DNA Lattice Nanostructures as Biointerface Materials for Electrochemical Biosensor Studies

Nanobiocomposite Biosensor

Murugan Veerapandian, [1] Chang-Hyun Jang[1], Guie-Sam Lim,[2] Sung Ha Park,[3] Min-Ho Lee[4] and Kyusik Yun,[1]*
[1]Kyungwon University, [2]LG Electronics, [3]Sungkyunkwan University, [4]Korea Electronics Technology Institute,
Republic of Korea Email: ykyusik@kyungwon.ac.kr

Abstract—**We demonstrate the use of well designed artificial DNA lattices as active biointerface material for electrochemical biosensing. Gold-PCB electrodes were modified with metalloid-polymer nanocomposite in presence/absence of DNA nanostructures and then characterized for its electrochemical response toward antigen, bovine serum albumin (BSA) and antibody, anti-bovine serum albumin (anit-BSA) interaction. The surface chemical modifications were achieved by utilization of suitable alkane dithiol and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride/*N*-Hydroxysuccinimde (EDC/NHS) coupling reaction, respectively. Furthermore, UV-visible spectral and Field Emission-Scanning Electron Microscope (FE-SEM) studies ensured the immobilization and morphological characteristics of the designed nanobiocomposite biosensor.**

Keywords-DNA cross tile; Metalloid-polymer; BSA/Anti-BSA; Biosensor; Biointerface

I. INTRODUCTION

DNA is well known as a biomolecular carrier of genetic information, but in recent years the application of DNA-based nanostructures are invaluable for material science and nanotechnology. Unique structural composition and physical-chemical properties makes them possible for diverse architectures [1]. The incorporation of DNA into nano-object design is of great interest to establish high selective and reversible interactions between the components of a nanosystem. Many researchers have highlighted the immobilization and characterization of DNA on surfaces including X-ray photoelectron spectroscopy (XPS) [2, 3], ellipsometry [2], neutran reflectivity [4], surface plasmon resonance (SPR) [5], Raman spectroscopy [6] and scanning tunneling microscopy (STM) [7]. However, relationship between different nanostructures and orientation of surface-tailored DNA, their biological adherence and the behavior of DNA modified electrodes are still remaining as fundamental issues for its exploitation in biosensor studies. By using suitable surface linking/ modifying agent optimal concentration of DNA can be easily immobilized on the electrodes. Generally, scanning probe microscopes (STM and AFM) are utilized to achieve imaging of DNA nanostructures [8, 9]. Zhang et al. studied the orientation of DNA on gold electrodes using electrochemical (EC)-STM [8]. Kelly et al. investigated the different applied potential effect on the orientation of self-assembled DNA helices on gold by EC in-situ atomic force microscope [10].

The target of the present research work is to extend the application of DNA-based nanostructures as biointerface materials. To this regard, we have utilized the artificially designed DNA lattices and modified at the interface of nanocomposite materials and gold-PCB electrodes and performed the biosensing of antigen-antibody interaction. The two different nanocomposite materials incorporated in the current experiments are poly(ethyleneglycol)-silica@silver core (PEG-SiO_2@Ag) and poly (*p*-dioxanone-co-caprolactone)-block-poly (ethylene-oxide)-block-poly-(*p*-dioxanone-*co*-caprolactone) (PPDO-*co*-PCL-*b*-PEG-*b*-PPDO-*co*-PCL)/ ABA-poly (ethyleneglycol)-silica@silver core (ABA/ PEG-SiO_2@Ag) [11]. DNA lattices are immobilized on gold-PCB electrodes by ethane dithiol activation (EDT). The electrochemical properties of the above nanocomposite (in absence of DNA lattices) and bionanocomposite (in presence of DNA lattices) structures were evaluated through biofunctionalization (using EDC/NHS coupling reaction) of model protein BSA and anti-BSA. The reaction mechanism and relationship at the bio-nano interface were briefly discussed for its possible application in biosensor studies. Fundamental determination of changes in the oxidation and reduction peak potential from the surface modified nanocomposite particles on before and after treatment with BSA and anti-BSA gives a considerable chance of sensing abilities. Further the morphological and wavelengths of maximum absorbance (λ_{max}) characterization of the immobilized nanostructures were obtained from FE-SEM and UV-visible spectroscopy, respectively. The two hybrid nanocomposite particles used in the current experiment has several distinct features such as polymer/copolymeric shell (PEG/ABA), metalloid core (SiO_2@Ag) and film forming ability for surface and interface studies [11].

