Journal of Biosensors & Bioelectronics
ISSN: 2155-6210

Montrose et al., J Biosens Bioelectron 2015, 6:3
DOI: 10.4172/2155-6210.1000174

Research Article Open Access

Novel Single Gold Nanowire-based Electrochemical Immunosensor for Rapid Detection of Bovine Viral Diarrhoea Antibodies in Serum

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Abstract

Bovine Viral Diarrheoa (BVD) is a worldwide disease with severe financial implications for the Bovine beef and dairy industries. A key challenge to BVD eradication is that the requirement to send samples to, and receive results from, specialized laboratories slows down the diagnostic process and leads to uncontrolled spread of the virus within a herd until diagnostic confirmation is received. Consequently, rapid identification of BVD is now critical for herd protection and prevention of costly herd outbreaks and new diagnostic devices, suitable for on-farm analysis, that deliver rapid and early identification of animal disease states, are required. We report, here, an electrochemical on-chip fully integrated nanowire based immunosensor device for detection of BVD in serum. The capture biomolecule, BVD virus, is covalently immobilized via a carboxylic terminated polymer firstly electrodeposited onto a single nanowire. Electrochemical characterization including faradaic electrochemical impedance spectroscopy and cyclic voltammetry is performed. Label free immunologic detection of antibodies (10 µg/mL, 20 min) is first demonstrated using a bovine serum albumin as a model antigen-antibody system. Then, the immunosensor is applied to detection of bovine viral diarrhoea antibodies (10 µg/mL, 20 min) in both buffer and serum. The sensor clearly discriminates between positive and negative infected bovine sera. This study clearly shows the potential of this chip nanowire-based electrochemical sensor for immunoassays application in serum with a view to developing portable devices for on-farm diagnosis or therapeutic monitoring in animal health applications.

Keywords: Immunosensor; Nanowire; Electrochemical impedance spectroscopy; Electrodeposited polymer; Bovine viral diarrhoea

Introduction

Bovine Viral Diarrhoea (BVD), is a major contributor to bovine respiratory disease and one of the most common diseases in cattle [1,2]. BVD virus is a pestivirus in the Flaviviridae family and has a worldwide distribution. Once BVD virus enters a herd, viral spread is rapid and full herd-infection can result within weeks. Economic implications of an outbreak are severe as diseased animals perform sub-optimally and decrease on-farm efficiency and profitability through waste feed, labor and increased veterinary costs. Delayed diagnosis of viral infections in animals can also lead to animal death.

European farmers €300 per cow in treatment/isolation costs [3]. Likewise, in the US this disease has a total economic cost to the cattle industry of over US $2 billion annually. Early identification of respiratory diseases are essential to achieve effective control, in that, appropriate intervention with removal from the herd or vaccines treatments can reduce clinical signs and reduce the number of carrier animals present within a herd [3]. To date, only a few European countries have achieved total eradication and in many countries national schemes have not been initiated as they are deemed too cost-prohibitive and time-consuming.

Detection of BVD is currently performed using high-end analytical instrumentation including: Reverse Transcription Polymerase Chain Reaction (RT-PCR), immunohistochemistry or Enzyme Linked Immunosorbant Assay (ELISA) by targeting whole BVD virus, specific antigen epitopes or specific antibodies [4-6]. Globally, these tests have sufficient sensitivity but can be time-consuming (a few hours). They also require dedicated laboratories, skilled personnel and have high instrument ownership and maintenance costs. Rapid detection and identification of BVD virus is critical for herd protection in order to prevent costly herd outbreaks. However current diagnostic approaches (based on laboratory-based methods) can slow down this diagnostic process as samples must be first sent to the designated laboratory; once received and analyzed results must then be communicated back to the key stakeholders (farmers and veterinarians), a process that can take days to weeks. These delays can lead to uncontrolled spread of the virus within a herd. Consequently there is now a growing need to develop new rapid diagnostic tools that will deliver rapid and early identification (<30 minutes) of animal disease state on-farm.

Electrochemical biosensors, though label-free detection methods, constitute a promising group of sensing devices that allow high sensitivity, short analysis times, affordability, miniaturized platforms, with low sample consumption and the possibility for measurements in complex samples [7]. Amperometry, potentiometry and electrochemical impedance spectroscopy (EIS) are examples of electrochemical techniques that have been used in immunosensors [8]. Of these, EIS represents a powerful method for the detection of target analyte at functionalized sensor surface via an immunochemical interaction in a label free manner, i.e., without the need for further costly or complex labeling techniques [9-11]. EIS uses periodic small AC voltage amplitudes that are applied to an electrode and a change of the electrode impedance is measured and can be correlated to the amount of analyte binding [12,13].

