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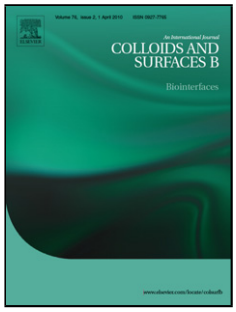

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Fabrication of Electrochemical Biosensor Composed of Multi-functional DNA Structure/Au Nanospike on Micro-Gap/PCB System for Detecting Troponin I in Human Serum

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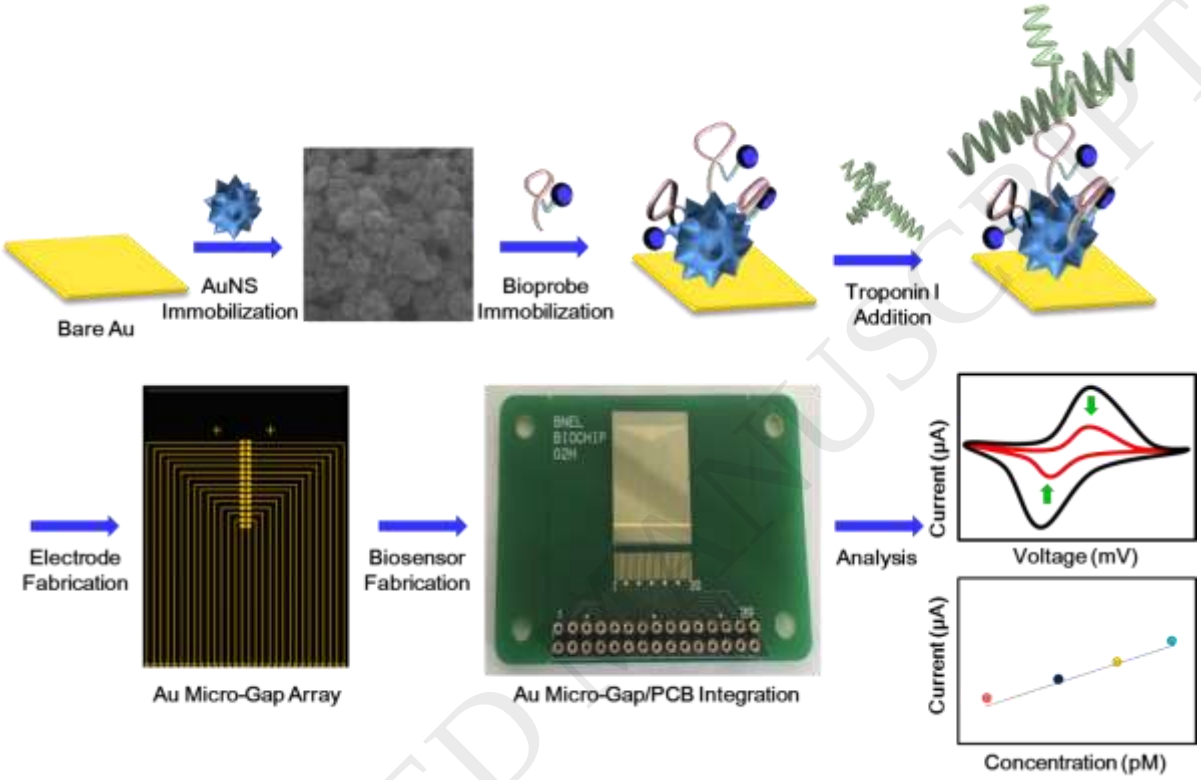

Highlights

- A troponin I biosensor composed of DNA 3WJ on Au nanospike was developed.
- The DNA 3WJ was rolled to recognition, EC signal transduction and immobilization.
- Au nanospike was prepared to increase the detection limit of troponin I.
- This study showed label-free EC detection elements for troponin I detection.

Abstract

Acute myocardial infarction (AMI) is one of the most serious diseases affecting human beings. In this study, in order to rapidly detect AMI disease, the authors fabricated a label-free electrochemical biosensor composed of a multi-functional DNA structure on Au nanospike (AuNS) with a fabricated Au micro-gap electrode which was incorporated with a PCB chip in order to detect cardiac troponin I (cTnI). As a bioprobe, the DNA 3 way-junction (3WJ) was introduced, because the DNA 3WJ has three arms for embodying the multi-functionality. Each piece of DNA was assembled to simultaneously form the DNA 3WJ for cTnI detection, signal transduction, and immobilization, respectively. The assembled DNA 3WJ structure was confirmed by Native-TBM PAGE. Moreover, in order to increase the electrochemical signal sensitivity, AuNS was prepared. The Au micro-gap array is fabricated with a printed circuit board (PCB) chip in order to control each micro-gap electrode panel selectively so as to detect low volumes of cTnI. Then, the DNA strucuture on pAuNS-modified electrode was prepared using the layer-by-layer (LbL) assembly method. FE-SEM and AFM were used to investigate the modified-surface morphology. The cyclic voltammetry (CV) was measured to confirm the cTnI binding to DNA 3WJ-modified electrode. cTnI was detected in the HEPES solution and human serum, respectively. The LOD result exhibited 1.0 pM in HEPES solution and 1.0 pM in 20% diluted human serum, respectively. In addition, the selectivity test was carried out with various proteins as the control experiment. The present study showed label-free, simple fabrication, and easy-to-tailor detection elements for cTnI.

Keyword: AMI detection; cardiac troponin i biosensor; Multi-functional DNA structure; Electrochemical Biosensor; Au nanospike

1. Introduction

Cardiovascular diseases (CVDs) are considered as the common cause of death in most developed countries [1]. The World Health Organization (W.H.O.) warned the numbers of victims of CVDs gradually increased in the developing countries too. Especially, the acute myocardial infarction (AMI) is one of the most serious CVDs that bring the death rate rapidly that needs to precise diagnosis and treatment [2,3]. AMI is closely related to heart attack and blood vessel injury such as blockage of a coronary artery that hamper the blood flowing in the vessels [4-6]. When the heart attack begins suddenly, the patient should be cared and treated within 60 mins. Like this, the rapid diagnosis of AMI is essential to treatment [7].

