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Immobilization of HIV GP41 antibodies on glass substrates for HIV biosensing

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ABSTRACT

Biological macromolecules such as antibodies, enzymes, proteins and aptamers have good molecular recognition ability which makes them good candidates for biosensing applications. In this study, glass substrates were treated with silane in order to immobilize HIV gp41 antibodies on their surfaces. The HIV pseudovirus was added to the treated substrates followed by addition of antibodies conjugated to nanoparticles. The surfaces were characterised by using water contact angle, atomic force microscopy (AFM) and Raman spectroscopy. Our preliminary data displayed that the antibodies were indeed immobilized on the glass substrates which made it possible for capturing the intact HIV pseudovirus. Further, Raman spectroscopy revealed the presence of disulphide bonds indicating successful conjugation of the HIV gp41 antibodies to the HIV pseudovirus.

Keywords: HIV gp41 antibodies, biosensing, glass substrates.

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1. INTRODUCTION

Acquired immune deficiency syndrome (AIDS) was first recognised in 1983 and is caused by the human immunodeficiency virus (HIV) [1]. The dreadful effects of HIV infection are known worldwide and can be considered among the greatest pandemics in human history [2]. Globally, at the end of 2018, an estimated 1.7 million people became newly infected with HIV and 37.7 million were living with HIV while 770 thousand people died of AIDS related illnesses [3]. As from June 2019, 24.5 million people infected with HIV had access to antiretroviral therapy (ART), compared to 23.3 million in June 2019. The region which is most widely affected by HIV is the Sub- Saharan Africa which accounts for approximately 70% of all HIV infection [2]. Due to the high prevalence of HIV infection and its epidemic, a large number of HIV diagnostics have been developed. The standard techniques that are used in HIV diagnostics usually include enzyme linked immunosorbent assay (ELISA), western blot technique and polymerase chain reaction (PCR) [4]. Although these techniques are sensitive and reliable, they have limitations such as being expensive, time consuming and unreliable during the window period and therefore alternative approaches need to be developed.

The use of biosensors have received a lot of attention over the past few decades because they allow for rapid, accurate and convenient testing that can be done at the point of care (POC) [5]. A biosensor has a recognition component which usually includes antibodies, enzymes, microorganisms, nucleic acids, cells, tissues and biomimetic materials. Regardless of the type of biosensor and the detection of the analyte either at solid-liquid interphase or in a solution involving the use of nanoparticles, the attachment of enzymes, antibodies, DNA or cells is inevitable [5]. Assay sensitivity of biosensors depends on the type of nanoparticles, conformation of the immobilised biomolecules such as antibodies and therefore performance of the system is greatly dependent of the surface chemistry used for immobilisation of biomolecules [6]. In this work, a silane layer was formed using (3-glycidoxypropyl)trimethoxysilane (GPTMS) for immobilizing HIV antibodies on glass substrates for HIV biosensing. The surfaces of the glass substrates were characterised by using water contact angle, atomic force microscopy (AFM) and Raman spectroscopy

2. METHODOLOGY

2.1 Cell culture

The 293T/17(ATCC, CRL, 11268) cell line was used for the production of HIV-1 Env pseudoviruses. The cells were cultured in Dulbecco's minimal essential medium (DMEM) (Sigma Aldrich, D5796) supplemented with 10% fetal bovine serum (FBS, FBS Superior, S0615) and 1% Penicillin-Streptomycin (Gibco, 15140122). Cells were incubated at 37 °C in 5% CO_2 and 85% humidity until they reached a confluency of 80%.

2.2 HIV-1 Env Pseudovirus production

The HIV1 pseudoviruses were produced by transfecting 293T/17 cells using the superfect transfection reagent as described by Malabi et al. [7] and Lugongolo et al. [8]. Briefly, a total concentration of $3x10^6$ cells were seeded in a T75 flask with 4µg of HIV-1 Plasmid and 8µg of env HIV-1 plasmid backbone. The supernatant containing pseudoviruses was harvested after 48 hours and filtered with a 0.45µm filtration system and used for the HIV biosensing experiments.

2.3 Preparation of glass substrates

Glass substrates were treated with piranha solution for 45 minutes at 75 °C in order to make the glass substrates hydrophilic. After treatment, the glass substrates were thoroughly washed is ultrapure water and blow dried using nitrogen. The glass substrates were then treated with GPTMS for 6 hours in vacuum at 80 °C. After silanization, the glass substrates were sonicated in toluene, methanol, ultrapure water and dried under a stream of nitrogen.

2.4 Immobilization of HIV antibodies

HIV antibodies were immobilized on the silanized glass substrates by incubating the glass substrates with HIV antibodies overnight. After overnight incubation, HIV pseudoviruses were added to the HIV coated glass substrates before adding HIV antibodies conjugated with nanoparticles.