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Received March 30, 2015; **Accepted** July 29, 2015; **Published** September 01, 2015

Citation: Montrose A, Creedon N, Sayers R, Barry S, O'riordan A (2015) Novel Single Gold Nanowire-based Electrochemical Immunosensor for Rapid Detection of Bovine Viral Diarrhoea Antibodies in Serum. J Biosens Bioelectron 6: 174. doi:10.4172/2155-6210.1000174

Recent developments in fabrication of robust and reproducible nanoelectrodes have opened the door to a new and exciting area of electrochemistry. Compared to traditional macroelectrodes, nanoscale electrodes, such as those based on single nanowires, have tremendous potential when employed in electrochemical analysis due to enhanced sensitivity. This enhancement arises from increased mass transport to the electrode (via radial diffusion) and low background charging currents (due to reduced surface area) relative to macroscale electrodes [10,14]. Reproducible wafer-scale fabrication (using nanoelectronic fabrication techniques) of fully integrated nanowire-based electrochemical devices have been previously developed [15,16] and the applicability of these devices for highly sensitive detection of (bio) molecules including: glucose and hydrogen peroxide [17], heavy metals [18] and explosives [19] have been demonstrated. These discrete nanowire sensor devices exhibit a 100-10,000 fold increase (analyte dependent) in sensitivity and can undertake analysis in milliseconds compared to minutes for commercial state-of-the-art electrodes [20,21].

To date, field effect transistor based semiconductor nanowire sensors have been successfully employed for detection of key biomolecules including DNA, viruses and disease biomarkers [22-24]. Although elegant, these approaches are limited in that they require application of a high back gate voltage (up to 40V DC), and many of their attributes such as reproducibility, product uniformity, potential for scaling up and cost effectiveness are not very well known [25]. Combining electrochemical impedance techniques with highly reproducible gold nanoelectrodes could, therefore, enable the development of new up-stream diagnostic devices. However, a critical challenge to developing such devices is that label-free electrochemical based detection of biomolecule binding events at the discrete nanostructures in buffer or bio-media has yet to be demonstrated.

In this paper, we address this challenge and demonstrate the first on-chip nanowire immunosensor device for the electrochemical-based detection of BVD antibodies in serum. Nanowires are fabricated using reproducible top-down nanofabrication approaches used in the nanoelectronics industry. Capture biomolecules are covalently immobilized onto an electrodeposited polymer layer pre-deposited on a nanowire sensor and each step of the modifying process is characterized using cyclic voltammetry and EIS. Immunoassays are developed using a bovine serum albumin antibody/antigen model system. The immunosensor is first applied to the detection of anti-BSA antibodies in buffer and then to BVD antibodies in both buffer and diluted serum. Finally the sensor is challenged to discriminate between disease positive and disease negative serum samples. BVD assay times are typically ~20 minutes demonstrating the potential of these nanowire electrochemical immunosensors for use in future portable devices required for on-farm diagnostic applications.

Identification of the agent

BVDV is a pestivirus in the family Flaviviridae and is closely related to classical swine fever and ovine border disease viruses. The two genotypes (types 1 and 2) are classified as separate species in the genus Pestivirus. A third putative genotype, BVDV type 3, has also recently been proposed. Although both cytopathic and non-cytopathic biotypes of BVDV type 1 and type 2 exist, non-cytopathic strains are usually encountered in field infections and are the main focus of diagnostic virus isolation in cell cultures. PI animals can be readily identified by a variety of methods aimed to detect viral antigens or viral RNA directly in blood and tissues. Virus can also be isolated by inoculation of specimens onto susceptible cell cultures followed by immune-labelling methods to detect the replication of the virus in the cultures. Persistence of virus infection should be confirmed by resampling after an interval of at least 3 weeks, when virus will again be detected. PI animals are usually seronegative. Viraemia in acute cases is transient and difficult to detect. Virus isolation in semen from bulls that are acutely or persistently infected requires special attention to specimen transport and testing. RNA detection assays are particularly useful because they are rapid, have very high sensitivity and do not depend on the use of cell cultures.

Serological tests

Acute infection with BVDV is best confirmed by demonstrating seroconversion using sequential paired samples, ideally from several animals in the group. The testing of paired (acute and convalescent samples) should be done a minimum of 21 days apart and samples should be tested concurrently in the same assay. Enzyme-linked immunosorbent assays and the virus neutralisation test are the most widely used.

