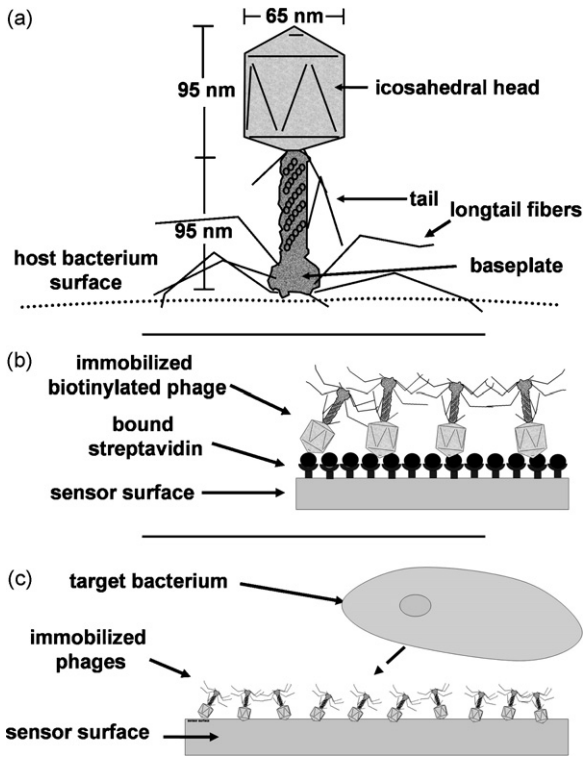

Fig. 1. (a) Bacteriophage T4 structure. The tails fibers contain proteins recognize and specifically bind to bacteria. The DNA contained in the icosahedral head is then injected into the bacteria through the core and base plate. (b) Proposed immobilization of biotinylated phages by chemical attachment of streptavidin onto the sensor surface. (c) Employment of the proposed chemical attachment of phages in biosensing platforms for bacterial detection.

particles and surfaces. In addition, as opposed to phages biotinylated by chemical procedures [23], the biotin is in these cases exclusively present on the phage capsid, and not on its tail, potentially allowing the oriented attachment of the phage onto surfaces.

We here describe such development of a surface attachment scheme that involves T4 bacteriophages that were similarly engineered to express a biotin binding domain on a capsid protein using a phage display technique. The constructed recombinant bacteriophage was reported to retain infectivity, burst size and latent period comparable to its wild-type counterpart. While the details of this biotinylation procedure is to be reported elsewhere [27], we here specifically report the streptavidin-mediated attachment of these recombinant biotinylated phages onto gold electrodes (Fig. 1(b)), as well as the use of this attachment in an impedance biosensing device (Fig. 1(c)). We observe that such streptavidin-mediated attachment of biotinylated phages improves by a factor of 15 the attachment of the viruses onto electrode surfaces, and significantly delays the growth of the host bacterium in comparison with electrodes functionalized using wild-type phages.

Such antibody-free chemical attachment of phages onto sensor surfaces could therefore be used in numerous other sensing transduction techniques, and enable the design of highly sensitive and highly specific platforms for the detection and identification of the host pathogenic organism.

2. Experimental

2.1. Materials

1-Hexadecanethiol, 11-mercapto-1-undecanol, 11-mercapto-undecanoic acid, cysteamine hydrochloride and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (St-Louis, MO, USA). Sulfosuccinimidyl-2-(biotinamido)-ethyl-1,3-dithiopropionate (sulfo-NHS-SS-biotin), tetramethyl rhodamine isothiocyanate (TRITC)-conjugated streptavidin and BupH phosphate buffered saline packs were purchased from Pierce (Rockford, IL, USA). *E. coli* was labeled using the SYTO BC bacteria stain from a bacteria counting kit purchased from Invitrogen (Carlsbad, CA, USA). Electric cell-substrate impedance sensing (ECIS) 8W1E chips were purchased from Applied Biophysics (Troy, NY, USA). *E. coli* ATCC 11303 and wild-type T4 bacteriophages were obtained from Biophage Pharma Inc. (Montreal, Canada). T4 bacteriophages with genetically biotinylated capsid heads were provided by Dr. M. Griffiths (University of Guelph, Canada).