There are many cardiac biomarkers related with AMI reported such as cardiac troponin complex (C,T and I), Creatine Kinase-MB, Pro-BNP, Myoglobin and MB isoenzyme [8,9]. In particular, the cardiac troponin I (cTnI) is mostly focused on AMI biomarker because of good relationship between cTnI level and AMI symptoms [10-12]. It showed a high specificity and sensitivity toward AMI that made a development of several detection methods. Several techniques reported to detect cTnI rapidly and accurately. enzyme-linked immunosorbent assay (ELISA) [13], surface plasmon resonance (SPR) [14], fluorescence-based technique [10, 15] and so on [16,17]. However, those approaches require multistep processing of samples, long diagnostic time and requirement of trained operator and high costs. To detect the cTnI rapidly and precisely, it is highly needed to develop the biosensor with simple detection step and high sensitivity. Among them, the electrochemical detection method provides the simple fabrication method, user-friendly measurement and short detection time [18-20]. It only required the redox

labeling process or redox buffer because cTnI does not have a redox center that hamper the commercilization of EC-based cTnI biosensor [12, 21-22].

To solve this problem, the present study proposed the multi-functional bioprobe-based biosensor composed of DNA structure that recognize the cTnI, immobilize on the substrate and gives the redox signal, simultaneously. To achieve the goal (target recognition, signal generation and immobilization) at once, the DNA 3 way-junction (3WJ) structure was used to multi-functional bioprobe because of three arms [23,24]. The troponin I aptamer was introduced to detect the cTnI and the Tro aptamer was connected to DNA 3WJa. For the electrochemical signal generation, the methylene blue (MB)-tagged DNA 3WJb introduced. The MB-3WJb provided the redox center that can use to determine the binding event between cTnI and the bioprobe. Finally, the thiol-modified DNA 3WJc (SH-3WJc) was used for the immobilization process. Each piece of DNA 3WJ was assembled for multi-functional bioprobe by annealing process. For increasing the sensitivity and reducing the enzymatic signal amplification step, the Au nanospike (AuNS) was synthesized. The AuNS showed the larger surface coverage that can facilitate the electron transfer between target and bioprobe [25]. Also, one important concern is loading small amount of clinical sample volume. To load the sample volume with small amount, the proposed biosensor was composed of Au micro-gap array for loading low volume of clinical sample. For controlling the Au micro-gap selectively, the Au micro-gap/PCB chip was prepared. Fig. 1 showed the schematic diagram of cTnI detection system.

2. Experimental details

2.1. Materials

Natural Cardiac Troponin I protein (cTnI, 23.9 kDa) was purchased from Abcam (UK). For the AuNS immobilization, the chemical linkers, cysteamine, 6-Mercaptohexanoic acid (6-MHA), myoglobin, hemoglobin, hemocyanin, bovine serum albumin, L-ascorbic acid, and poly(vinylpyrrolidone) were purchased from Sigma-Aldrich (USA). For the clinical test, the human serum from human male AB plasma (USA origin) was purchased from Sigma-Aldrich (USA). The three fragments of DNA 3WJ were synthesized by HPLC method by Bioneer (South Korea). The sequence information of cTnI aptamer was followed by Shim's group report [20]. The Tro6 aptamer was connected to DNA 3WJ for integrating the functionality. The sequence of Tro6 aptamer-tagged 3WJ-a (Tro6/3WJa) is 5'-CGC ATG CCA AAC GTT GCC TCA TAG TTC CCT CCC CGT GTC CTT GCC ATG TGT ATG TGG G-3'. The methylene blue-tagged 3WJ-b (MB/3WJb) is MB-5'- TTT GGG TAG GGC GGG TTG GG C CCA CAT ACT TTG TTG ATC C -3', and Thiol-tagged 3WJ-c (SH/3WJc) is SH-5'-GGA TCA ATC ATG GCA A-3', respectively. All oligonucleotides were diluted in the nuclease free water. The bare Au electrode (50 nm)/Cr (2 nm) on SiO_2 (5 mm) and micro-gap Au electrode were fabricated by the National Nanofab Center (South Korea). The 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was used as the buffer for electrochemical experiment. Hydrogen tetrachloroaurate (III) hydrate was purchased from Kojima Chemicals Co. (Japan). Silver nitrate, hydrogen peroxide (30%) were purchased from Junsei (Japan). All chemicals were used as received.

2.2. Synthesis of AuNS

Hollow and spike-like gold NPs (gold-spikes) were synthesized by the GRR method between AgNPs and $HAuCl_4$ [26]. First, AgNPs as sacrificial materials were prepared as follows: first, 10 mL of 0.025 M AgNO3 was added to 80 mL of boiling water, followed by adding 1 wt % trisodium

citrate dehydrate. The mixture was boiled for 20 min and cooled to room temperature. Then, AuNS was prepared with the as-made AgNP solution, as follows: 5 mL of AgNPs was dispersed in 25 mL of 3 mM $HAuCl_4$, and 5 mL of 10 mM ascorbic acid was injected into the resulting solution. To enhance the dispersion stability in the aqueous phase during storage, 0.1 g/mL of PVP was added into the final solution at 50 °C for 6 h.

2.3. Assembly of multi-functional DNA 3WJ nanostructure

For constructing the multi-functional bioprobe, the DNA 3WJ assembled well which retain the multi-functionality. For the cTnI recognition part, Tro6 aptamer-tagged DNA 3WJa (TROapt/3WJa, 5'-CGC ATG CCA AAC GTT GCC TCA TAG TTC CCT CCC CGT GTC CTT GCC ATG TGT ATG TGG G-3') was introduced. For the electrochemical signal generation part, the MB/ 3WJb was introduced (MB-5'- TTT GGG TAG GGC GGG TTG GG C CCA CAT ACT TTG TTG ATC C -3'). For, the immobilization part, thiol-modified DNA 3WJc was prepared (SH/3WJc, SH-5'-GGA TCA ATC ATG GCA A-3'). Those sequences were chemically synthesized, respectively, from Bioneer (South Korea). The TROapt/MB/SH-3WJ was assembled by annealing three corresponding DNA strands at equal molar ratio in TMS buffer (40 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl) by heating at 80ºC for 5 min, followed by slowly cooling down to 4ºC at a rate of 2ºC/min on a T100™ Thermal Cycler (Bio-rad). The multi-functional DNA 3WJ assembly was confirmed by 8% native polyacrylamide gel prepared in TBM buffer (89 mM Tris, 200 mM boric acid, 5 mM $MgCl_2$, pH 7.6). About 0.2 ug of DNA samples were loaded into each lane. After running at 80 V for 80 mins, the DNA assembly were visualized by ethidium bromide staining [27].