Requirements for vaccines

There is no standard vaccine for BVD, but a number of commercial preparations are available. An ideal vaccine should be able to prevent transplacental infection in pregnant cows. Modified live virus vaccine should not be administered to pregnant cattle (or to their sucking calves) due to the risk of transplacental infection. Live vaccines that contain cytopathic strains of BVDV present a risk of inducing mucosal disease in PI animals. Inactivated viral vaccines are safe and can be given to any class of animal but generally require booster vaccinations. BVDV is a particularly important hazard to the manufacture of vaccines and biological products for other diseases due to the high frequency of contamination of batches of fetal calf serum used as a culture medium supplement.

Materials and Methods

Materials

O-aminobenzoic acid (o-ABA), N-hydroxysuccinimide (NHS), N-ethyl-N-(dimethylaminopropyl)-carbodiimide (EDC), phosphate buffered saline (PBS) solution, Dubelcco's phosphate buffered saline 10 × (PBS10) solution, tween 20, sodium chloride (NaCl), sodium hydroxide (NaOH), bovine serum albumin (BSA), anti-bovine albumin antibody (BSA Ab), and ferrocene monocarboxylic (FcCOOH) were purchased from Sigma-Aldrich. The acetate buffer (10 mM; pH 4) and ethanolamine-HCl (1mM) were obtained from Sierra Sensors GmbH (Germany). Deionized water (18.2 MΩ cm) was obtained using an ELGA Pure Lab Ultra system. BVD virus and monoclonal antibodies BVD specific to the envelop glycoprotein (Erns) of the virus were purchased from Animal and Plant Health Agency (APHA Scientific, UK). All reagents were used as received. Positive and negative bovine serum samples were provided by Teagascbiobank (Moorepark, Ireland).

Methods

Fully integrated nanowire based electrode fabrication: Gold nanowires electrodes, on-chip gold counter and platinum pseudo-reference electrodes were fabricated on wafer-scale silicon substrates using hybrid e-beam/optical lithography, metaldeposition and lift-off techniques, as previously described in detail by Wahl et al. [19]. Before use, chips were cleaned using a mixed solvent clean process and dried under a stream of nitrogen.

Electrode modification: A schematic diagram of the immunosensor

is shown in Figure 1. All the modification steps were performed in a bespoke electrochemical cell. On-chip nanowire electrodes were first modified using electrodeposited o-ABA. The electrodeposition of the polymer (10 mM o-ABA in 10mM acetate buffer pH 4) was undertaken by cyclic voltammetry (25 cycles) in the applied potential ranging of -0.2 to 0.8V (versus on-chip pseudo platinum electrode) at a scan rate of 50 mV/s followed by careful rinsing of the electrodes with DI water to remove any remaining monomers. The carboxylic terminated polymer was activated using a fresh mixture of EDC/NHS (10 mM/10 mM) for 30 min. Then, capture molecules, either BSA (10 mg/mL) or BVD virus (100 µg/mL), were covalently attached to the surface (3 hours at 4°C). Following this immobilization, the electrodes were well rinsed with PBS10 solution containing 0.1% Tween-20 (PBS10-T), PBS and DI water to remove any unbound proteins and the un-reacted active sites were blocked by immersing in ethanolamine for 30 min.

Finally, the antigen modified electrodes were exposed to known concentrations of antibody solution in PBS or to 1% diluted serum samples for 20 min at room temperature. The electrodes were rinsed carefully with PBS10-T, NaCl (1 M), PBS, NaOH (10 mM) and DI water to remove non-specifically bound target biomolecules prior to measurement.

Electrochemical measurements: Electrochemical experiments were carried out using an Autolab Potentiostat/ Galvanostat PGSTAT128N (Metrohm Ltd, Utrecht, The Netherlands) controlled by the Autolab NOVA software. All experiments employed a standard three-electrode cell configuration using a single gold nanowire as the working electrode, versus the on-chip gold counter electrode and the on-chip platinum pseudo-reference electrode.

Cyclic voltammetry (CV) and faradaic electrochemical impedance spectroscopy (EIS) were performed in a 10 mM PBS solution (pH = 7.4) containing 1 mMFcCOOH. For CV, the potential was cycled from - 0.2 to 0.6V (versus on-chip platinum pseudo reference electrode) at a scan rate of 100 mV/s. The impedance spectra were recorded in a frequency range of 100 mHz to 100 kHz at 150 mV, the equilibrium potential of the FcCOOH redox couple. The amplitude of the alternating voltage