Luria Bertani (LB) media was purchased from Quelabs (Montreal, Canada) and prepared by dissolving 25 g of LB powder into 1 l of distilled water. LB-agar medium was prepared by adding 6 g of granulated agar in 400 ml of LB media. SM buffer was prepared using Sigma reagents by diluting 5.8 g NaCl, 2.0 g $MgSO_4{\cdot}7H_2O$, 50 ml 1 M Tris–HCl at pH 7.5, 1 ml 10% (w/v) gelatin in 1 l of distilled water. The LB medium and SM buffer were autoclaved. Phosphate buffered saline solution (PBS) was prepared by mixing one BupH phosphate buffered saline pack to 500 ml of MilliQ water yielding a solution of 0.1 M phosphate, 0.15 M NaCl, pH 6.9–7.2.

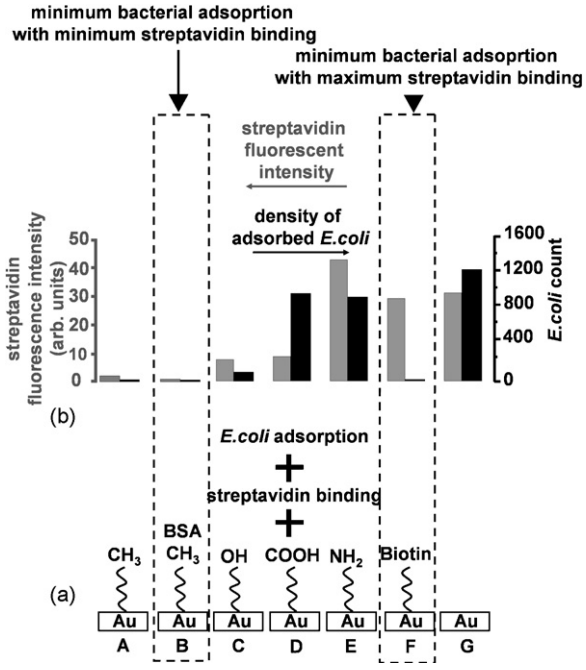

Fig. 2. Development of a streptavidin chemical functionalization process. (a) Seven gold substrates (A–G) were terminated with various functional groups described in the material section and at the bottom of the figure. The surfaces are then treated with TRITC-conjugated streptavidin, and finally put into contact with SYTO BC *Escherichia coli*. (b) Streptavidin fluorescent intensity (in gray) and bacterial count (in black) for each of the seven surfaces following streptavidin attachment and bacterial adsorption. The bacterial count is performed over a 665 μm × 890 μm area. Sample F, a biotin-terminated alkyl chain, shows high streptavidin binding with minimal non-specific bacterial adsorption, proving to be the optimal for the immobilization of biotinylated bacteriophages.

2.2. Development of streptavidin-immobilization chemistry

Our phage immobilization procedure leverages the presence of biotin on the phage capsid, and the natural affinity of the biotin/streptavidin system (Fig. 1(b)). A series of binding procedures have therefore been investigated in order to optimize the attachment of streptavidin onto gold while minimizing the non-specific binding of the *E. coli* host. Substrates were fabricated with a 3 in. silicon (1 1 1) wafer by first sputtering 5 nm of a chrome adhesion layer followed by 50 nm of gold. The coated wafer was then cleaved into 5 mm × 20 mm rectangles using a diamond tip pen.

