

Development of a highly sensitive, field operable biosensor for serological studies of Ebola virus in central Africa

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Received 16 April 2006; accepted 3 July 2006

Available online 14 August 2006

Abstract

We describe herein a newly developed optical immunosensor for detection of antibodies directed against antigens of the Ebola virus strains Zaire and Sudan. We employed a photo immobilization methodology based on a photoactivatable electrogenerated poly(pyrrole-benzophenone) film deposited upon an indium tin oxide (ITO) modified conductive surface fiber-optic. It was then linked to a biological receptor, Ebola virus antigen in this case, on the fiber tip through a light driven reaction. The photochemically modified optical fibers were tested as an immunosensor for detection of antibodies against Ebola virus, in animal and human sera, by use of a coupled chemiluminescent reaction. The immunosensor was tested for sensitivity, specificity, and compared to standard chemiluminescent ELISA under the same conditions. The analyte, anti-Ebola IgG, was detected at a low titer of 1:960,000 and 1:1,000,000 for subtypes Zaire and Sudan, respectively. While the same serum tested by ELISA was one order (24 times) less sensitive.

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Keywords: Optical fibers; Immunosensor; Ebola virus; Poly(pyrrole-benzophenone); Electropolymerization

1. Introduction

Infection with Ebola virus causes an acute hemorrhagic fever in human and some non-human primates resulting in high mortality rates. There are four species of Ebola virus that have been identified: Zaire, Sudan, Ivory Coast, and Reston. All four species differ in their virulence; thus acute mortality in human outbreaks is typically greater than 80% for Ebola virus Zaire strain and greater than 50% for Sudan. Ebola Zaire and Sudan viruses are the most widespread and have been responsible for the majority of the recent hemorrhagic fever outbreaks in humans [1–3]. The last large outbreak occurred in 2000–2001

in the Gulu district of Uganda. In total, there were 425 cases with 224 deaths. This case fatality rate of 53% was similar to that observed for two previous outbreaks of Ebola virus Sudan in 1976 and 1979 in which 53% and 66% of cases died, respectively [4–6].

Ebola virus has affected populations primarily throughout central Africa. Although it has only affected a limited geographic region its effects have reverberated world-wide due to its extremely high mortality rate. In addition, since the reservoir for this virus has not been identified it has evoked much fear in populations living outside central Africa and has become a biodefense concern [1,2,7].

The ELISA was proved as a more sensitive and specific test for Ebola virus than the indirect fluorescent antibody test (IFAT) [8]. Therefore the ELISA has been the most commonly used serologic technique for Ebola virus serology studies [3–5,8–11].

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Recently other assays for Ebola virus have been developed and described. They include reverse transcriptase polymerase chain reaction (RT-PCR) [12,13], antibody phage indicator assay [14], and immunohistochemical assay [15].

In this study we present a newly developed optical immunosensor for detection of antibodies to Ebola virus strains Zaire and Sudan, by using a photoimmobilization methodology based on a photoactivable electrogenerated polymer film. This newly developed immunosensor relies on luminescence-based technology that couples antigen targets to a fiber-optic and employs chemiluminescence for detection of minute quantities of specific antibodies.

In the field of optical fiber immunosensors, we have previously demonstrated that optical fibers, which are made of doped silica and hence are electrically inert, can be modified by an electrically conductive layer [16–19]. This fiber-optic electroconductive surface modification was achieved by deposition of a thin layer of indium tin oxide (ITO). ITO is an indium oxide (In_2O_3)-based material that has been doped with tin oxide (SnO_2) to improve its electrical quality. Tin acts as a cationic dopant in the In_2O_3 lattice and substitutes indium sites to bind with interstitial oxygen. The presence of SnO_2 results in n doping of the lattice because the dopant adds electrons to the conduction band [20]. We must take care to produce thinner films of indium tin oxide even though there is a correlation between thicker films and lower resistivity, as lower light transmission may occur due to absorption by the film [21]. Since sputtering is one of the most extensively used techniques for deposition of transparent conducting oxide films, it was selected as the deposition technique. This method renders far better control over the stoichiometry, obviating, in most cases, the heat treatment step, which is usually performed after deposition of the film. Highly transparent and conductive films of ITO have been previously deposited by this technique [16,22].

After deposition of the ITO film, monomers of pyrrole-benzophenone were electropolymerized onto the conductive metal oxide surface. The advantage of the electrochemical polymerization method is that the film can be prepared easily in a rapid, reproducible, and well controlled one-step procedure, that enables production of a thin homogeneous layer with well defined thickness of the desired polymer [18,23]. Modification of an optical fiber with pyrrole-benzophenone film allows the photoactivable linking of a biological receptor, inactivated Ebola virus, to the tip of the fiber-optic surface (Fig. 1). This innovative photoelectrochemical method for immobilization of

biological macromolecules combines the advantages of photolithography with those of the electrochemical deposition of polymer films.