2.4. Fabrication of Au Micro-gap array/PCB Chip

The P-type 4 inch Si substrate (100) with a thermally oxidized SiO_2 was initially cleaned using a piranha solution composed of H_2SO_4 (Daejung Chemical and Metals Co. Ltd., South Korea) and H_2O_2 (Daejung Chemical and Metals Co. Ltd., South Korea) with a volume ratio of 4:1 at for 5 min at room temperature, and baked on the hot plate at 200°C for 20 min for adhesion promotion with photoresist. The metal deposition of pre-pattern was prepared by the standard photolithographic process using negative photoresist. Su-82 photoresist was spin-coated onto the substrate with a 3000 rpm, then, it was baked at 65°C for 1 min and 95°C for 1 min. After exposure of 110 mJ/cm^2 (i-line) by MA6 mask aligner (SUSS MicroTec, Germany), substrate was post-baked by the same steps with pre-baking and developed for 70s. Metal deposition of Cr (2 nm) and Au (50 nm) was carried out with an electron beam evaporator. Finally, a lift-off process was performed using remover PG at 70°C for 2 hr. The fabricated micro-gap array was cleaned by piranha solution with the same conditions. The designed Au micro-gap array which is a square with a side-length of 1 mm, consists of 30 individually addressable electrodes, and the gap size between working electrode and counter electrode is 10 um, respectively.

For the electrochemical analysis, the working chamber was prepared by PDMS linkage and attached to the micro-gap. After attachment of the chamber on the electrode, substrates were washed by ethanol and DI water to remove organic contaminant, which could be induced in the curing process of PDMS at high-temperature chamber [28]. The mask and pattern of PCB was followed by Salvo's group work [29]. The PCB was manufactured by Purax (Japan). The PCB fabrication was followed by micro-etching, surface preparation and the gold layer deposition on

standard PCBs (Material: FR4, Size: 40 X 40 mm, Thickness: 2 mm), with nickel as adhesion promoter. Then, the prepared Au micro-gap array was attached to the PCB epoxy treatment. For the attachment effectively, the prepared Au micro-gap/PCB was baked in 80 °C for 5 mins. To control the micro-gap electrode, the Au bonding process conducted by Young-Sung Technology (South Korea). For connecting the micro-gap and PCB electrode, the Au-wire bonding process and epoxy treatment process were conducted. Fig. 2a-f showed the schematic diagram, CAD image and the optical image of fabricated electrode and real picture of fabricated biosensor

2.5. Fabrication and investigation of multi-functional DNA 3WJ/AuNS heterolayer

To fabricate a multi-functional DNA 3WJ/AuNS on Au micro-gap array, the fabricated electrode was treated with acetone solution with a sonication for 5 min, washed with ethanol and deionized water, and dried under N_2 gas to remove surface residues. The 3 µl of 2% 6-MHA was dropped onto the Au surface for 3 hrs, then, the thiol group was anchored to Au substrate and carboxyl group was exposed to layer interface. And, the 3 µl of 2% cysteamine was added onto the modified substrate that reacted with amine group of cysteamine and carboxyl group of 6-MHA that exposed to thiol group layer interface for 3 hrs. Then, 3000 ppm of 3 µl of AuNS was immobilized onto the modified surface for 3 hrs. Then, 3 µl of 10 uM multi-functional DNA was added onto the AuNS surface for 3 hr. The thiol group of the multi-functional DNA molecule was immobilized onto the AuNS surface by covalent bonding. The excess biomolecule was removed by DI water and N_2 gas stream [30].

2.6 Surface morphology analysis

The surface analysis of multi-functional DNA on AuNS-modified electrode was investigated by tapping-mode AFM using Nanoscope IV / Multimode (Digital Instruments, USA). The bare Au substrate, AuNS-modified substrate, multi-functional DNA-modified substrate and multi-functional DNA on AuNS-modified substrate were investigated for comparison. The n-type doped Si phosphorous (RTESP, Bruker, USA) tip was introduced with resonance peaks (230-305 kHz). The surface roughness and sectional analysis of the each image was determined using the offline procedure provided by Nanoscope software. And field-effect scanning electron microscopy (FE-SEM) (Auriga, Carl Zeiss, Germany) was used to analyze the particle morphology of AuNS immobilized electrode. The image size was 700 nm x 700 nm, and a scan rate is 1.0 Hz. The sectional analysis for surface roughness and vertical distance measurement of the each image obtained using the Nanoscope software. The root mean square (RMS) roughness R_q is the standard deviation of height. The roughness max (R_{max}) is the highest peak of the the surface. The roughness average R_a is the arithmetic mean of the absolute values of the height of the surface profile this R_a most widely used amplitude roughness parameter. Vertical distance provides the immobilization of DNA and protein on the substrate.

2.7. Electrochemical analysis of multi-functional DNA/AuNS heterolayer on Au micro-gap array/PCB

Cyclic voltammetry (CV) experiments were carried out with a modified-three-electrode system. The DNA 3WJ/pAuNP/Au micro-gap electrode was used as the working electrode. As the counter electrode, another side of Au micro- electrode was introduced. Then, Ag/AgCl electrode was

mounted on the top of fabricated electrode used as reference electrode, respectively. Fig. S1 showed the fabrication process of biosensor. All Experiments were performed using a Versastat3 potentiostat (Princeton Applied Research, USA) [27]. All EC experiments were repeated with 8 samples.