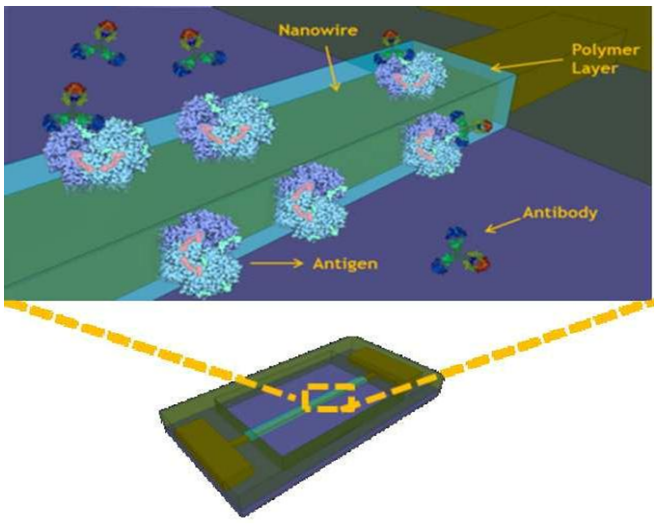

Figure 1: Schematic of an immunoassay at a single gold nanowire. A polymer layer is first electrodeposited at a single nanowire electrode. Capture biomolecule, virus or antigens, are then covalently attached on the electrodeposited polymer layer and used to specifically capture and bind to target antibodies which are subsequently detected electrochemically.

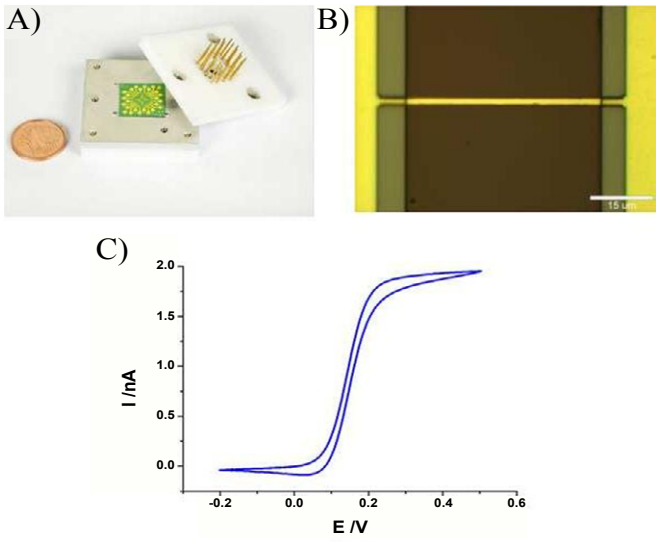

Figure 2: (A) Photograph of the electrochemical cell and the chip containing 12 electrodes and on chip platinum reference and gold counter electrodes; (B) Optical photography of a single gold nanowire of 700 nm width; (C) Typical Cyclic voltammogram with an applied potential ranging from -0.2 to 0.5 V obtained for a single nanowire in 1 mMFcCOOH in 10 mM PBS (pH 7.4).

was 5 mV. All experiments were performed at room temperature in a Faraday cage.

ELISA measurements: ELISA tests were performed using a commercial BVDV p80 Ab detection kit for the detection of specific antibodies directed to bovine viral diarrhea virus (IDEXX, UK) following the short (1 h) protocol. Briefly, the p80 modified ELISA plate of the kit was exposed to the serum samples diluted (10%) in the commercial dilution solution. Following 1h incubation at room temperature, plates were rinsed with diluted commercial wash buffer using an ELISA plate washer (DIASource, Belgium). Then the conjugate was diluted (with the provided solution) and incubated in the plate for 30 min at room temperature. After washing, a chromogenic substrate was added for 20 min in dark room at room temperature. Finally, the reaction revealing the conjugate was stopped using the commercial stop solution and the absorbance was read at 450 nm using an ELISA plate reader (DIASource, Belgium). Blank and controls using positive and negative provided control solutions were also performed. All samples were analyzed in triplicate.

Results and Discussion

Characterization of gold nanowire electrode

Fabricated devices were mounted in an electrochemical cell set-up (Figure 2A) and contain twelve separate electron-beam fields containing nanowire electrodes, an on-chip gold counter electrode, an on-chip platinum pseudo-reference electrode and peripheral contact pads to facilitate direct electrical and electrochemical probing. Nanowire electrodes were 700 nm width and 45 µm long (Figure 2B). Electrochemical functionality of the nanowire electrode was characterized using cyclic voltammetry in presence of a redox probe. Figure 2C shows a typical voltammogram obtained in 1 mMFcCOOH with an intensity current of 2 nA, at a single gold nanowire electrode. As expected, a quasi-steady-state response was observed, typical of single electron oxidation occurring at a discrete nanowire [26]. The magnitude of the current confirms that electrochemistry only occurs at

the nanowire electrodes and the passivation layer successfully prevents unwanted electrochemistry occurring at on-chip metallisation. Nanowire devices that exhibited lower or no electrochemical current were discarded and not used for further experiments as they revealed a defect in the nanowire or an incomplete removal of the passivation layer.