II. EXPERIMENTAL

A. Fabrication of nanocomposite particles and DNA lattice structures

poly(ethyleneglycol)-silica@silver core (PEG-SiO_2@ Ag) and poly(*p*-dioxanone-co-caprolactone)-block-poly

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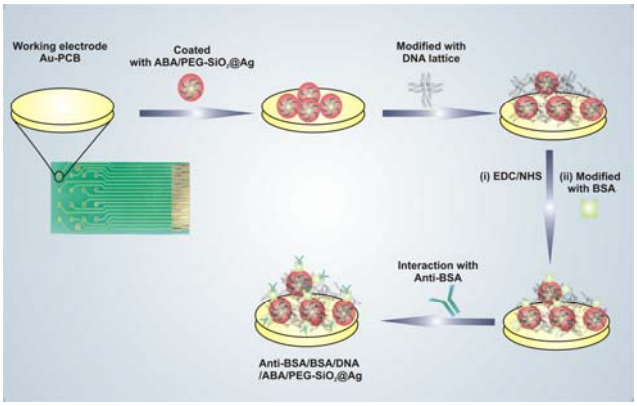
(ethyleneoxide)-block-poly-(*p*-dioxanone-*co*-caprolac -tone) (PPDO-*co*-PCL-*b*-PEG-*b*-PPDO-*co*-PCL)/ABA-poly (ethyl eneglycol)-silica@silver core (ABA/PEG-SiO_2@Ag) were prepared by our previous report [11]. DNA nanostructures of double cross over tiles (DX1 and DX2) [12] were successfully self assembled for DNA lattice growth. These DNA nanostructures are fairly stiff and considerably stable at room temperature [13] sequenced by multiple DNA crossovers. Final concentration of DNA lattice sample utilized in electrode preparation is about 200 nM. The details of DNA base sequence can be found from Table 1. DX1 cross tiles and DX2 cross tiles are composed of individual strands such as DX1#1, DX1#2, DX1#3, DX1#4 and DX2#1, DX2#2, DX2#3, DX2#4, respectively.

B. Electrode pretreatment and immobilization of nanocomposite particles/DNA lattice structures

Platinum and Ag/AgCl electrodes were used as counter and reference electrodes, respectively. Surface of working electrode (gold-PCB) were cleaned by soaking the electrode surface under acetone and ethanol for 5 m separately and rinsed thoroughly with D.I H_2O. Prior to surface functionalization of prepared colloidal PEG-SiO_2@Ag and ABA/PEG-SiO_2@Ag nanocomposite particles the surface of gold-PCB electrode was made hydrophilic by oxygen-plasma coating for 300 s. After plasma cleaning, the electrode surface were drop coated with EDT (1 mM) and kept under room temperature for 12 h. EDT activated electrode surface were then drop coated with 4 µL of above nanocomposite particles, separately. DNA lattice (200 nM) modification on the nanocomposite/gold-PCB surface was prepared by same procedure. Biofunctionalization of BSA and anti-BSA on the surface of these nanocomposite/ (in presence and absence of DNA lattice) gold-PCB electrode were done via EDC/NHS coupling reaction [14]. Cyclic voltammetry scans were recorded under 5 mL PBS (10 mM; pH 7.4) solutions in the potential range of -0.1 to 0.450 V and at the scan rate of 80 mV/s. Each CV scans were repeated for three times. To observe the CV measurements, three-electrode configuration from BioLogic science instrument SP-300 was utilized in the current experiment.

III. RESULTS AND DISCUSSION

The schematic representation of immobilization of nanocomposite particles and its surface modification is explained in Scheme 1. Colloidal solution of nanocomposite particles (ABA/PEG-SiO_2@Ag) is self assembled onto the surface of oxygen plasma treated gold-PCB electrodes. Further a thin film of DNA lattice structures is developed on the nanocomposite particle surface through drop casting method. Evaporation induced self assembly result in the deposition of biointerface membrane. It is demonstrated that



Scheme 1. Fabrication of biocompatible nanobiocomposite biosensor. (unpublished mateiral).

the development of thin structures of polymer film or biomolecules such as DNA or peptides are feasible for number of applications in biosensor studies. For instance, DNA microarrays on polymers such as poly (methyl methacrylate) (PMMA) [15], the synthesis of star shaped poly (ethylene glycol) (PEGs) [16] and immobilization of genetically engineered proteins on gold-MWNT films for biosensor platforms [17]. Further the confinement of sub 100 nm thin films has been reported to have influence in the