3. Results and Discussion

3.1. Construction of multi-functional DNA 3WJ

For contructing multi-functional bioprobe, the cTnI aptamer was integrated into 3WJa motif (TROapt/3WJa). And the MB/3WJb motif for EC signal generation. Finally, The thiol-modified 3WJc was prepared for direct immobilization. The TROapt/3Wja and target troponin I reactivity was confirmed by 8% native TBE-PAGE with EtBr staining (Fig. S2). Fig. 3a depicted the expected Troapt/MB/SH-DNA 3WJ 2D structure. The assembly of TROapt/MB/SH-DNA 3WJ was confirmed by 8% native TBM-PAGE. Fig. 3b shows the PAGE gel result of Troapt/MB/SH-DNA 3WJ assembly. The gel shows the TROapt/3WJa (lane 2), MB/3WJb (lane 3), SH/3WJc (lane 4) and Troapt/MB/SH-DNA 3WJ (lane 5) well. The result showed the designed multi-functional bioprobe can be easily integrated without loss of functionality well. Moreover, the designed bioprobe should retain the target recognintion. For confirming this binding event, the taret cTnI was reacted with assembled TROapt/MB/SH-DNA 3WJ. Lane 7 showed the migration change and the band diminisment was originated from bioprobe-target binding, presumably. Also, as the negative control, the myoglobin was reacted with assembled TROapt/MB/SH-DNA 3WJ. (lane 9). Because, the myoglobin is one of AMI biomarker that is also affect the AMI diagnosis. In this case, there is no migration change compared to lane 5. That means the assembled

TROapt/MB/SH-DNA 3WJ did not react with myoglobin. Based on the combined results, the designed bioporbe is successfuly assembled and it can detect the cTnI with high specificity.

3.2. Investigation of fabricated cTnI/multi-functional DNA on AuNS heterolayer

The immobilization process of cTnI and TROapt/MB/SH-DNA 3WJ on AuNS-modified electrode was investigated by AFM and FE-SEM. Fig. 4a displayed AFM result of AuNS-immobilized Au electrode. AuNS-modified electrode formed the 100-120 nm size was that could be assumed that separated by deep valleys. Fig. 4b showed the troponin I/TROapt/MB/SH-DNA 3WJ immobilized on AuNS/Au electrode. The small lumps were formed on AuNS surface. When the troponin I was immobilized, the AFM morphology was slightly changed with large biomolecule (30-50 nm size) compared to other substrates (Fig. 4c). Also, the AuNS-modified electrode was investigated by FE-SEM that showed the large amount of the AuNS immobilization well (Fig. 4d).

In addition to, the sectional surface roughness analysis and vertical distance analysis was carried out. The surface roughness of the AuNS, the TROapt/MB/SH-DNA 3WJ on AuNS, the troponin I/TROapt/MB/SH-DNA 3WJ on AuNS were shown in Fig. 4e. In case of the AuNS, the R_a value was 2.940 ± 0.479 nm and RMS roughness (R_q) was 7.256 ± 1.443 nm, R_{max} was 10.943 ± 1.984 nm and the vertical distance (VD) between substrate and top of AuNS was 76.426 ± 10.312 nm. In case of the TROapt/MB/SH-DNA 3WJ on AuNS-modified electrode, the R_a, R_q, R_{max}, VD were 7.908 ± 1.113 nm, 16.416 ± 3.175 nm, 55.883 ± 7.616 nm and 81.717 ± 9.752 nm, respectively. The change of surface roughness and VD showed the functional DNA molecule was immobilized well on AuNS. In case of the troponin I addition to the TROapt/MB/SH-DNA 3WJ on AuNS-

modified electrode, the surface roughness values (R_a: 7.642 ± 1.778 nm, R_q: 17.719 ± 2.986 nm and R_{max}: 44.578 ± 6.582 nm) were similar to TROapt/MB/SH-DNA 3WJ on AuNS-modified electrode. However, the VD value increased 87.576 ± 11.333 nm. Presumably, the increment of VD could be elucidated the binding event of TROapt/MB/SH-DNA 3WJ and troponin I. Also, the surface roughness and vertical distance analysis were carried out (Fig. 4e). Based on the results, the prepared electrode was well prepared for troponin I.

3.3. Electrochemical properties of fabricated layer

To confirm the electrochemical signal enhancement effect of AuNS, the cyclic voltammetry (CV) experiment was carried out on TROapt/MB/SH-DNA 3WJ and TROapt/MB/SH-DNA 3WJ with the cTnI protein immobilized on Au substrate (0.7 x 2 cm). As shown in Fig. 5a, it is hard to determine the oxidation peak and reduction peak of TROapt/MB/SH-DNA 3WJ (Green line) and TROapt/MB/SH-DNA 3WJ with troponin I (Blue line). However, when the AuNS was introduced to the electrode (Fig. 5b), the clear redox peaks of TROapt/MB/SH-DNA 3WJ were shown (Green line). The redox property of the TROapt/MB/SH-DNA 3WJ showed a quasi-reversible redox peaks because of the redox center of MB facilitated the electron transfer. The cathode and anode potential values are 315.9 ± 5.383 mV and 129.9 ± 9.162 mV (vs. Ag/AgCl). The cathode and anode current values are 3.392 ± 0.895 μA and -2.627 ± 0.758 μA. When the troponin I was added to TROapt/MB/SH-DNA 3WJ/AuNS-modified electrode, the redox peaks were decreased. The cathode and anode potential values are 261.4 ± 2.492 mV and 93.2 ± 3.999 mV (vs. Ag/AgCl). The cathode and anode current values are 691.3 ± 9.058 nA and -731.8 ± 7.920 nA. This redox

current signal decrement effect could be applied to use of troponin I detection. Also, this effect can be elucidated by the electron transfer facilitation of AuNS. And, the AuNS provided the higher surface roughness and active area which yields to a higher coverage [31]. Based on the combined results, the AuNS-modified electrode can strongly facilitate the electron transfer that can be successfully applied to the signal-enhanced electrochemical troponin I detection biosensor platform.

Moreover, as the feasibility test, the CV was carried out on TROapt/MB/SH-DNA 3WJ and TROapt/MB/SH-DNA 3WJ with the troponin I protein immobilized on Au micro-gap array/PCB for confirming the electrochemical signal enhancement effect of AuNS. Fig. 5c depicted the cyclic voltamogram of TROapt/MB/SH-DNA 3WJ (Green line) and TROapt/MB/SH-DNA 3WJ with troponin I (Blue line). In this case, it is hard to determine the redox signal without AuNS same as Au substrate. In case of TROapt/MB/SH-DNA 3WJ/AuNS-modified Au micro-gap array (Fig. 5d), the clear redox peaks of TROapt/MB/SH-DNA 3WJ were obtained (Green line). The cathode and anode potential peak values are 312.4 ± 2.293 mV and 72.9 ± 1.504 mV (vs. Ag/AgCl). The cathode and anode current values are 2.017 ± 0.104 nA and -2.627 ± 0.758 nA. When the troponin I was added to TROapt/MB/SH-DNA 3WJ/AuNS-modified electrode, the redox peaks were considerably decreased. The cathode and anode potential values are 297.3 ± 4.932 mV and 21.2 ± 2.781 mV (vs. Ag/AgCl). The cathode and anode current values are 577.9 ± 4.923 nA and -621.2 ± 7.780 nA. The result showed the clear decrement of redox currents values when the troponin I was added.