Application of the biofunctionnalization process to a model BSA antigen-antibody

Electrodeposition of o-ABA: The modification process is based on the electrodeposition of an o-ABA polymer layer which is then used to immobilize the antigen onto a single gold nanowire electrode. Poly o-aminobenzoic acid is selected since it is a carboxyl functionalized aniline polymer capable of self-doping. This bio-compatible polymer is emerging as a promising polyaniline derivative for biosensor applications. After polymerization, the free carboxylic acid functional groups can be used as a matrix material for subsequent immobilization though covalent binding with biomolecules such as proteins and antibodies [27-31].

A typical CV obtained for the electrodeposition of o-ABA on the single nanowire. The first cycle of the electrodeposition displays an oxidative peak around 0.5 V corresponding to the formation of the polymer on the gold electrode. This peak disappears with subsequent scans and a small reversible peak around 0 V, characteristic of the doping-dedoping of the polymer, and a second dedoping peak at 0.3 V in the cathodic scan appear with increasing number of cycles. The emergence of these peaks confirms the electrodeposition of o-ABA on the gold surface [31].

Detection of BSA antibody in buffer: To confirm suitability of on-chip nanowires for immunoassay applications, the immobilization process and the immunologic detection of BSA antibody onto the o-ABA modified nanowire was first demonstrated. Figure 3A shows a schematic of the layer-by-layer build up on a nanowire surface. Each step of the assays was characterized using both cyclic voltammetry and impedance spectroscopy. The CV and the Nyquist spectra for the detection of 10 µg/mL BSA Ab are presented in Figures 3B and 3C, respectively.

The CV of the cleaned gold nanowire electrode shows a typical quasi-steady-state response characteristic of the redox couple (Figure 3B, curve a) and the associated nyquist spectrum (Figure 3C, curve a) displays a semi-circle with an estimated charge transfer resistance around 58 ± 16 MΩ. After the electrodeposition of o-ABA layer, the current intensity in the CV decreases (Figure 3B, curve b) while nyquist semi-circle increases (Figure 3C, curve b). This indicates an increase in charge transfer resistance and in the global capacitance at the nanowire. These changes may arise from the electrodeposited polymer partially blocking the electrode and therefore limiting the electron transfer from FcCOOH to the electrode. The negative charges of the carboxylate terminated polymer can also induce an electrostatic repulsion of bulk FcCOOH ions. Covalent immobilization of the BSA is undertaken though the amide bond formed by the EDC-NHS coupling. No major change is visible on the voltammogram (Figure 3B, curve c) but the nyquist spectrum (Figure 3C, curve c) reveals a decrease in the semi-circle. This change could be attributed to the covalent immobilization of BSA at the carboxylate activated sites of the polymer rather than the simple adsorption on the electrode as the accessible surface for the oxidation and reduction of the FcCOOH remains constant. This decrease in measured impedance could be attributed to a decrease in charge density following binding (arising from displacement of solvent counter ions) and an increase in the thickness of the modifying layer leading to a decrease in the global capacitance at the nanowire. After ethanolamine blocking step, no significant difference (from the BSA curve) is visible in the CV (Figure 3B, curve d) or the nyquist spectrum (Figure 3C, curve d) suggesting that BSA is bound to all accessible active sites.

For the immunologic detection, anti-BSA antibodies (BSA Ab; 10 µg/mL) in buffer solution (PBS; pH 7.4) are deposited on the fully blocked BSA-modified nanowire and allowed to incubate for 20 min. The binding of BSA Ab leads to a strong decrease in current intensity (Figure 3B, curve e), and an increase in the impedance (Figure 3C,