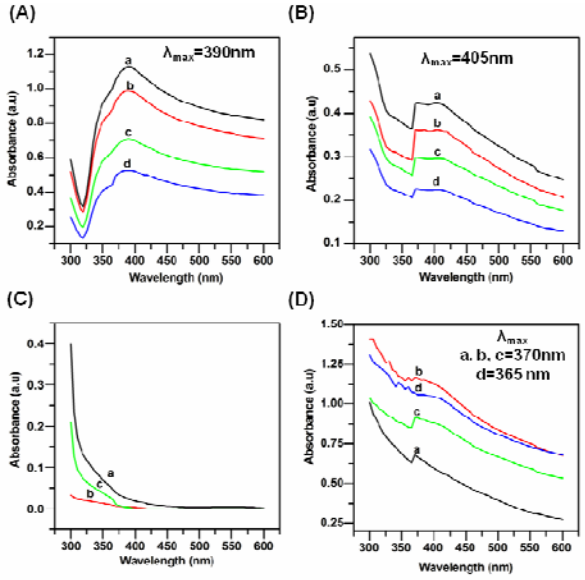


Figure 1. UV-visible spectral studies. (A) PEG-SiO_2@Ag and (B) ABA/PEG-SiO_2@Ag nanocomposite particles of different concentration (100 µL, 80 µL, 60 µL and 40µL (a-d) in D.I water used for analysis). (C) BSA (a), anti-BSA (b) and mixture of BSA/anti-BSA (c). (D) BSA/ABA/PEG-SiO_2@Ag (a), Anti-BSA-BSA/ABA/PEG@SiO_2@Ag (b), BSA/DNA/PEG-SiO_2@Ag (c) and Anti-BSA-BSA/DNA/ABA/PEG-SiO_2@Ag (d).(unpublished material).

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diffusion of polymer molecules comprising the film, as well as diffusion of small, lower-molar-mass tracer molecules and other altered properties [18].

Figure 1 (A) and (B) denotes the maximum absorbance spectrum of two nanocomposite particles. As seen from the images the curve peak indicates the presence of SPR from the core silver particles. Further the increased wavelength of maximum absorbance (from (A) 390 to (B) 405 nm) indicates the successful surface modification of ABA triblock copolymer on the PEG/SiO_2@Ag core. This is in-

TABLE I. THE SEQUENCE DETAILS OF ALL STRANDS FROM 5` to 3` (UNPUBLISHED MATERIAL)

Strand	No. of Base	Sequence(5' to 3')
DX1#1	26 MERS	**TGCTA** CTACCGCA CCAGAATG **CTAGT**
DX1#2	48 MERS	CATTCTGG ACGCCATA AGATAGCA CCTCGACT CATTTGCC TGCGGTAG
DX1#3	48 MERS	CAGTAGCC TGCTATCT TATGGCGT GGCAAATG AGTCGAGG ACGGATCG
DX1#4	26 MERS	**CATAC** CGATCCGT GGCTACTG **TCACT**
DX2#1	26 MERS	**GTATG** GGCAATCC ACAACCGC **AGTGA**
DX2#2	48 MERS	GCGGTTGT CCAACTTA CCAGATCC ACAAGCCG ACGTTACA GGATTGCC
DX2#3	48 MERS	GCTCTACA GGATCTGG TAAGTTGG TGTAACGT CGGCTTGT CCGTTCGC
DX2#4	26 MERS	**TAGCA** GCGAACGG TGTAGAGC **ACTAG**

consistent with our previous report [10]. On the other hand the BSA, anti-BSA and mixture of both doesn't give a significant absorbance on this specific region (Figure 1(C)). Notably, after surface modification of final nanocomposite particles (in presence/absence of DNA lattice) with BSA and anti-BSA, there is a change observed in the wavelength of maximum absorbance and shape of the peak. For instance in presence of anti-BSA, both the normal BSA-nanocomposite particles and BSA/DNA-nanocomposite particles shows broad peaks. Whereas, in absence of anti-BSA the other two nanocomposite (a: BSA/ABA/PEG-SiO_2 @Ag and c: BSA/DNA/ABA/PEG-SiO_2@Ag) particles shows a short but intense peak, which attributes the surface characteristics of the reacted/unreacted nanostructured surface, respectively.

Biocompatibility, biointegration and functionality are the crucial factors for biomedical devices and implants, but also applicable for biosensor in various forms. For instance, immobilization of biomolecules for biosensing studies needs a suitable biocompatible interface material [18]. The key strategy demonstrated here is the use of hybrid nanocomposites (PEG-SiO_2@Ag and ABA/PEG/SiO_2@Ag) as an electrochemical substrate based on their excellent electrical properties and large surface areas for the immobilization of bioreceptors and to fabricate a sensing platform by using an advanced DNA lattice nanostructures as suitable interface material specifically for binding on the surface of nanocomposite-gold-PCB electrode, which adheres the antigen BSA and significantly interfere with anti-BSA through the antigen-antibody interactions.