Also, the surface concentration (Γ) of immobilized TROapt/MB/SH-DNA 3WJ on the fabricated substrate could be estimated as 1.731×10^{-8} mol/cm^{-2} ·V^{-1} · s^{-1} based on the following equation [10]:

$$\Gamma = Q/nFA\,v \quad (1)$$

Where, Γ is the surface coverage of the DNA on the electrode, Q is the consumed charge (area under the reduction peak), v is the scan rate (0.05 V.s^{-1}), n is the number of electrons (n=1), F is the Faraday constant (96,500 C/mol· e) and, A, is the area of the electrode (0.01 mm^2). Based on the combined results, the fabricated biosensor composed of multi-functional DNA on Au micro-gap array can be applied to troponin I detection effectively.

3.4. Detection performance and Clinical Test

For analyzing the biosensor performance considering LOD and dynamic range, CV experiments were conducted in HEPES solution with 9 samples. And the fabricated biosensor was tested with troponin I in the diluted human serum for the clinical test. The troponin I was added to the DNA 3WJ/AuNS-modified biosensor for 3 hrs at RT. After the washing step, the LOD was measured. The EC signal was obtained corresponding to the cTnI concentration (0 pM to 100 nM). As shown in Fig. 6a (Red line: cTnI in HEPES buffer, Green line: cTnI in 20% diluted human serum) and 6b (Red line: cTnI in HEPES buffer, Green line: cTnI in 20% diluted human serum), the redox peaks of TROapt/MB/SH-DNA 3WJ decreased with increasing the cTnI concentration. This phenomenon indicated relevance of cTnI binding to the TROapt/MB/SH-DNA 3WJ that inhibit

the electron transfer due to conformational change of bioprobe and hindrance effect of electron transfer of MB. In this range, the good linear relation obtained between the target concentration and redox value. The LOD of fabricated biosensor was estimated to 1.0 pM (24 pg/ml) in HEPES buffer. The regression equations were Δipc (μA) = -1E-07 lnC (pM) + 2E-06, R^2 = 0.981 and Δipa (μA) = 8E-08 lnC (pM) - 1E-06, R^2 = 0.969, respectively. In case of clinical test, LOD test was carried out in the diluted human serum. The regression equations were Δipc (μA) = -1E-07 lnC (pM) + 2E-06 R^2 = 0.982 and Δipa (μA) = -1E-08 lnC (pM) - 2E-06, R^2 = 0.962, respectively. The LOD of fabricated biosensor was estimated to 1.0 pM (24 pg/ml) in 20% dilluted human serum. Furthermore, the specificity test of fabricated biosensor was carried out by introducing other proteins such as hemocyanin, myoglobin, hemoglobin, and albumin as the negative control experiments. There is little EC signal change observed to other proteins in the human serum (Fig. 6c, 6d). A sensor performance comparison between present cTnI biosensor and other biosensors reported is summarized in Table 1. The combined result can be elucidated the proposed biosensor can be succesfuly detected the cTnI.

4. Conclusions

The detection of cTnI is getting important in AMI diagnosis and treatment for public health and medical industry. Here, this study presented the EC-based label-free cTnI detection method using multi-functional DNA 3WJ/AuNS on Au micro-gap/PCB system. Each pieces of functional DNA (cTnI detection, EC signal generation and immobilization, respectively) assembled well without loss of functionality. The assembly results were confirmed by TBM-PAGE experiments. And the AuNS was easily prepared. This unique nanomaterial was introduced to Au micro-gap

electrode for the EC signal enhancement. AFM used to analyze the surface morphology. The electrochemical experiment (CV) was conducted to confirm the cTnI binding to DNA 3WJ/AuNS-modified electrode. The LODs of cTnI were determined in the HEPES buffer and human serum, respectively. The present study provided the label-free, simple fabrication and easy-to-tailored detection bioprobe for AMI disease. And, the results showed the successful application of multi-functional DNA/AuNS on micro gap/PCB detection system. For our next study, this system can be integrated portable EC measurement system with Arduino or Rasberry Pi for constructing field-ready AMI biosensor in the near future that can be a great alternative to other conventional diagnosis system.

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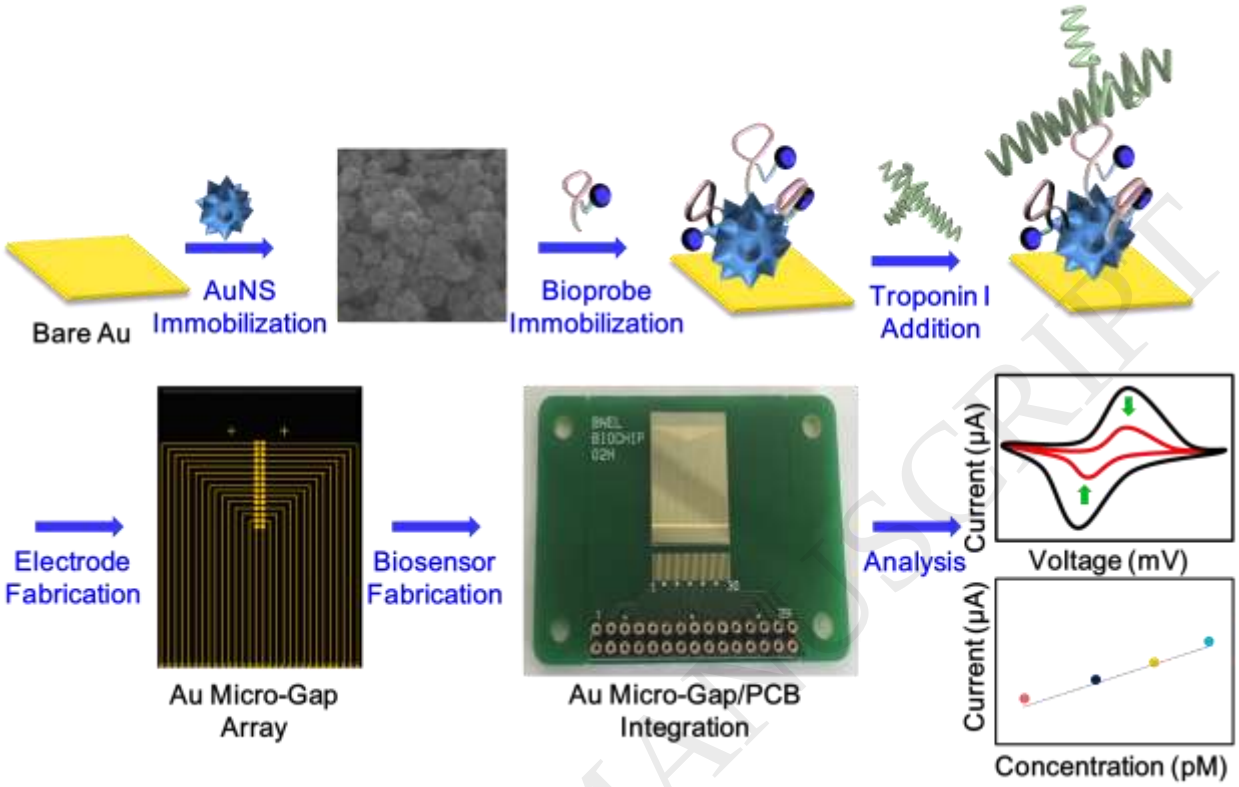