The immunosensor we constructed was tested for sensitivity, specificity, and compared to standard luminescence ELISA under the same conditions. Our results suggest that the detection of antibodies to the Ebola virus using our newly developed immunosensor will contribute significantly to serological and epidemiological studies in central Africa by increasing the sensitivity of the tests dramatically. When modified into an “easy-to-use” procedure, this technology might be used in the future in a field operable clinical tool for Ebola virus antibody screening. In addition, our newly developed fiber-optic immunosensor can be reversed and transformed into an antigen detection biosensor for viral agents.

The production of an ultrasensitive immuno-biosensor for the Ebola virus is indeed extremely important since: (1) the reservoir of this virus is unknown and it can therefore suddenly reemerge from the environment; (2) the virus has an extremely high mortality rate and it is therefore important to diagnose potential victims as soon as possible; (3) the development of an environmental sensor for Ebola is important as an early warning of a potential outbreak, especially since Ebola is a biodefense concern [1]. We present herein a study that employs an immunosensor for evaluation of sera samples obtained in the field from survivors of Ebola and close contacts. This work will serve as a blueprint for future biosensors, especially viral biosensors, and is important for biodefense and control of this and other viruses.

2. Experimental

2.1. Reagents

The pyrrole monomer with a benzophenone functional group was prepared as previously described [23]. Acetonitrile, 97% (CP, BioLab Ltd.), and lithium perchlorate (99.99%, Aldrich) were used as received for electropolymerization.

Bovine serum albumin (BSA, A4503, fraction V) and polyoxyethylene-sorbitan monolaurate (Tween[®] 20, P7949) were purchased from Sigma.

Luminescence measurements were carried out using the Western blot chemiluminescence reagent plus kit from NENTM Life Science Products (NEL105, containing enhanced luminol reagent and oxidizing reagent).

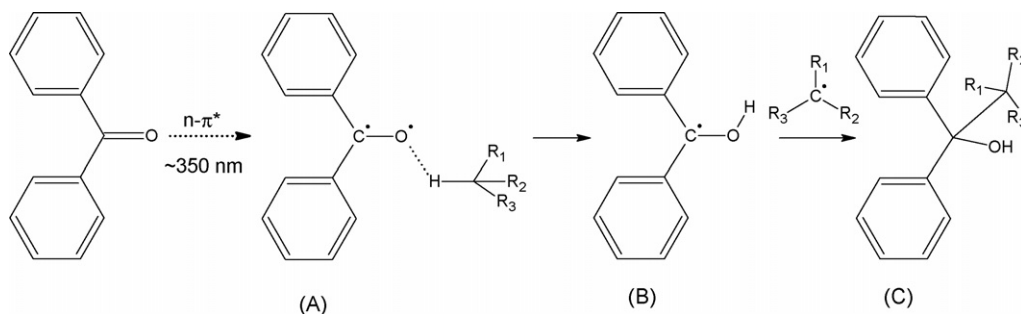


Fig. 1. A mechanism for photochemical reaction of benzophenone with a C–H bond of an amino acid side chain.

2.2. Virus growth and antigen preparation

All work with infectious Ebola viruses was performed in the BSL-4 facility of the Institute of Virology, Philipps University of Marburg, Germany, by Elke Mühlberger and the BSL-4 laboratory at Vector Laboratories, Koltsovo, Novosibirsk, by Alex Chepurnov. For preparation of viral stocks, vero cells were infected with Ebola virus Sudan, strain Maridi, or Ebola virus Zaire, strain Mayinga, respectively, at an m.o.i. of 0.1 pfu per cell. Following an adsorption period of 1 h, cells were incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% (v/v) fetal calf serum for 3 days at 37 °C, 5% CO₂. Subsequently, culture fluid was clarified by centrifugation in a minifuge at 6000 rpm for 10 min at 4 °C and used either as virus inoculum or further purified for isolation of antigen.

Clarified culture supernatant was centrifuged through a 20% (w/v) sucrose cushion in TNE (16.67% (v/v) of 1.0 M Tris–HCl (pH 7.5) and 3.3% (v/v) of 0.5 M EDTA in 5 M NaCl) buffer at 25,000 rpm for 2 h at 4 °C in a SW28 rotor (Beckmann). Pelleted virus was resuspended in TNE and further purified by gradient centrifugation through 0–40% (w/v) potassium tartrate, 30–0% (w/v) glycerol in TNE at 27,000 rpm for 16 h at 4 °C. The virus band is isolated, diluted in TNE, and pelleted by centrifugation at 38,000 rpm for 20 min at 4 °C in a SW40 rotor (Beckmann). The virus pellet is resuspended in phosphate buffered saline (PBS). For the immunosensor assay and ELISA tests the virus was inactivated with 1% (w/v) SDS and boiled according to standard techniques [1]. This inactivated viral preparation was the Ebola antigen used for all our assays.