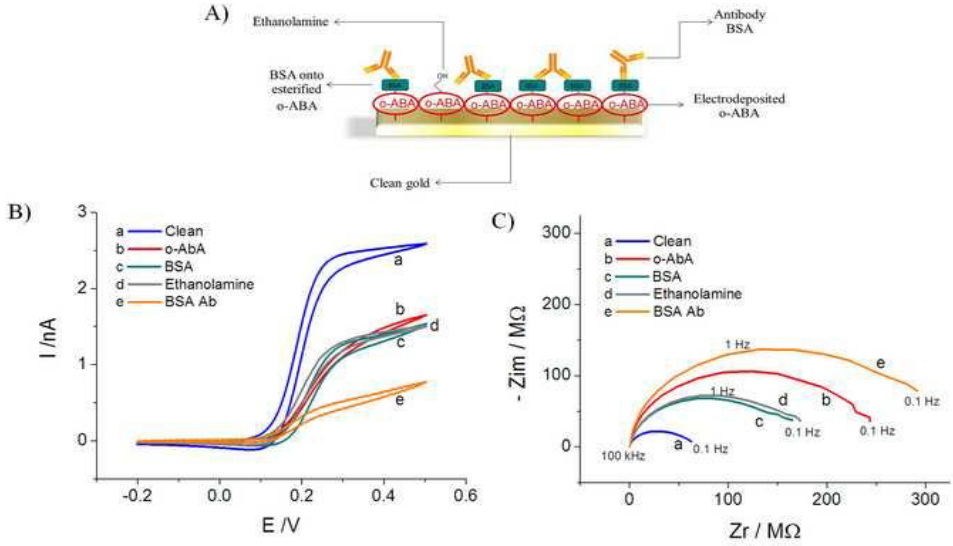

Figure 3: (A) Schematic of the layer-by-layer build up on a nanowire sensor for the BSA antigen/antibody model system; (B) Cyclic Voltammograms and (C) Nyquist plots of (a) pristine gold nanowire electrode;(b) o-ABA modified electrode; (c) BSA (10 mg/mL) modified electrode; (d) ethanolamine blocked electrode; (e) Antibody BSA binding (10 µg/mL). Solution composition: 1 mMFcCOOH in 10 mM PBS. CV scan rate: 100 mVs^{-1}. EIS frequency range: 0.1 Hz to 100 kHz; E=150 mV and ΔV=5 mV.

curve e). These changes may be attributed to the anti-BSA orienting in a manner so as to block the FcCOOH from reaching the electrode surface preventing electronic transfer to the electrode.

The detection of anti-BSA antibodies (10 µg/mL) in buffer in 20 min in this way demonstrates the potential of these nanowire sensors for use as rapid and label-free immunosensors. To confirm this potential, electrochemical-based detection of BVD antibodies in both buffer and diluted serum samples using virus as capture biomolecules was then assessed.

Detection of BVD antibodies in buffer

The functionalization process previously described was then applied to the covalent immobilization of BVD virus (100 µg/mL) onto the o-ABA polymer electrodeposited on the nanowire electrode. Figure 4A shows a schematic of the layer-by-layer build up on a nanowire surface. The detection of BVD antibodies (BVD Ab) is firstly performed in buffer (PBS; pH 7.4) for a concentration of 10 µg/mL. As previously described, CV and nyquist spectra were recorded in presence of FcCOOH as redox probe (Figures 4B and 4C).

O-ABA was again electrodeposited onto pristine gold nanowires (Figure 4B and 4C, curve a) leading to a decrease in electron transfer as expected (Figures 4B and 4C, curve b). BVD virus was then immobilized on the o-ABA modified electrode resulting in an important decrease in the current intensity (Figure 4B, curve c) and an increase in the semi-circle diameter i.e. increase of the charge transfer resistance and the global capacitance (Figure 4C, curve c). These changes are mainly attributed to the large BVD virus (40-60 nm), capture molecule, attached on the nanowire preventing FcCOOH to reach the electrode surface. Following the blocking step, slight changes are visible but the CV and nyquist spectrum remain very similar to the BVD virus layer (Figures 4B and 4C, curve d).

After binding of the antibodies to the multiple epitopes on the peptide envelop of the virus, a strong increase in the current intensity is observed in CV (Figure 4B, curve e) with a corresponding decrease in the measured impedance (Figure 4C, curve e). This suggests that the binding may cause a re-orientation or re-arrangement of the virus layer forming channels between virus particles through which FcCOOH molecules may pass to reach the electrode. It also suggests that electronic transfer between BVD antibody and the electrode may be occurring and probably a combination of both.

After successful demonstration of BVD antibodies detection in buffer, the BVD virus modified immunosensor was then applied to detection of BVD antibody in bovine serum of known disease state i.e. with BVD antibody positive and negative, obtained from cows.

Detection of BVD antibodies in serum

To evaluate the behavior of the electrochemical nanowire-based immunosensor in complex matrices, a serum dilution of 1% was chosen. This dilution corresponds to the maximum dilution permitting the BVD Antibody detection with the ELISA after 1 h incubation of the positive infected serum sample. The CV and nyquist spectra were recorded in presence of FcCOOH for each step of the virus immobilization and the detection of BDV antibodies in 1% positive infected serum (Figures 5A and 5B).