Six of these gold surfaces (A–F) were then immersed in 1 mM self-assembled monolayer solutions for 24 h. More specifically, samples A and B were immersed in 1-hexadecanethiol diluted in ethanol. Additionally, sample B was also immersed in bovine serum albumin (BSA) dissolved in phosphate buffer pH 7.2 with a concentration of 1 mg/ml. BSA is used to block the surface to potentially improve non-specific adsorption of the bacteria to the 1-hexadecanethiol. Sample C was immersed in 11-mercapto-1-undecanol diluted in ethanol. Sample D was immersed in 11-mercapto-undecanoic acid diluted in ethanol. Sample E was immersed in cysteamine hydrochloride diluted in ethanol. Sample F was immersed in sulfo-NHS-SS-biotin diluted in MilliQ water. Sample G consists of a control surface of bare Au that was not functionalized by any monolayer. The samples were then rinsed in MilliQ water. A diagram of these seven samples is shown in Fig. 2(a).

The seven samples were then treated with a 25 μg/ml TRITC-conjugated streptavidin solution in milliQ water for 15 min. TRITC has absorbance maxima between $\lambda = 515$–520 and 550–555 nm and an emission maximum at $\lambda = 570$ nm. Fluorescence microscopy has been performed using an approach similar to the procedure reported by Huang et al. [10]. For this purpose, the substrates were first placed within a durable silicon gasket. An imaging chamber was formed by pressing a square patterned silicon gasket against a microscope slide. The imaging chamber was sealed with a coverslip before taking the fluorescent images. An Olympus IX81 (Tokyo, Japan) equipped with a TRITC filter and a Roper Scientific CoolSnaps HQ CCD camera (Duluth, Georgia, USA) was then used for recording the fluorescence images. Fluorescence intensity has been acquired randomly on 10 different locations for each sample, and averaged. The substrates were then treated with SYTO BC labeled *E. coli* for 15 min at a concentration of 10^8 colony forming units (cfu)/ml, and observed again with the fluorescence microscope. SYTO BC stain has an excitation maximum at $\lambda = 480$ nm and an emission maximum at $\lambda = 500$ nm.

2.3. Immobilization of biotinylated bacteriophages onto streptavidin-derivitized gold

Amplification of the phages was performed by using established methods. Briefly, 10^5 plaque forming units (pfu) of phage preparation was mixed with 10^6 cfu of fresh log-phase *E. coli* ATCC 11303 bacterial culture. After 15 min of incubation at room temperature, LB medium was added to the mixture followed by a 6 h incubation in a shaking incubator at 37 °C. The solution was then centrifuged at 4000 *g* to pellet the bacteria. The supernatant was filtered using a 0.22 μm filter to remove any remaining bacteria. The filtered supernatant solution was then concentrated by centrifugation, and the phage pellet was resuspended in 1 ml SM buffer. Enumeration of bacteria was performed by the plate count technique and expressed in cfu/ml. Enumeration of phage was performed by the soft agar overlay technique and expressed in pfu/ml [28].

Similarly to the protocol used above, gold substrates were treated with 1 mM sulfo-NHS-SS-biotin in MilliQ water for 24 h, and then immersed in 25 μg/ml TRITC-conjugated streptavidin in milliQ water for 15 min. Substrates were then immersed overnight into 10^9 pfu/ml solutions of either wild-type or biotinylated phages. The surfaces were then observed with a Hitachi S-4800 (Tokyo, Japan) scanning electron microscope (SEM).

2.4. Employment of phage attachment in impedance biosensing device

The attachment procedure described above was then used to investigate the effect of a bacteriophage terminated surface on the growth of the bacteria. This assessment was conducted in an ECIS device. This device monitors the growth of bacteria onto gold electrodes by tracking the increase in impedance induced by such growth. This increase of impedance is mainly related to a constriction of the ionic current allowed to flow as the bacteria increasingly cover the test electrode. Additional impedance signal is however also expected from the growth of the bacteria in the supernatant culture medium itself (Fig. 3).