2.3. Sera

The sera of Ebola immunized animals, provided by Dr. Alex Chepurnov (Vector Laboratory, Koltsovo, Novosibirsk), was used for IgG tests on the optical fiber biosensor and comparative chemiluminescence ELISA. For Ebola virus Zaire and Sudan subtypes, goat and rabbit anti-Ebola antisera were used, respectively. The human sera samples were taken from 10 survivors (in 2004) of the Ebola Sudan outbreak in Gulu, Uganda 2000–2001 and from 10 healthy volunteers that live in this district but were not clinically ill with Ebola. All patients were healthy at the time of phlebotomy. All animal sera tested negative for Ebola virus and human survivors were healthy at the time of phlebotomy and tested negative for the Ebola virus by PCR.

Horseradish peroxidase (HRP)-labeled polyclonal secondary antibodies were used for sera antibody detection. Goat anti-rabbit IgG conjugated HRP (A-6154, Sigma) and donkey anti-goat IgG conjugated HRP (Jackson Laboratories) were used for anti-Ebola Sudan and Zaire calibration curve construction, respectively. For human samples, testing was performed with goat anti-human IgG conjugated HRP secondary antibody (Jackson Laboratories).

2.4. ELISA detection of antibodies to Ebola virus

ELISA was performed according to standard procedures. A volume of 75 µl of inactivated viral preparation diluted 1:400

(this material was used for all our assays), was added to each well of a 96-well microtiter plate (MaxiSorp, Nunc). The plate was sealed and allowed to incubate overnight at 4 °C. After incubation, the coating solution was removed and the plate was washed in PBS (pH 7.2). A blocking step involved adding 50 µl per well of a blocking solution (BSA 5% (w/v) in PBS (pH 7.2)), which reduced overall background and increased the sensitivity of the assay. The plate was then incubated for 1 h at 37 °C and the wells washed thrice with PBS Tween[®] 20, 0.1% (v/v) (PBS-T), pH 7.2. Then 75 µl per well of diluted serum solutions (at dilutions ranging from 1:500 to 1:960,000 for Ebola Zaire strain and from 1:500 to 1:1,000,000 for Ebola virus Sudan) were added, along with control serum samples, in triplicate and the plate incubated 1 h at 37 °C. Additional empty wells and non-coated wells were used to check the level of the background signal. The wells were then washed thrice with PBS-T and 75 µl per well of a secondary antibody solution was added and the plate incubated 1 h at 37 °C. Donkey anti-goat IgG and goat anti-rabbit IgG antibodies labeled HRP were used for the Ebola virus Zaire and Sudan ELISA, respectively. After incubation, the plate was washed three times with PBS-T and then read in a luminometer.

The microtiter plate was read with a standard luminometer (Thermolabsystems-Luminoskan Ascent 2.5) where, before each well measurement, oxidizing reagent and enhanced luminol reagent solutions were injected into the well with an automatic dispenser in a 1:1 ratio. A total of 20 measurements were performed in a kinetic measurement type with a gap of 1 s between each reading. The data were collected with the Luminoskan Ascent Software (version 2.6); the mean and the standard deviation of the triplicates were calculated for each point.

2.5. Optical fiber tip preparation

All fibers used were SFS400/440B silica fibers (Fiberguide Industries, NJ, USA) with an original numerical aperture (NA) of 0.22. Their core was 400 µm in diameter (refractive index of 1.457 at 633 nm) and was surrounded by a 40 µm silica cladding (refractive index of 1.44 at 633 nm), followed by a 150 µm thick silicon buffer and finally a 210 µm thick black TEFZEL jacket. The detailed protocol of the optical fiber tip preparation is described by Konry et al. [18].

2.6. Indium tin oxide sputtering

The depositions were carried out in a radio frequency (r.f.)—sputtering Varian system. The sputtering target was a 4 in. diameter circular disk of hot pressed powder 99.999% purity ITO (90% wt. In₂O₃ + 10% wt. SnO₂), made by Testbourne Ltd. (Hampshire, UK). The sputtering chamber was evacuated to a pressure lower than 10^{−6} Torr. Argon and oxygen gases were introduced into the sputtering chamber with partial pressures of 2 × 10^{−3} and 2.5 × 10^{−4} Torr, respectively. The r.f. supply was then switched on and stabilized to a power of 400 W. The fiber tips were exposed to the sputter beam for 1–1.5 h. As a result, the fiber core end-face (the important area where electro-polymerization must occur) and at least one side

of the cylindrical surface (for contacting and voltage supply) were covered with the ITO coating.