2.5 Water contact angle

The water contact angle measurements were performed to measure the hydrophilic properties of the glass substrates before and after piranha treatment using the Skuur drop shape analyser. A water droplet was deposited on the glass substrates and the contact angle was measured within 5 seconds.

2.6 Atomic force microscopy

The surface morphology of glass substrates were analysed using atomic force microscopy (AFM). The AFM images were captured using a Vecco AFM system (Digital Instruments, USA) using a silicon cantilever tip. The tip has a curvature radius of 10 nm and is n doped silicon, with a resonance frequency of 204-497 kHz and a force constant of ≈10-130 N/m. The images acquired by the AFM instrument were analysed using the Nanoscope software.

2.7 Raman Spectroscopy

A schematic of the custom built Raman optical setup used to analyse the surface of the substrates is shown in figure 1. The Raman excitation source was a 527nm single mode diode laser (Evolution Nd:YLF, diode-pumped, Q-switched) of 1 KHz repetition rate with 5 µs pulse duration. The laser beam was expanded using two lenses telescope and delivered to the sample through 100X a microscope objective (NA = 1.25). A dichroic mirror was used to direct the laser beam to the back of the microscope objective lens and remove the Rayleigh scattering. The spot size of the beam at the sample was approximately 1 µm with a power of 10 mW. After passing through a notch filter (to further remove the Rayleigh scattering), the backscattered Raman signal was collected by a spectrograph (Andor Shamrock spectrometer) and a deep cooling CCD camera (Newton, Andor CCD camera) through an optical fiber. A grating with 1200 g/mm and 500 nm as blazing wavelength was chosen to disperse the Raman signal.

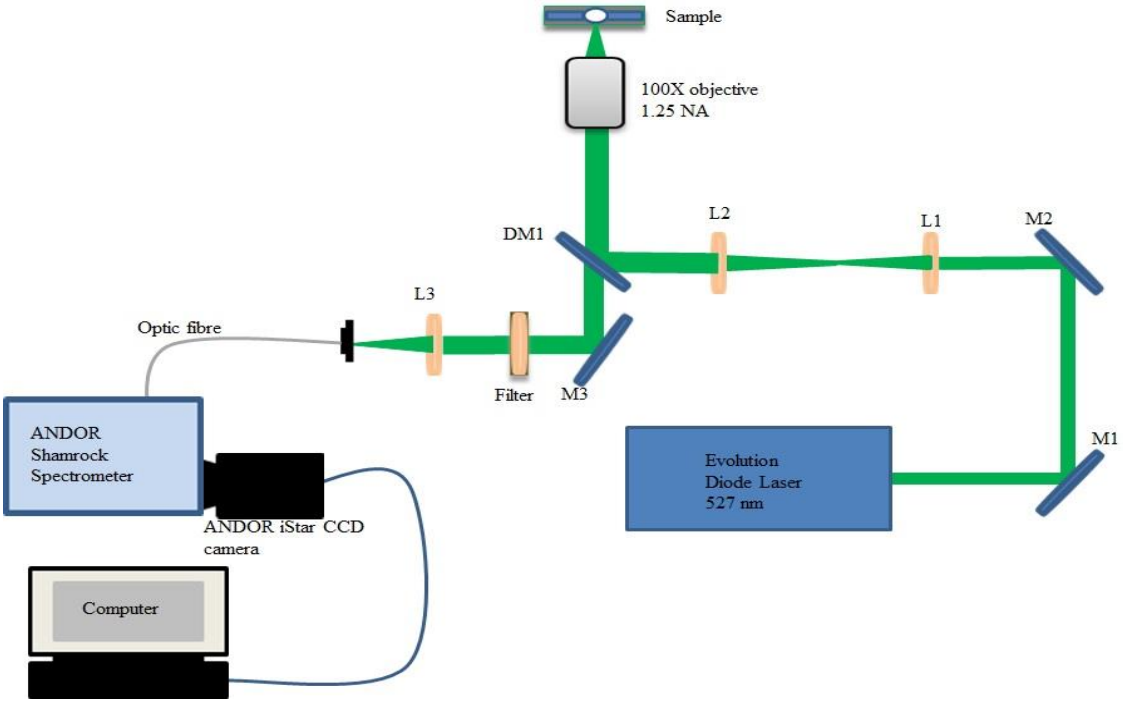

Figure 1. Schematic representation of the Raman spectroscopy set-up.

3. RESULTS AND DISCUSSION

The immobilisation of biomolecules such as antibodies on the surface of various substrates has become a subject of great interest for many scientists and researchers in the biosensing field. The chemical and physical stabilities of antibodies immobilized on substrates still cause many problems because upon immobilization, their binding activity decreases due to random orientation of the antibodies on the surface [9]. Various technologies have been developed for immobilizing antibodies on glass substrates and silanization using organosilanes offer added advantages over other technologies because of its simplicity and sensitivity. In this study, HIV antibodies were immobilized on glass substrates using GPTMS in order to capture the HIV pseudoviruses and the substrates were characterized using various techniques.