Fig. 1. Schematic image of the fabricated electrochemical biosensor for troponin I detection

Fig. 1. Lee, et al.

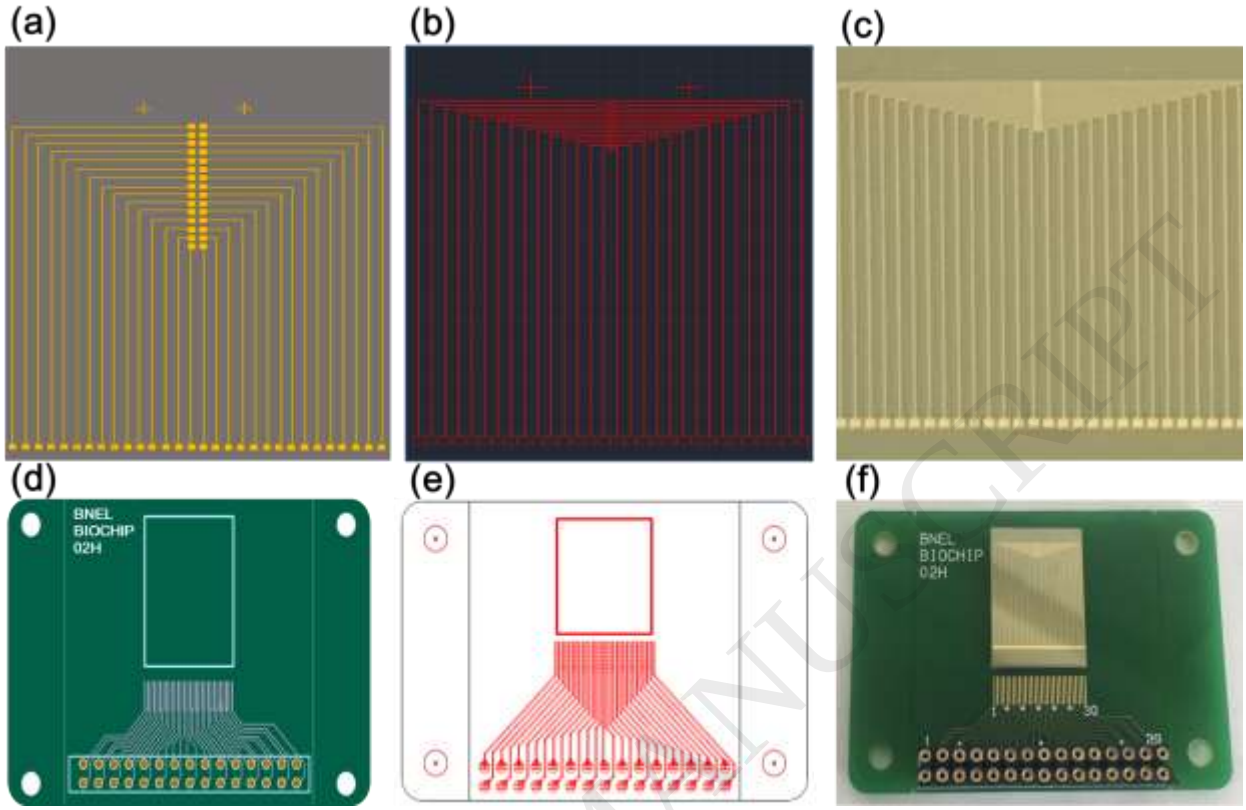


Fig. 2. (a) Schematic image of the Au micro-gap array, (b) CAD image of Au micro-gap array, (c) Real picture of fabricated Au micro-gap array, (d) Schematic image of the PCB, (e) CAD image of PCB, (f) Real picture of fabricated Au micro-gap array integrated with PCB

Fig. 2. Lee, et al.