2.7. Electropolymerization of pyrrole-benzophenone on the optical fiber surface

All electrochemical experiments were performed with an Electrochemical Instruments Autolab with PGSTAT30 (Eco Chemie BV) operated by General Purpose Electrochemical System (GPES) for Windows (version 2.4) software. The electropolymerization of pyrrole monomers and characterization of the resulting polymers on the modified electrode surfaces was carried out in a conventional three-electrode cell at room temperature. The poly-pyrrole films were prepared by controlled potential oxidation of monomers (2 mM) in 0.1 M LiClO₄ in CH₃CN. The working electrode was the ITO coated optical fiber; an electrical contact was established with a steel wire above the acetonitrile electrolyte solution. A platinum wire (50 μ m diameter) was used as a counter electrode and Ag/Ag⁺ of 10 mM AgNO₃ in electrolyte solution was used as a reference electrode. Electropolymerization of pyrrole-benzophenone was accomplished by controlled potential at 0.85 V until the appearance of the characteristic apex in an amperometric plot.

2.8. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) micrographs were taken by a JEOK JSM 7400F system with 129 eV resolution of detector and exposure time of 100 s. The specimens were analyzed using energy dispersive X-ray spectrometry microanalysis analytical equipment installed in the scanning electron microscopy.

2.9. Immobilization of antigen

The optical fibers coated with poly(pyrrole-benzophenone) were soaked in diluted solution containing inactivated Ebola virus antigen (approximately 7.5 μ g/ml, the concentration was determined by Micro BCA Protein assay kit, PIERCE) and irradiated with UV light. Benzophenone and most of its derivatives absorb a photon at around 345 nm resulting in the promotion of one electron from a nonbonding sp²-like n-orbital on oxygen to an antibonding π^* -orbital of carbonyl group. The actual electron-deficient oxygen n-orbital becomes electrophilic and therefore interacts with weak C–H σ -bonds, resulting in a hydrogen abstraction to complete the half-filled n-orbital. When amines or similar heteroatoms are in the vicinity of the excited carbonyl, an electron-transfer step may occur, followed by proton abstraction from an adjacent group. In biological systems, the most effective H-donors include backbone C–H bonds in amino acids; thus, methylene groups of amino acid side chains are good candidates providing abstractable hydrogens through the general mechanism shown in Fig. 1 [24].

2.10. Immunoassay rationale and design

The steps carried out for the construction of the immunosensor are visualized in Fig. 2. A poly-pyrrole with a benzophenone functional group was applied for photo immobilization of viral antigens on the optical fiber tip surface. In order to produce the desired activation radiation we used a 1000 W Xe lamp (Oriel 6271) mount connected to a light condenser (Oriel 66021). The light was reflected through a dichroic mirror (Oriel 66226). The spectrum radiation was then condensed into the monochromator (Oriel 77250) using the appropriate lenses (Oriel, plano-convex

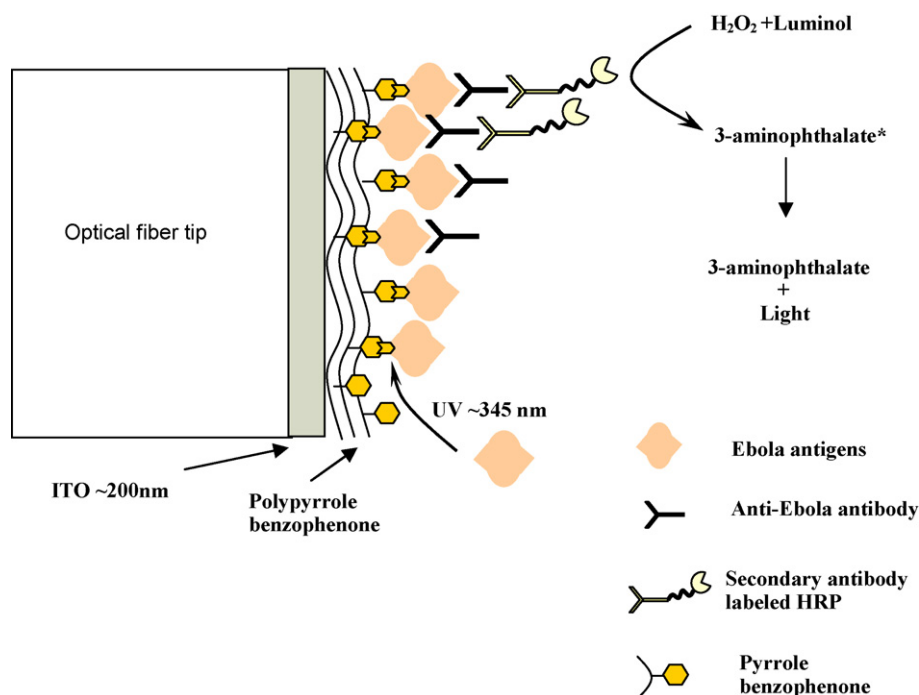


Fig. 2. The biosensor scheme describing the various steps involved in the immunoassay using ITO–poly(pyrrole-benzophenone)-coated optical fibers for the detection of anti-Ebola virus in sera samples.

lenses). After this, a 345 nm wavelength light output, with a light intensity of 80 mW cm^{-2} (as measured by an Ophir Optonics power-meter Nova reader, PD300-UV) was projected for 7 min into the far end of the optical fiber that was coated at its near-end with poly(pyrrole-benzophenone) and soaked in 120 μl of an inactivated Ebola virus solution during irradiation. The excited benzophenone radicals could then bind to neighboring proteins in the solution, in our case Ebola virus antigens (approximately $7.5 \mu\text{g/ml}$).