3.1 Water contact angle measurements

The contact angle measurements were performed to determine the hydrophilic properties of glass substrates before and after treatment with piranha solution. There were changes in the contact angle for the water droplet after treatment with piranha solution. Before treatment with piranha, the contact angle was 70° which is in line with the properties of a hydrophobic substrate. After treatment with piranha solution the contact angle decreased significantly and it was difficult to measure. This observation indicates that the treatment with piranha introduced hydrophilic groups to the surface of the substrates and made the glass substrate hydrophilic which is important for immobilizing biomolecules on the surface of the substrates. The results correlates with that of Karma et al.[10] where piranha solution introduced hydrophilicity on glass substrates for covalent immobilization of molecularly imprinted polymer nanoparticles using an epoxy silane.

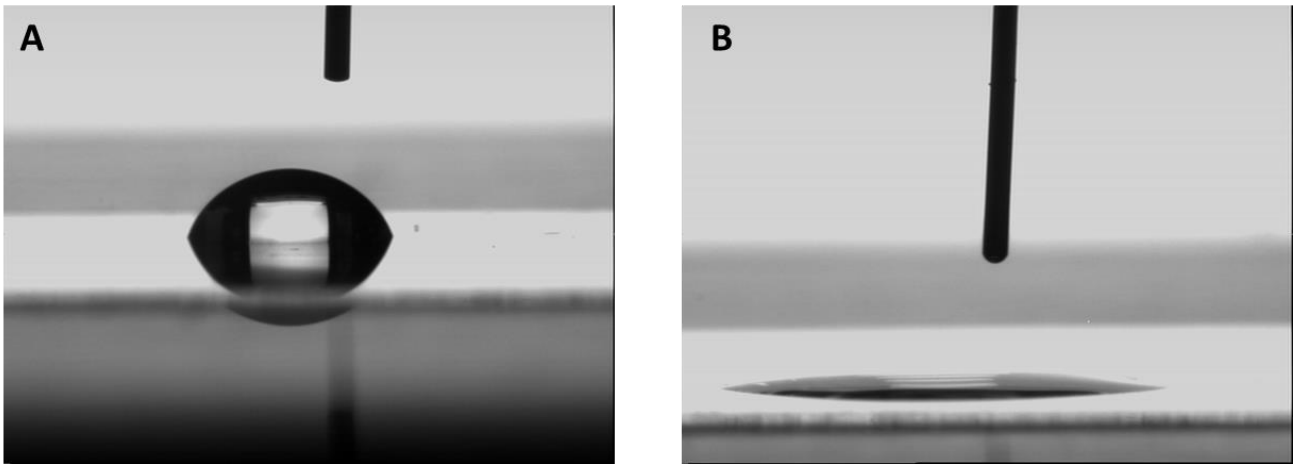

Figure 2. A display of water contact angle measure before (A) and after (B) treatment with piranha solution.

3.2 Atomic force microscopy

The images taken using AFM is shown is figure 3 and each of the scan represent a 5μMx5μm lateral area that was scanned. AFM was used to characterize surfaces that were coated with HIV antibodies, followed by addition of HIV pseudovirus and antibody conjugated to nanoparticles. The roughness of the surface increased on the surfaces which had HIV antibodies conjugated to nanoparticles in the presence of the HIV pseudovirus. The root mean square roughness increased to 2 nm the presence HIV pseudovirus. This results show that immobilisation of the HIV antibodies was achieved and the antibodies were able to capture the pseudovirus which bound to the antibody conjugated to nanoparticles.