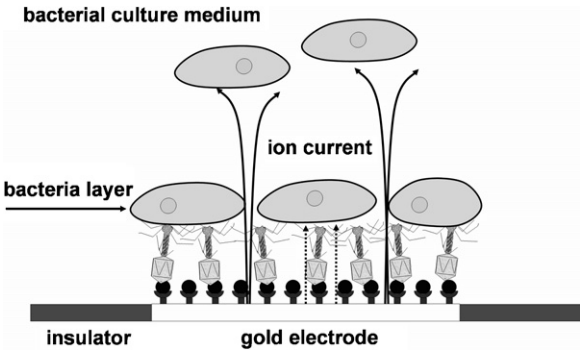

Fig. 3. Schematic transversal view of an ECIS chip's 250 μm gold dot electrode. The electrode is terminated with sulfo-NHS-SS-biotin, streptavidin and biotinylated T4 phages. *E. coli* binds to the surface and grows in an increasingly thick layer that restricts the flow of ion current and increases the measured impedance. Additional impedance signal is also expected from the supernatant culture medium itself.

As was employed for sample F described above, modified ECIS wells were first treated with 1 mM sulfo-NHS-SS-biotin in MilliQ for 24 h and TRITC-conjugated streptavidin for 30 min. Some of the devices were then treated for 30 min with 10^8 pfu/ml of genetically modified biotinylated phages (T4B), and some with wild-type phages (T4W). The devices were then washed twice in PBS and twice in LB. The T4W control experiment was performed to demonstrate the improved performance of the biotinylated phages over the wild-type phage in the presence of the streptavidin-terminated surface. The wells were then filled with the *E. coli* suspension (10^4 cfu/ml), incubated at 37 °C, and the growth of the bacteria monitored by measuring impedance for 24 h.

3. Results and discussion

Fig. 2(b) shows the fluorescent microscopy data of the seven gold samples following exposure to TRITC-streptavidin and SYTO BC *E. coli* solutions, respectively. Relative intensity of streptavidin fluorescence as well as bacterial count for each of the seven samples, are plotted. Streptavidin and *E coli.* adsorb readily to bare gold (sample G), demonstrating the need of surface chemistry to prevent non-specific adsorption. Hexadecanethiol treated with BSA (sample B) resulted in minimal *E. coli* adsorption coupled with minimal streptavidin adsorption, making it a good candidate for a blocking surface. Bare hexadecanethiol (sample A) was also shown to be a good inert surface but to a lesser extent than sample B. The carboxyl- and amine-terminated SAMs (samples D and E) are expected to form peptide bonds with proteins. This induced strong attachment of the streptavidin. However, these two surfaces also featured significant *E. coli* binding activities that would impede on the specificity of the eventual sensing platform.

In comparison, the hydroxyl terminated SAM (sample C) had much lower adsorption of both streptavidin and *E. coli*. Finally, the biotin-terminated SAM (sample F) showed high streptavidin binding with minimal non-specific *E. coli* adsorption, proving to be the optimal candidate for the immobilization of biotinylated phages. This procedure was used in the following experiments unless specified.

Having identified the optimal procedure for streptavidin attachment, biotinylated phages were then bound to the gold surfaces. Fresh gold surfaces were functionalized with streptavidin using the biotin-SAM protocol described above, and exposed to the biotinylated phage. Fig. 4 shows three micrographs respectively showing (a) the attachment of biotinylated phages onto a streptavidin-coated surface, (b) the attachment of wild-type phages onto the same streptavidin-coated surface, and (c) the attachment of wild-type phages onto a bare Au surface. The density surface-attached phages reaches 4.4 phage/μm^2 for biotinlyated phages attached to the streptavidin-coated surfaces (Fig. 4(a)). It significantly drops below 0.3 phage/μm^2 when wild-type phages are rather used on the same streptavidin-coated surface (Fig. 4(b)), or onto bare Au (Fig. 4(c)). The attachment of biotinylated phages onto streptavidin-coated surfaces therefore results in at least a 15-fold improvement of attachment when compared to the simple attachment of the wild-type phage.