Later, the optical fibers were rinsed profusely and washed with PBS-T, pH 7.4 and transferred to blocking solution (5% (w/v) BSA in PBS-T) for 30 min to prevent the nonspecific binding of anti-Ebola IgG. The fibers were then rinsed and washed with PBS-T for 10 min, before being incubated in 120 μl of antibody solution for 20 min. Next the antigen conjugated fibers were placed in diluted serum solutions, ranging from 1:960,000 for Ebola virus Zaire and from 1:1,000,000 for Sudan up to 1:500. Sets of three replicas were prepared for each concentration measured and a series of fibers were set aside for use as controls. Thereafter, all fibers were rinsed and washed once with PBS-T for 10 min. Subsequently, the optical fiber tips were dipped into 120 μl of a solution containing the HRP labeled secondary antibody for 20 min, and finally, rinsed and washed once with PBS-T for 10 min. All materials were disposed according to standard biosafety regulations.

2.11. Chemiluminescence measurements

The instrument set-up for the chemiluminescence measurements has been previously described [25]. A Hamamatsu HC135-01 Photo Multiplier Tube (PMT) Sensor module was used for chemiluminescence measurements, combining the sensitivity of a photomultiplier tube with the intelligence of a microcontroller. The detector was optimized to the blue light region and included a 21 mm diameter active area convenient for gathering light radiation without any optical focusing elements for luminescence measurements. The instrument set-up and the chemiluminescence's procedure were fully described by Konry et al. [18].

3. Results and discussion

3.1. Immunosensor behavior and comparison with ELISA for Zaire strain of Ebola virus

The detection of anti-Ebola virus antibodies was achieved using a sandwich fiber-optic immunoassay (Fig. 2). For this purpose the prepared tip of an optical fiber was coated with an ITO transparent semiconductor film that afterwards enables electropolymerization of the photoactive polymer—poly(pyrrole-benzophenone) and photoimmobilization of viral antigens. Fig. 3 shows SEM micrographs of ITO coated optic fiber tip conjugated with poly(pyrrole-benzophenone) film.

Animal anti-Ebola virus antisera were serially diluted down to 10^{-6} and a fiber-optic luminescence assay was performed along with a standard chemiluminescence ELISA for comparison. The subsequent binding of peroxidase-labeled secondary

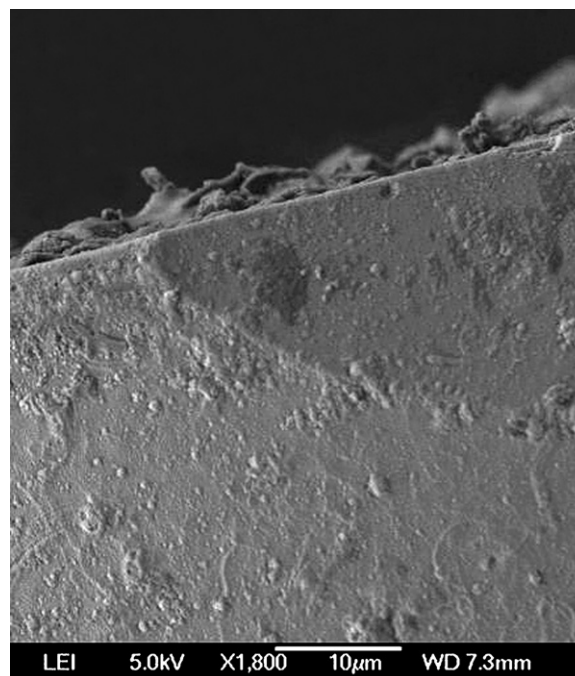


Fig. 3. SEM micrograph of poly(pyrrole-benzophenone) film formed on an ITO-coated fiber-optic.

antibodies enabled detection via a chemiluminescence reaction by adding an enhanced luminol solution. The light, emitted as a side reaction, is transduced by the optical fiber to the measuring instrument.