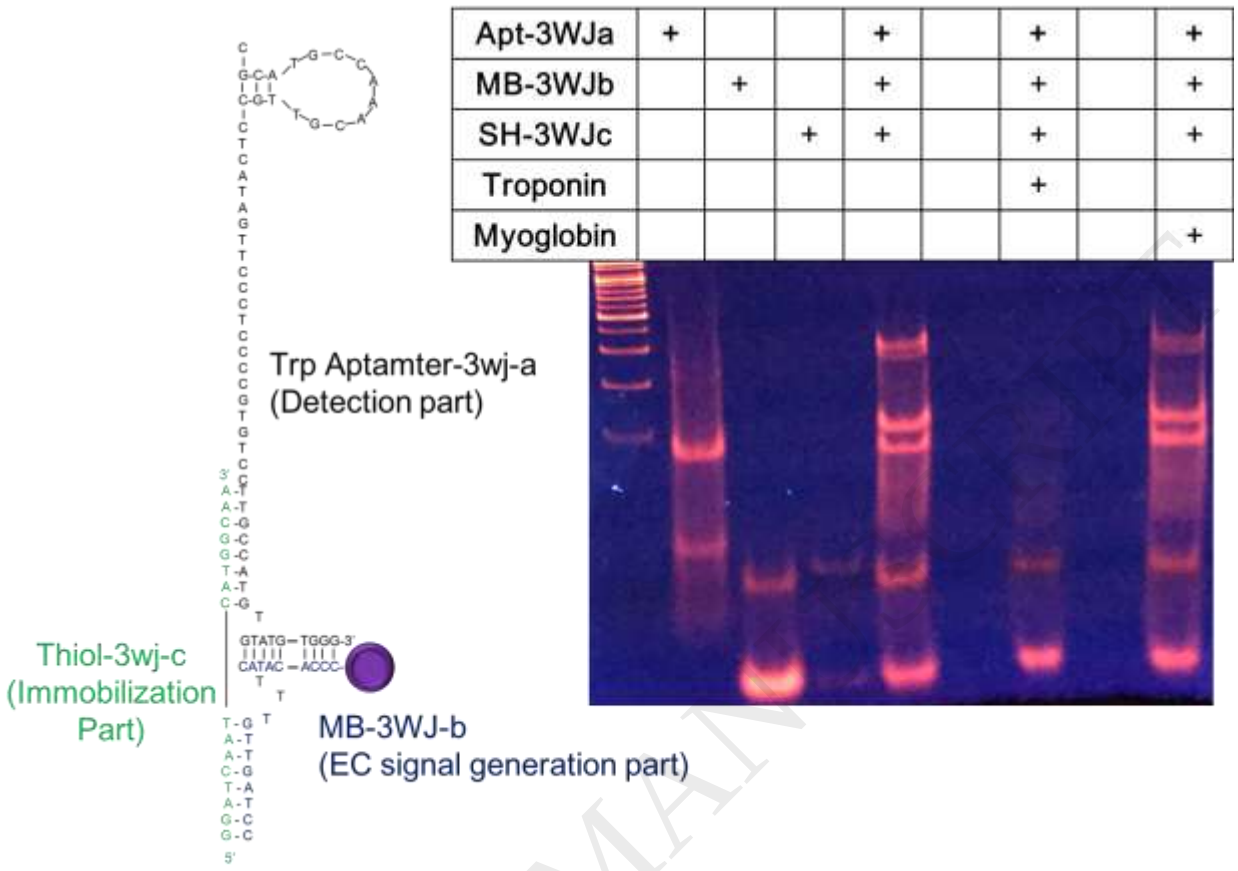
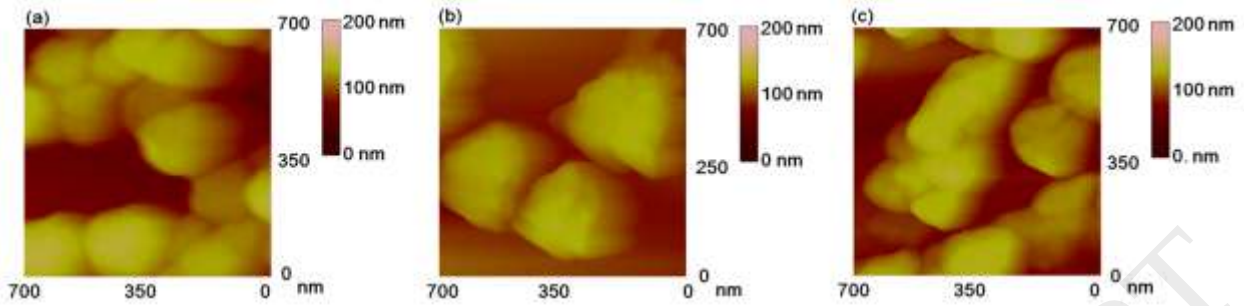
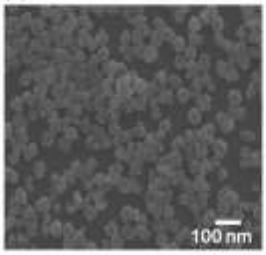


Fig. 3. (a) Schematic diagram of expected multi-functional DNA structure, (b) TBM PAGE gel result of multi-functional DNA 3WJ, troponin I and myoglobin

Fig. 3. Lee, et al.



(d)



(e)

	AuNS	AuNS/3WJ	AuNS/3WJ /Troponin I
Roughness Average (Ra) (nm)	2.940 ± 0.479	7.908 ± 1.113	7.642 ± 1.778
RMS Roughness (Rq) (nm)	7.256 ± 1.443	16.416 ± 3.175	17.719 ± 2.986
Maximum Height (Rmax) (nm)	10.943 ± 1.984	55.583 ± 7.616	44.578 ± 6.582
Vertical Distance (nm)	76.426 ± 10.312	81.717 ± 9.752	87.576 ± 11.333

Fig. 4. (a) Surface morphology of AuNS-immobilized Au substrate by AFM, (b) surface morphology of TROapt/MB/SH-DNA 3WJ on AuNS-immobilized Au substrate by AFM, (c) Surface morphology of Troponin I on TROapt/MB/SH-DNA 3WJ-modified AuNS-immobilized Au substrate by AFM, (d) Surface morphology of AuNS-immobilized Au substrate by FE-SEM, (e) Surface roughness analysis of the AuNS, TROapt/MB/SH-DNA 3WJ-modified AuNS-immobilized Au substrate and Troponin I on TROapt/MB/SH-DNA 3WJ-modified AuNS-immobilized Au substrate.

Fig. 4. Lee, et al.