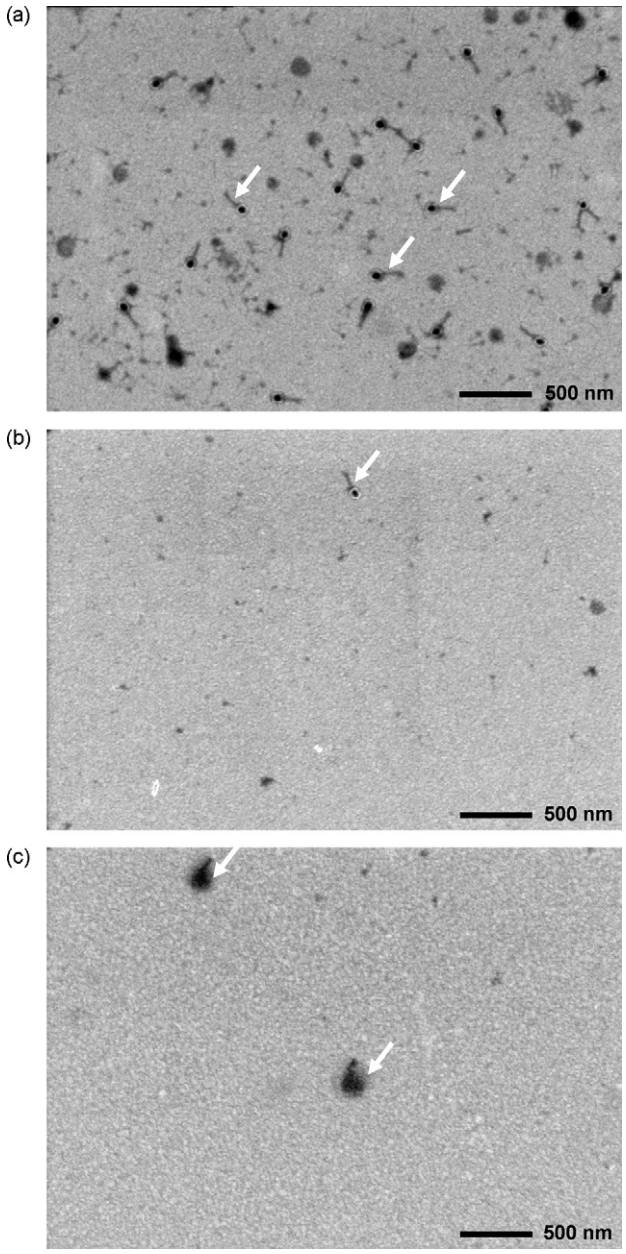

Fig. 4. Scanning electron micrographs showing (a) the attachment of biotinylated phages onto a streptavidin-coated surface, (b) the attachment of wild-type phages onto the same streptavidin-coated surface, and (c) the simple physisorption of wild-type phages onto a bare Au surface. Some of the phages are indicated by arrows.

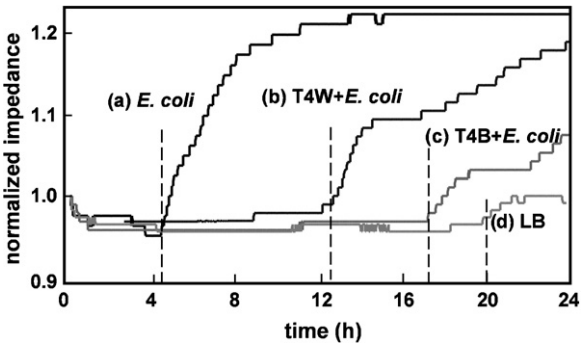

Fig. 5. ECIS Impedance measurement obtained from the growth of *E. coli* on four differently terminated gold electrodes. All electrodes were first functionalized with sulfo-NHS-SS-biotin and streptavidin. (a) Growth of *E. coli* without prior attachment of bacteriophages. This shows a normal bacterial growth curve rising at 4.5 h and peaking at 14 h. (b) The gold electrode terminated with the wild-type phage (T4W) shows a 13 h delay in bacterial growth. (c) The gold electrode terminated with the biotinylated phage (T4B) shows a 17.2 h delay in bacterial growth. (d) The LB negative control shows a flat line until 18 h.