The standard curve for the goat anti-Ebola subtype Zaire sandwich immunoassays was constructed by collecting data in triplicate at sera titers ranging from 1:500 to 1:960,000. Fig. 4 shows the typical sigmoidal behavior for the standard calibration curve. For the characterization of the immunosensor calibration curve, the linear range curve fit was carried out using the equation of the form $y = A + Bx$, where x is the dilution of serum sample and y is the corresponding response signal obtained. The dynamic linear range of the curve most useful in the quantization of titers was found to be from 1:1000 to 1:80,000, exhibiting in this range an acceptable square correlation coefficient, $R^2 = 0.94$ and a satisfactory sensitivity of 6219.4 photocounts/s

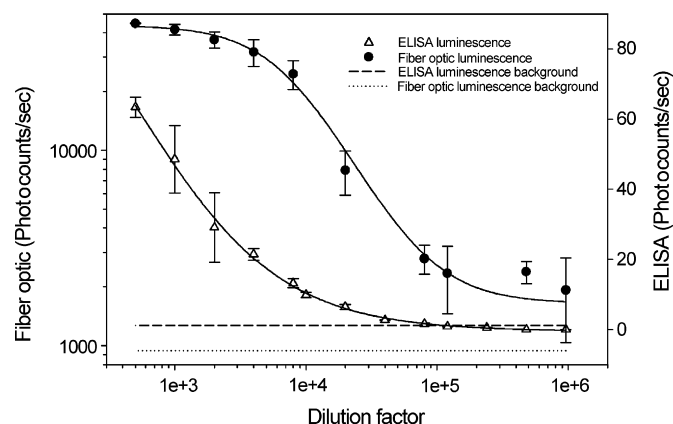


Fig. 4. The calibration curve obtained from the optical fiber immunosensor and ELISA for the detection of anti-Ebola subtype Zaire antibodies.

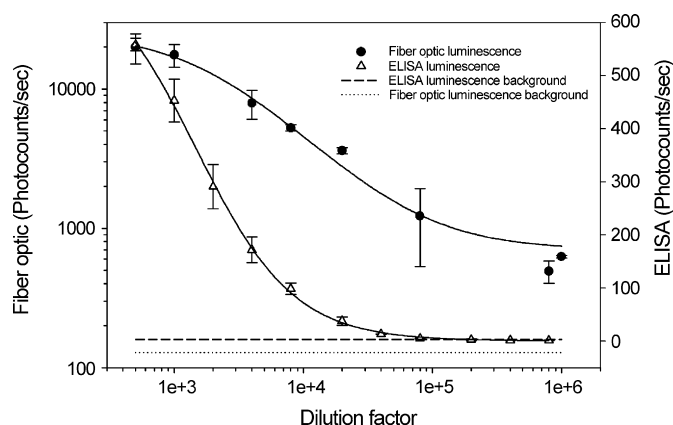


Fig. 5. The calibration curve obtained from the optical fiber immunosensor and ELISA for the detection of anti-Ebola subtype Sudan antibodies.

(determined within the linear range of the biosensor as the slope B , of the calibration curve). At higher concentrations, the curve levels off with a response saturation observed from titers 1:1000 and up. As small inaccuracies in such measurements occur at such low dilutions, these measurements can lead to large errors in prediction, so such samples should be diluted for accurate quantization.

The lower detection limit of the immunosensor, defined as the amount (or concentration) of the analyte that gives a response (YDL) that is significantly different, is three standard deviations (SDBR) from the background of the analysis that is itself obtained from negative sera (YBR). Therefore, the lower detection limit (YLDL) was calculated using Eq. (1):

$$\text{YLDL} > \text{YDL} \quad (1)$$

The background signal recorded for the blank (in the absence of the analyte) and its calculated standard deviation enable quantization with dilutions as high as 1:960,000 for the immunosensor and 1:40,000 for the ELISA. Tests of samples were performed in triplicate and under identical conditions, with the same materials, in both immunosensor and ELISA assays. Therefore, this enables a direct comparison of results, and reveals that the immunosensor is much more sensitive than chemiluminescence ELISA and is likely capable of recognizing virus specific antibody presence in the sera of patients in earlier stages of disease. Finally, the optical fiber immunoassay showed a greater lower detection limit than ELISA. Since ELISA uses absorption as the immobilization methodology for the antigen, a change in protein conformation may occur. Therefore the loss of putative epitopic binding sites would reduce their potential for binding to antibodies, while the covalent immobilization would offer a reduction in such a loss.

3.2. Immunosensor behavior and comparison with ELISA for Sudan strain of Ebola virus

The same experiment was repeated with the Sudan subtype of Ebola virus (Fig. 5). In this case diluted sera of rabbits immunized with Ebola virus Sudan was used as the analyte for antibody detection. As expected, the behavior of the assay was

similar to the first one. The linear range fit for the immunosensor curve was also found to be in the titer range between 1:1000 and 1:80,000 with an acceptable square correlation coefficient $R^2 = 0.95$ and a satisfactory sensitivity of 3208.4 photocounts/s (determined within the linear range of the biosensor as the slope B , of the calibration curve). The detection limit of the immunosensor for the Sudan strain of the virus was calculated using the same statistics methods as in the previous case.