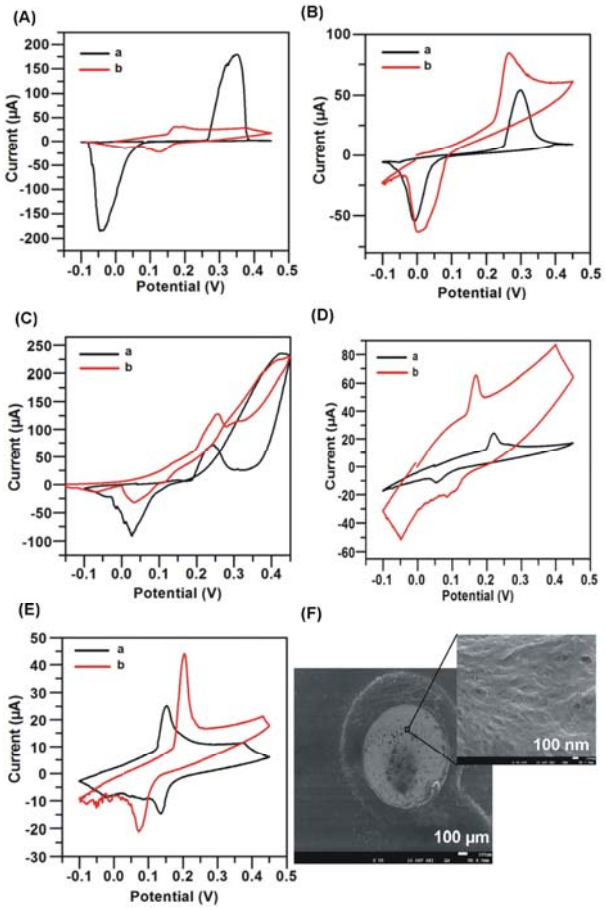

Figure 2. Shows the cyclic voltammagrams of nanocomposite particles-Au-PCB electrode at different conditions (Note: (a) PEG-SiO_2@Ag: black trace and (b) ABA/PEG-SiO_2@Ag: red trace) such as bare (A) (a: E_{pc}=0.34; E_{pa}= -0.04, b: E_{pc}=0.19; E_{pa} =0.12), BSA modified (B) (a: E_{pc}= 0.30; E_{pa}= -0.007, b: E_{pc}= 0.26; E_{pa} = 0.0003), BSA-Anti-BSA modified (C) (a: E_{pc}= 0.24; E_{pa}=0.02: b: E_{pc}= 0.25; E_{pa} =0.03), DNA-BSA modified (D) (a: E_{pc}=0.22; E_{pa}=0.05, b: E_{pc}=0.16; E_{pa} =0.08) and DNA-BSA-Anti-BSA modified (E) (a: E_{pc}= 0.15; E_{pa}=0.13, b: E_{pc}=0.20; E_{pa} =0.07). F: depicts the FE-SEM image of Au-PCB electrode coated with nanocomposite (b) particles and DNA lattice structures. (unpublished material).

Figure 2 shows the cyclic voltammagrams and its relevant cathodic peak potential (E_{pc}) and anodic peak potential (E_{pa}). As seen from the images, it is clear that there is a possibility of sensing the significant changes occur between the antigen-antibody interactions that taken place on the hybrid nanocomposite and bionanocomposite surfaces immobilized on the gold-PCB electrode. Figure 2 (F) represents the FE-SEM image of gold-PCB electrode surface modified with nanocomposite particles-DNA lattice structures. As observed from the magnified inset image the well aligned DNA lattice structures and nanocomposite particles are

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densely packed with large surface areas. Incorporation of DNA lattice nanostructures as interface material acted both as a suitable surface linker/compatible material for integration of biomolecules on the hybrid environment.

IV. CONCLUSION

In conclusion, we have developed a nanobiocomposite biosensor platform for antigen-antibody interaction. A well defined DNA lattice nanostructure shows significantly a biocompatible environment for both nanomaterial and biomolecules (such as model protein BSA) utilized in the study. Furthermore, we demonstrated the cyclic voltammetry response for different surface modified biosensor platform in presence and absence of DNA lattice. A detailed study is in progress to unravel the underlying efficiency for concentration dependant biosensing abilities. Further Bio-AFM based investigations of orientation of these hybrid nanoenvironments are underway. We believe that the concept of integration of DNA lattice nanostructures as interface materials with nanocomposite particles will be sure opens a new insight into other nanobiotechnology. It is expected that a fundamental study performed in the present research will allows us to exploit the present situation of DNA nanotechnology towards a facile construction of new nanomaterial platform for many other biomedical applications.

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