This attachment procedure was then used to investigate the effect of a biotinylated phage-terminated surface on the growth of the host bacteria. This assessment was conducted in an ECIS device (Fig. 3). Fig. 5 shows the measured impedance caused by the growth of the *E. coli* host in the presence of phages immobilized onto the gold electrodes. Trace (a) corresponds to the growth of *E. coli* in the presence of streptavidin-terminated gold electrodes without any immobilization of phages. Trace (b) corresponds to the bacterial growth in the presence of streptavidin-terminated gold electrodes with immobilized wild-type T4 phages (T4W). Trace (c) corresponds to the growth in the presence of streptavidin-terminated gold electrodes with immobilized biotinylated T4 phages (T4B). Trace (d) corresponds to a well with streptavidin-terminated gold electrodes and filled with LB solution without any *E. coli* growth, thus providing a background control for comparison purposes. At the early stages of the incubation (i.e. first 4 h on Traces (a)–(c)), there was limited bacterial growth on the electrode and in the solution and the impedance remained constant. After 4–18 h of incubation, depending on the preconditioning of the surface ((a) without phage, (b) with T4W phages, and (c) with T4B phages), an increase in the impedance signal is detected, indicating that the growth rate of bacteria on the surface and in the solution was not the same in all the samples. The devices devoid of any immobilized phage began to show measurable growth at 4.5 h (Trace a). This is a typical time for detecting an *E. coli* concentration of 10^4 cfu/ml as used in this experiment. In comparison, the devices onto which wild-type phages were immobilized began to show growth after 13 h (Trace b). This indicates that there were phages adsorbed non-specifically to the surface that inhibit bacterial growth by capture and subsequent lysis of bacteria, thus delaying the detection of bacterial growth. Interestingly, the devices onto which biotinylated phages are immobilized begin to show growth after 17.2 h (Trace c).

This greater delay indicates that the electrodes with the biotinylated phages delayed the growth of the bacteria in a more efficient manner, indicating that more phages capable of infecting and destroying bacteria were present on the surface. As was observed by SEM in the control surfaces (Fig. 4(a) and (b)), the biotinylated phage T4B indeed attaches in greater numbers to the streptavidin-terminated surface compared to the wild-type phage. In addition, given that biotin is present only on the head capsid, the chemically attached phages are expected to mostly bind to the surface through their head, leaving the tail fibers free to attach to bacteria receptors. This overall larger amount of infective phages on the initial surface therefore resulted in a greater number of lysed bacteria and therefore in a longer delay of bacterial growth both on the surface of the electrode and in the surrounding solution.

This difference in bacterial growth delay could have alternatively been explained by a difference of burst or latent time of the biotinylated phage compared to the wild-type. However, we do not believe this to be the case as such genetic biotinylation has not been found to affect the viral activity of the T4 bacteriophages [27]. As was observed in Fig. 4, the functionalization scheme of biotin-SAM and streptavidin therefore improved the binding of biotinylated phages onto the electrode surface, and delayed bacterial growth with greater efficiency than for devices that were initially treated with non-biotinylated phages.

4. Conclusions

We reported the streptavidin-mediated attachment of capsid-biotinylated phages onto gold electrodes, as well as the employment of such an attachment in an impedance biosensing system. The functionalization of gold surfaces with a biotin-terminated alkyl chain optimizes the attachment of streptavidin while minimizing the non-specific adsorption of the host *E. coli*. These streptavidin-fuctionalized surfaces were then used to capture bacteriophages with biotinylated capsid heads. The capture efficiency of these surfaces was 15 times higher when exposed to biotinylated phages instead of the wild-type ones. This indicates that the usage of biotin-streptavidin interaction chemistry significantly improves the attachment of the viruses onto the gold surfaces. These streptavidin modified surfaces were then used to mediate the attachment of biotinylated phages onto an impedance biosensing device. Such procedure significantly delayed the growth of the host bacteria, indicating that phages were efficiently bound to the functionalized surface. Devices onto which biotinlyated phages have been bound showed a greater delay in the growth of bacteria compared to devices onto which wild-type phages were employed. This was again a result of the improved efficiency of the attachment of the biotinylated phages onto the streptavidin-terminated gold. Such chemical attachment of phages onto sensor surfaces could in turn be leveraged in highly sensitive and more rapid transduction platforms such as SPR, QCM, and microcantilevers.