3.3. The advantages of the immunosensor

The very low background signals allow measurements of very low concentrations of Ebola specific antibodies in the analyte solution. This is significant as following the emergence of Ebola hemorrhagic fever there is an undetectable or very negligible elevation in the concentration of the IgG antibodies in the sera using conventional techniques. As a result, the immunosensor has great potential for clinical and serological studies of Ebola hemorrhagic fever. In addition, the fiber-optic immunosensor assay is less time consuming than standard ELISA (approximately 2 h versus 4 h, respectively, after antigen binding, thus constituting a real gain in time for high-throughput diagnosis). Therefore the immunosensor will enable an earlier detection of the production of antibodies, whereas the standard ELISA will only detect them at a more advanced stage, when the level of the antibodies reaches a high concentration [3,8,26–28].

To assess the precision of our technique, the coefficient of variance (CV) was calculated for the replicates measured in triplicates. The fiber-optic biosensor shows a lower CV percentage and better precision between replicates, than does the ELISA assay for nearly all the presented ranges of concentrations used.

In addition, the natural host for Ebola virus, presumed to be an animal, has not been identified [1,29,30]. A straightforward approach that might be used to determine animal contact with the Ebola virus, or identification of an animal reservoir, is to assess the presence of specific antibodies in the serum. Finally, many studies have demonstrated the long-term persistence of IgG antibodies against filoviruses in sera, which suggests that this new serologic tool will be useful for field studies of the Ebola virus [3,8].

3.4. Cross-reactivity between strains

There is some evidence that some cross-reactivity between Ebola subtypes is observed in ELISA assay tests [14,31,32]. Fig. 6 demonstrates the serological cross-reactivity between two different Ebola strains, Zaire and Sudan, using the optical fiber immunosensor. In this experiment immobilized antigen of Sudan strain was placed in a serum sample of goat immunized against the Zaire strain of Ebola virus and vice versa. The signals received under such condition were very low and close to the background of the assay, however they indicate a true cross-reactivity. This data demonstrates the high sensitivity of the biosensor and its ability to identify very low levels of cross-reactivity between the two strains of the Ebola virus, Zaire and Sudan.

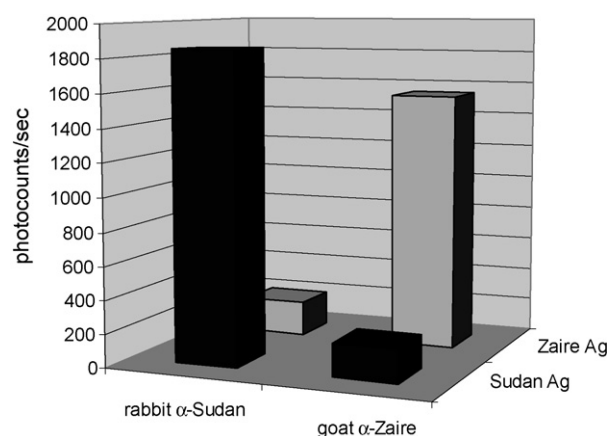


Fig. 6. Examination of cross-reactivity between two different strains, Zaire and Sudan, of Ebola virus using the optical fiber immunosensor.

3.5. Serological study

To demonstrate further the practical application of the immunosensor, we tested the human sera samples from the survivors of the outbreak in the Gulu district of Uganda, 2000–2001. All sera samples, 10 from Ebola survivors and 10 from healthy volunteers serums from the same geographical region, were initially checked by PCR and found to be currently negative for the virus. As a valid negative control for this test we used the serum of a healthy individual from Israel, and in addition, the sera samples of healthy volunteers from the Gulu district of Uganda. Although these data are limited by the number of sera samples available for verification, one of the healthy volunteers from the same geographical region showed a positive reaction to inactivated Ebola virus when tested with the immunosensor. An ELISA test for the same person was negative due to relatively low sensitivity of the method as was revealed in standard calibration curves for ELISA and immunosensor (Figs. 4 and 5). Also due to limited number of sera samples, the one negative serum (in ELISA) that was converted to positive by our method could be a false positive result.

This data may indicate the presence of IgG antibodies in a healthy individual due to exposure to antigen in an endemic area without any clinical evidence of infection (or perhaps a subclinical case). In addition, the prevalence of this seropositivity within the population, in areas of prior epidemics, could be found among the latest surveys performed using ELISA assays [8,9,33]. Using a more sensitive detection method, such as the immunosensor presented herein, will enable serological studies among the population of endemic areas that may strengthen the hypothesis that one can develop some immunity to the Ebola virus without developing clinical illness. Consequently the Ebola virus might leave its footprint in some individuals without causing an active infection. Finally, our sensitive immunosensor can be used effectively for tracking the reservoir of Ebola.