As before, immobilization of the BVD virus on the nanowire leads to the limitation of electron transfer of FcCOOH to the electrode i.e. decrease in current intensity and increase in impedance (Figures 5A and 5B, curves a-c) with no major changes being observed after ethanolamine blocking step (Figures 5B and 5A, curve d). The BVD virus immunosensor was then exposed to 1% positive infected serum incubated for 20 min and washed to remove any unbound material. The CV and EIS obtained following the binding of BVD antibodies from the positive serum evolve in the same way as in buffer i.e. an increase in the CV and a decrease in the impedance (Figure 5A and

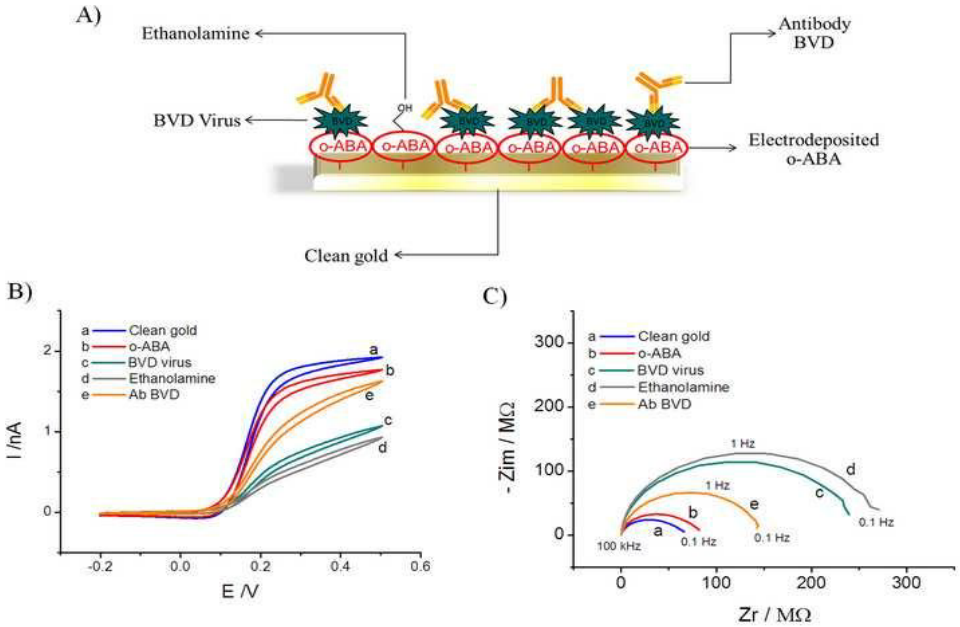

Figure 4: (A) Schematic of the layer-by-layer build up on a nanowire sensor for BVD antibody detection; (B) Cyclic Voltammograms and (C) Nyquist plots of (a) pristine gold nanowire electrode; (b) o-ABA modified electrode; (c) BVD virus (100 µg/mL) modified electrode; (d) ethanolamine blocked electrode; (e)Antibody BVD (10 µg/mL) binding in buffer. Solution composition: 1 mMFcCOOH in 10 mM PBS. CV scan rate: 100 mVs^{-1}. EIS frequency range: 0.1 Hz to 100 kHz; E=150 mV and ΔV=5 mV.

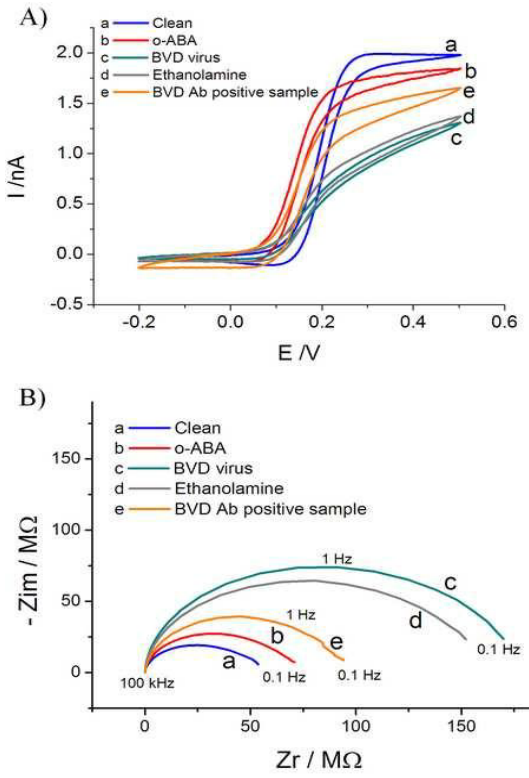

Figure 5: (A) Cyclic Voltammograms and (B) Nyquist plots obtained of (a) pristine gold nanowire electrode;(b) o-ABA modified electrode; (c) BVD virus (100 µg/mL) modified electrode; (d) ethanolamine blocked electrode; (e) Antibody BVD binding in 1% bovine serum positive. Solution composition: 1 mMFcCOOH in 10 mM PBS. CV scan rate: 100 mVs^{-1}. EIS frequency range: 0.1 Hz to 100 kHz; E=150 mV and ΔV=5 mV.