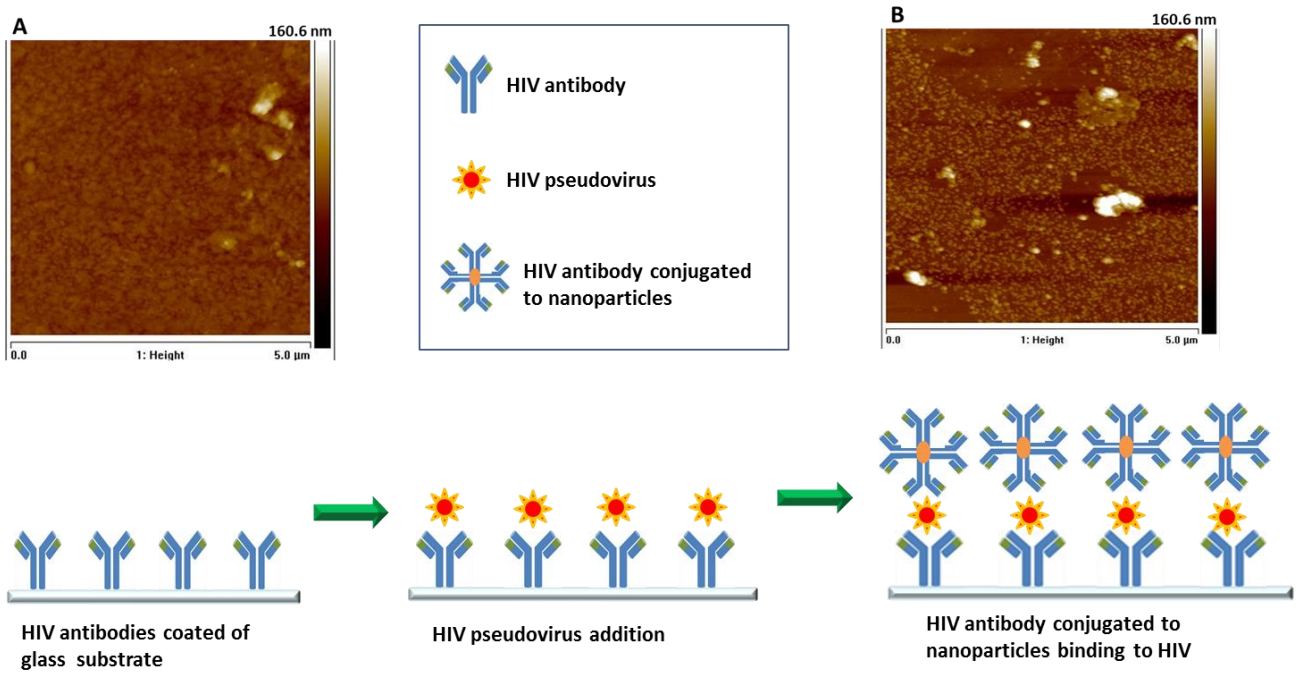

Figure 3. Illustration of immobilising of HIV antibodies on glass substrates, followed by addition of HIV pseudovirus which subsequently capture HIV antibody conjugated to nanoparticles. (A) Atomic force microscope (AFM) image of HIV antibodies immobilized on glass substrates. (B) Image of HIV antibody conjugated to nanoparticles captured on glass substrates coated with HIV antibodies and HIV pseudovirus.

3.3 Raman spectroscopy

Raman spectroscopy measurements play an important role in the identification and structural characteristics of molecules and were measured using a custom built Raman spectroscopy system. Figure 4 shows Raman spectra of HIV antibodies immobilized on the glass substrates, HIV pseudovirus and HIV antibodies conjugated to nanoparticles. As shown in figure 4, seven significant peaks were observed at ca. 545 (S-S stretching), 590 (Tyrosine), 680 (Tyrosine), 900 (N-C-C bond), 1200 (C-N bond), 1240 (amide III bond), and 1400cm^{-1} (Tryptophan). The HIV pseudovirus comprises of two or three parts namely; DNA/or RNA which is a protein coat responsible for protecting the viral vehicle and lipids [11]. The peaks that were obtained in the Raman spectra are associated with the HIV viral enzymes, glycoproteins or lipids. These results suggest that HIV biosensing was possible using the custom built Raman system.

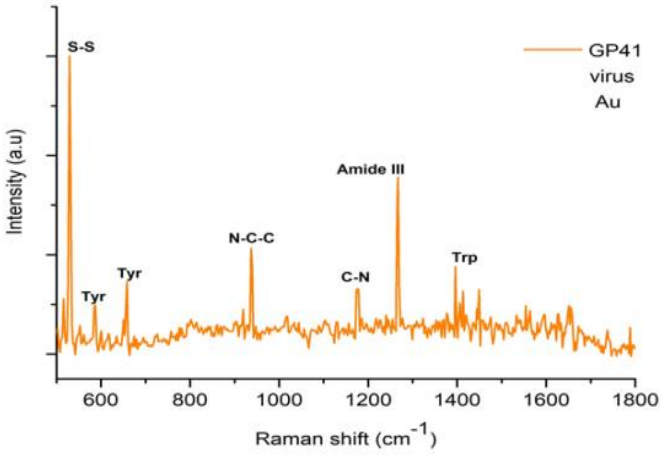

Figure 4. Raman spectra of HIV antibodies immobilized on glass substrates for capturing HIV pseudovirus.

CONCLUSION

The method used in this study for immobilising antibodies on the surface of glass substrate and capturing of the analytes can be used in a variety of biosensing applications. The AFM results showed that we were able to immobilize HIV antibodies on the surface of the glass substrates which in turn captured the HIV pseudoviruses. The Raman spectra confirmed the presence of HIV pseudoviruses on the surface of the glass substrates which confirms that the custom built Raman spectroscopy system can be used for HIV biosensing. Further studies to determine the limit of detection of the system are required.

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