4. Conclusions

We have demonstrated herein that one can electropolymerize the conductive surface of a fiber-optic tip with a photosensitive

layer, which will enable a one-step immobilization procedure of proteinaceous biospecific entities such as inactivated Ebola virus. The model analyte, anti-Ebola IgG, was detected at a low titer of 1:960,000 and 1:1,000,000 for subtypes Zaire and Sudan, respectively. Even though our ability to evaluate the sera of known infections with this group of viruses was limited by the availability of appropriate sera, we believe that the results are encouraging and strongly suggest that this immunosensor will prove a preferable alternative to ELISA for measurement of antibodies to Ebola viruses.

Acknowledgments

The authors thank the North Atlantic Treaty Organization (NATO), Yakov Yuzhelevski from the Department of Physics at BGU for his technical assistance in running the ITO r.f. sputtering instrumentation, and Luba Burlaka from the Center for Meso, Nanoscale Science and Technology, Department of Chemical Engineering (BGU) that carried out the SEM measurement.

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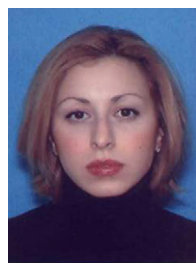
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Biographies



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Dr. Ilya Trakht, PhD, is an accomplished scientist, with extensive experience in immunology and biochemistry. Dr. Trakht is a world leading expert in developing technologies for the immortalization and proliferation of human immune cells through hu-hybridoma technology. Dr. Trakht has been conducting his research at Columbia University since 1990. Prior to coming to the United States, Dr. Trakht was a Senior Research Scientist in the Laboratory of Molecular and Cellular Cardiology at the USSR Cardiology Research Center, Moscow. Dr. Trakht has a DSc in immunology from the Institute of Cardiology, USSR Cardiology Center and a PhD in Biochemistry from the Institute of Molecular Biology, USSR Academy of Sciences.



Dr. Julius J. Lutwama, PhD, FRES, is a Senior Research Officer in the Department of Arbovirology at Uganda Virus Research Institute; Dr. Lutwama is trained as an Entomologist and in arbovirological techniques. His research work involves outbreak investigations and confirmation, and routine arbovirus investigations. He has over 25 publications in International and National peer reviewed journals.



Dr. Elly B. Rwaguma has a Master of Veterinary Medicine degree and is the Head of the Arbovirology Department at Uganda Virus Research Institute. He is an Arbovirologist and carries out research investigations on arbovirus outbreaks from which he has several publications in International and National peer reviewed Journals. He has been involved in outbreak investigations including the Ebola outbreak in Uganda in 2000.

Dr. Alex Chepurnov, PhD, heads the Biosafety Level 4 Virus Laboratory at Russia's State Research Center of Virology and Biotechnology in Novosibirsk, commonly referred to world-wide as “Vector”. He is one of the leading Russian experts on the Ebola virus and has studied the host response to this virus as well as methods for treatment. Most of his own research focuses on Ebola Zaire, the deadliest strain of Ebola.



Dr. Elke Mühlberger received her PhD in Biological Science with qualification for tenure professorship in Virology from the University of Marburg, Germany. Currently she works as Assistant Professor and group leader of the Department of Virology in University of Marburg, Germany. Her current research is concentrated on replication and transcription of filoviruses, host–virus interactions of filoviruses and interaction of SARS–coronavirus with the type I interferon system.



Dr. Leslie Lobel, MD PhD, a graduate of Columbia College of Columbia University where he received BA degree summa cum laude. His graduate education was in the Medical Scientist Training program at the College of Physicians and Surgeons of Columbia University. He graduated with the MD, PhD degrees in 1988. Dr. Lobel is now in the Department of Virology in the Faculty of Health Sciences of Ben Gurion University of the Negev in Israel. His current work focuses on immunovirology and he studies the human humoral immune response to a variety of infectious diseases that include smallpox,

Hepatitis C, Ebola and West Nile virus. He has isolated a library of fully human monoclonal antibodies from patients immunized against smallpox and from patients that were infected with Hepatitis C. Currently Dr. Lobel focuses on more deadly diseases, such as Ebola, for development of immunotherapeutic tools and synthetic vaccines.



Dr. Robert S. Marks received his BSc in Biological Sciences and his MSc in Cell Biology and Physiology from the University of California, Santa Barbara, USA. He then completed his PhD in Chemical Immunology at the Weizmann Institute of Science, Rehovot, Israel, in 1992 on an enhanced mucosal vaccination method using silica microparticles coated with synthetic peptides. From 1992 to 1994, he was a research associate at the University of Cambridge, UK designing a fluorescent adiabatically tapered optical fiber immunosensor.

In 1995, he moved to the Ben Gurion University of the Negev, where he is now a tenured Senior Lecturer and heads a biosensor group with projects on bioluminescent whole-cell bioreporter biosensors, chemiluminescent fiber-optic immunosensors to viral antigens, phagocyte fiber-optic biosensor, amperometric biosensors, biomaterials, enzyme nanolithography and other projects.