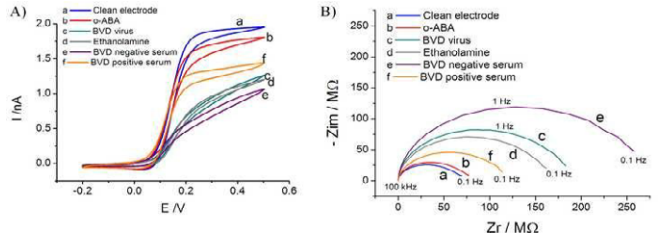

Figure 6: (A) Cyclic Voltammograms and (B) Nyquistplots of (a) pristine gold nanowire electrode; (b) o-ABA modified electrode; (c) BVD virus (10 µg/mL) modified electrode; (d) ethanolamine blocked electrode;(e) negative BVD Antibody serum; (f) positive BVD Antibody serum. Solution: 1 mMFcCOOH in 10 mM PBS. CV scan rate: 100 mVs^{-1}. EIS frequency range: 0.1 Hz to 100 kHz; E=150 mV and ΔV=5 mV.

5B, curve e). These changes are attributed to immunologic binding of BVD antibodies present in the serum to the virus immobilized on the nanowire.

To confirm these results, control experiments were undertaken using BVD antibody negative serum samples. Following these control experiments, serum positive infected samples aliquots were then dispensed on the sensors to (i) confirm bio-functionality and efficacy of the virus capture probe and (ii) demonstrate the specificity of the sensor against BVD antibodies. Figure 6 presents typical CV and EIS obtained first for BVD antibody negative and then for positive serum samples on the same immunosensor. Figure 6 presents typical CV and EIS obtained first for BVD antibody negative and then for positive serum samples on the same immunosensor.

A decrease in charge transfer and an increase in capacitance are again observed, following covalent coupling of the BVD virus to the gold nanowire and blocking with ethanolamine, compared to pristine gold nanowire electrode (Figures 6A and 6B, curves a-d). Incubation of negative infected serum sample leads to a decrease in the current intensity (Figure 6A, curve e) and an increase in the impedance (Figure 6B, curve e) indicating a further reduction of electron transfer (versus the ethanolamine curve). This result is contrary to those observed during BVD Ab binding experiments and suggests the formation of a more insulating layer on the electrode further preventing FcCOOH molecules from reaching the electrode surface.

Immediately after these assays, BVD antibody positive serum was deposited onto nanowire immunosensor and allowed to incubate for 20 min. An increase in the current intensity and a decrease in the impedance (Figures 6A and 6B, curve f), consistent with results presented in Figures 4 and 5, are observed following binding of BVD antibodies present in serum samples to the immobilized virus. These results strongly suggest that the observed changes in both CV and nyquist spectra are attributed to the presence of BVD antibody in sera sample. Furthermore, it strengthens the arguments of re-orientation or re-arrangement of the virus layer allowing ingress of FcCOOH molecules to the electrode when BVD antibodies are bound. Finally, this result highlights the fact that immobilized virus are fully functional, not degraded during the experiments and demonstrate the specificity of the sensors to the BVD antibodies. In all cases, electrochemical results were in full agreement with results obtained from the commercial ELISA kit.

The electrochemical based immunologic detection of BVD antibodies in 1% serum in 20 min demonstrates the suitability of this sensor device for potential use in future on-farm diagnostic devices and opens the door for detection of a wide variety of other diseases.

Conclusion

This paper presents the first electrochemical-based on-chip nanowire immunosensor device. Using an electrodeposited o-ABA to covalently immobilize the capture biomolecules, electrochemical immunosensing (10 µg/mL) on the on-chip nanowire is validated in buffer using first a model BSA antigen-antibody and then BVD virus-antibody. The nanowire-based BDV virus immunosensor allows the specific detection of BDV antibodies in serum in 20 min permitting the discrimination between BVD infected and non-infected bovine sera obtained from cattle. Work is still ongoing to optimize the modification process to increase the performance of the immunosensor.

Acknowlegement

The authors would like to thank Caoimhe Robinson for undertaking ELISA experiments. This work was supported by Science Foundation Ireland under the US-Ireland "Agri-Sense" project (12/US/I2476) and the Irish Higher Education Authority PRTLI programs (Cycle 3 "Nanoscience" and Cycle 4 "INSPIRE").

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