Acknowledgments

This work was supported by the National Institute for Nanotechnology (NINT), a joint venture between the National Research Council of Canada, the University of Alberta and the province of Alberta. The SEM imaging of the bacterial phage-coated surfaces was performed at the NINT Electron Microscopy facilities. Fabrication of gold substrates was undertaken in the University of Alberta Nanofab facilities. The authors would like to thank Dr. Ken Westra and the Nanofab staff for valuable discussions and help.

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Biographies

Luc Gervais received his Bachelor of Engineering degree in Computer Engineering in 2004 from Concordia University in Montreal. After a year at National Taiwan University, where he learned the intricacies of the Chinese language, he is currently pursuing a Masters of Science in Electrical and Computer Engineering at the University of Alberta. His interests are the integration of biological and electronic systems, bionanotechnology, and bioMEMS sensors and devices used in medical diagnostics.

Murat Gel received his BS degree in Physics from Middle East Technical University, Turkey in 1997. He received MS and PhD degrees in Mechano-Informatics from the University of Tokyo, Japan in 2000 and 2003, respectively. He worked as a Researcher in the Center for International Research on MicroMechatronics in Tokyo (2003–2005), also, he worked as Postdoctoral Fellow in National Institute for Nanotechnology, Canada (2005–2006). His research interests include the development of microfabricated cantilevers for sensitive force measurements as well as the biofunctionalization of these devices for biosensing applications. Dr. Gel is also interested in applications of MEMS-based tools for single-molecule and single-cell level studies.

Beatrice Allain obtained her PhD in Virology from the University of Lyon in 1994, studying the DNA replication of HIV virus. During her post-doctorate at the University of Montreal, she specialized in HIV protein expression by looking at the importance of accessory proteins in the viral replication. Then she joined the Biophage research team in 1999 as a Research Scientist to work on bacteriophages. She is now Director of the Therapeutic Division at Biophage Parma Inc. where she is developing an integrated approach for the management of bacterial contamination comprising the selection of phages for use in detection tools (biosensors) and as therapeutic agents (phage therapy). She has also developed complementary assays for bacterial detection based on impedance biosensors. These biosensors can be used alone or in conjunction with phage therapy for intelligent treatments. She is also author of numerous papers, presentations and patents.

Mona Tolba graduated in 1998 from the Faculty of Veterinary Medicine, Cairo University, Egypt with a PhD in Veterinary Science. She was then Lecturer for a Food Microbiology course for Department of Food Hygiene, Faculty of Veterinary Medicine, Cairo University. Her initial Master and PhD work concentrated on the modeling growth, survival and control of *Listeria moncytogenes* in milk and dairy products. She immigrated to Canada in 2002 where she obtained a scholarship (2003) from the Department of Food science, University of Guelph to do another PhD. The focus of her PhD research is to develop site-specific immobilized bacteriophage for the detection and/or control of bacteria in food.

Lubov Brovko obtained her degrees of PhD (1980) and DSc (1991) in Biochemistry and Biotechnology from Lomonosov Moscow State University, Moscow, Russia. She is currently a Senior Research Associate at Canadian Research Institute for Food Safety, and Adjunct Professor at the Department of Food Science, University of Guelph, Ontario, Canada. Dr. Brovko has published over 100 papers in peer-reviewed journals and 4 book chapters on basic mechanisms of bioluminescence and its applications in biochemistry, microbiology, immunology and biotechnology. Her current research projects include bioluminescent cell-based pathogenicity biosensors for high throughput assay, paper-based biosensors for pathogen detection, immuno-modulating properties of fermented milk and its components, antimicrobial photodynamic treatment for surface sanitation, and rapid phage-based methods for the detection pathogens in food. Dr. Brovko is a member and Scientific Advisor of International Society for Bioluminescence and Chemiluminescence. She recently submitted tutorial book on applications of bioluminescence for food and environmental safety for publication.

Mohammed Zourob received his PhD in 2003 from Department of Instrumentation and Analytical Science (DIAS) from The University of Manchester Institute of Science and Technology (UMIST). From 2003–2005 Dr. Zourob worked as a Researcher at the University of Manchester working in biosensors and lab on a chip for life science applications. Then he moved to the Department of Biomaterials Science of the same university to work in developing high throughput screening platforms for "Omics" applications. At the end of 2005 Dr. Zourob moved to the Institute of Biotechnology at the University of Cambridge where his research focus on optical sensing and lab on a chip. Recently he was appointed as a Director of Biosensors Division at Biophage Pharma Inc. Dr. Zourob is currently editing a book "Technologies for the detection of bacteria (transducers, recognition receptors and microsystems)". Dr. Zourob research interest includes the development of chemo/biosensors, highthroughput screening for "Omics" applications, platform for therasonstics applications, BioMEMS and lab on a chip for life sciences. He has a number of patents and many peer-reviewed articles. Dr. Zourob serving on the editorial board of a number of journals.

Rosemonde Mandeville is an entrepreneur, a scientist and a seasoned manager, and the founder and acting president and CSO of Biophage Pharma Inc. She serves on several boards of directors, most importantly Investment Quebec. As a Former Professor, she has over 200 publications. Located at the National Research Council Biotechnology Research Institute (Montreal, Canada), Biophage employs 18 people, including a team of 14 researchers. Biophage has secured an impressive portfolio of promising new diagnostics and therapeutic applications of phages and possesses an extensive library of phages.

Mansel Griffiths received his BSc degree in Applied Biology at North East London Polytechnic and his PhD from Leicester University where he studied the biochemistry of thermophilic microorganisms under the supervision of Sir Hans Kornberg. Dr. Griffiths was appointed to the staff of the Hannah Research Institute, Ayr, Scotland in 1974 and, in 1980, he was appointed Head of the Dairy Microbiology group. In 1990 Dr. Griffiths was appointed Chair in Dairy Microbiology in the Food Science Department at the University of Guelph. Dr. Griffiths' position is funded jointly by the Dairy Farmers of Ontario and the Natural Science and Engineering Research Council of Canada (NSERC). Dr. Griffiths is Program Chair for the MSc in Food Safety and Quality Assurance programs being offered at Guelph and is the Director of the Canadian Research Institute for Food Safety. His current research interests include rapid detection of foodborne pathogens; factors controlling growth and survival of microorganisms in foods; beneficial uses of microorganisms. Dr. Griffiths has authored more than 250 peer-reviewed articles and appears on http://www.ISI HighlyCited.com.

Stephane Evoy received a PhD in Applied Physics from Cornell University in 1998. He is an Assistant Professor of Electrical and Computer Engineering at the University of Alberta, with cross-appointment as Leader of the Devices and Sensors Group of the National Institute for Nanotechnology. His current research includes the development of micro/nanomechanical devices for biosensing applications, as well as the integration of nanostructures for the development of chemical sensors. Dr. Evoy has also recently co-edited "Introduction to Nanoscale Science and Engineering", a textbook supporting the teaching of nanoscale technologies at the undergraduate level. He is currently serving as member of the Executive Committee of the Nanoscale Science and Technology Division of the American Vacuum Society. Since October of 2005, he is also serving on the editorial board of "Review of Scientific Instruments".