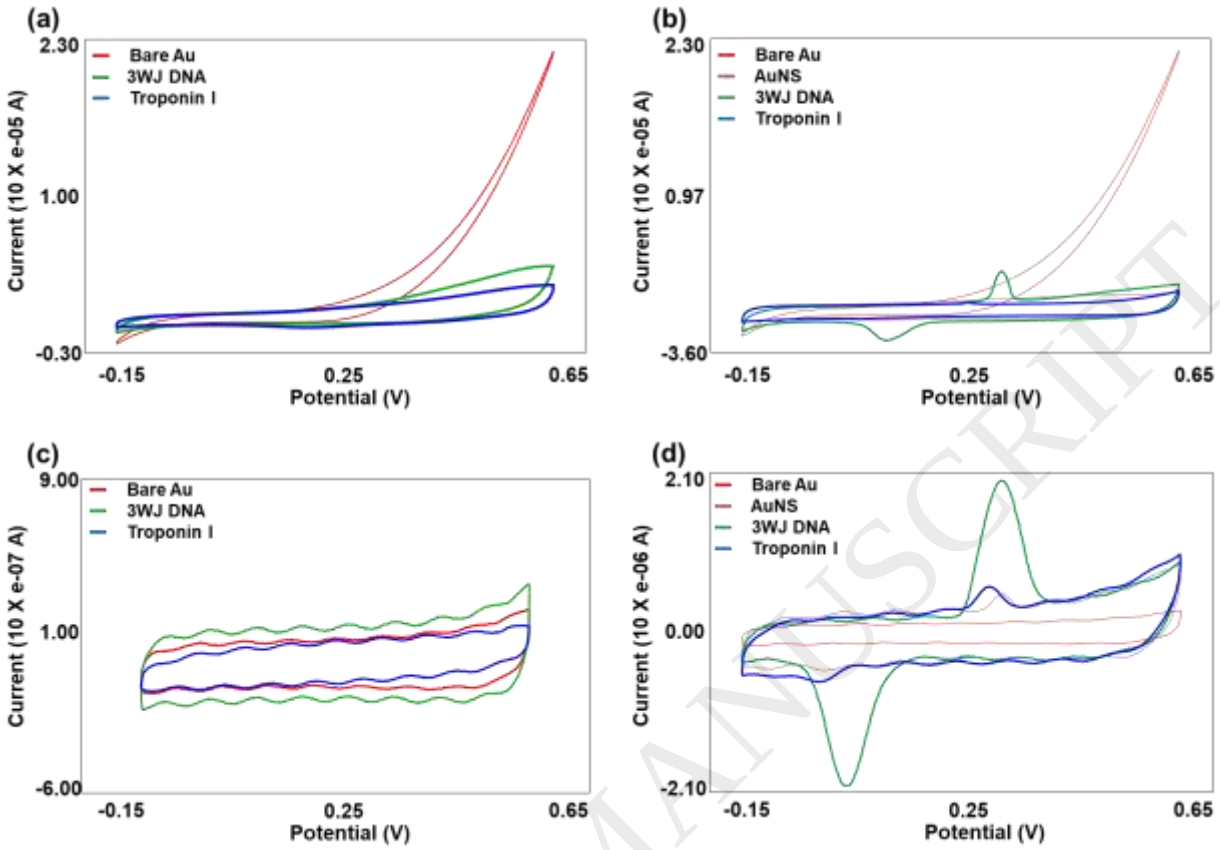
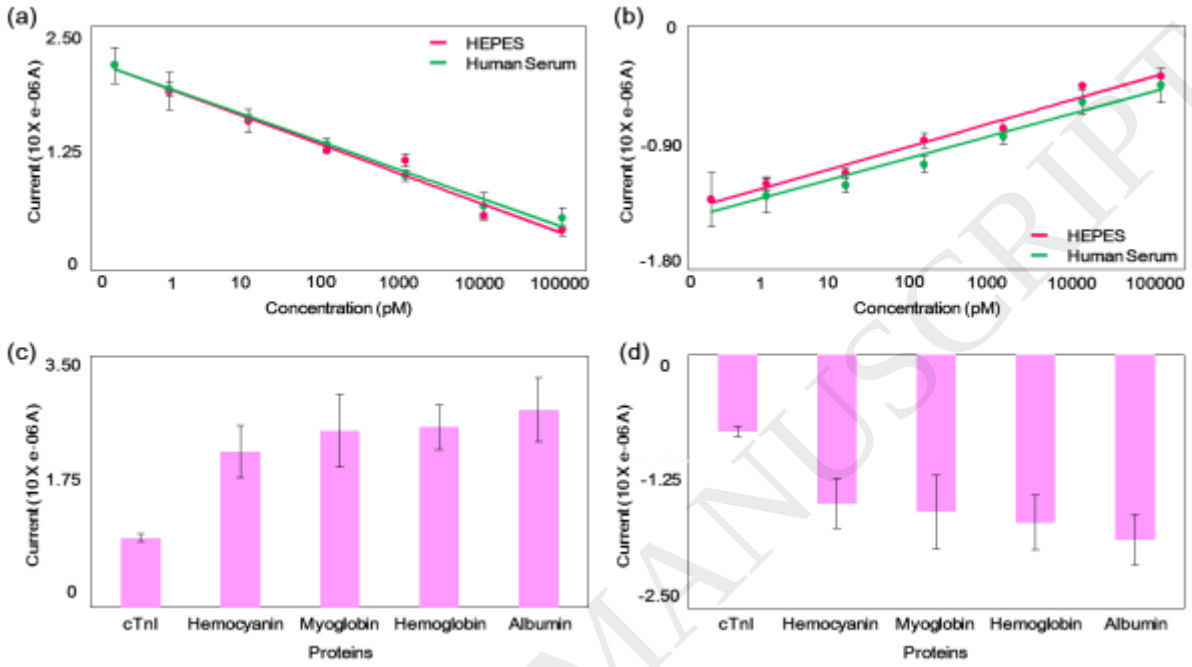


Fig. 5. (a) Cyclic voltamogram of Troponin I and 3WJ-modified Au substrate, (b) Cyclic voltamogram of Troponin I and 3WJ-modified Au substrate with AuNS, (c) Cyclic voltammogram of 3WJ immobilized on Au micro-gap array/PCB (d) Cyclic voltammogram of 3WJ/AuNS immobilized on Au micro-gap array/PCB

Fig. 5. Lee, et al.



(a) Reduction potential of the fabricated biosensor to various concentrations of cTnI in HEPES buffer and human serum (0 pM to 100 nM), (b) Oxidation potential of the fabricated biosensor to various concentrations of cTnI in HEPES buffer and human serum (0 pM to 100 nM), (c) Reduction potential of the fabricated biosensor to various target proteins in HEPES buffer (cTnI, Hemocyanin, Myoglobin, Hemoglobin, Albumin), (d) Oxidation potential of the fabricated biosensor to various concentrations of target HA protein in HEPES buffer (cTnI, Hemocyanin, Myoglobin, Hemoglobin, Albumin). Error bar represents relative standard deviation of 9 independent experiments.

Fig. 6. Lee, et al.

Table 1. Comparison of the fabricated biosensor with other biosensors for troponin I detection in terms of materials, detection method, sensitivity (LOD) and labeling step

No.	Materials	Detection Method	Detection Limit	Label-free or not	References
1	Antibody	Fluorescence	0.1 ng/mL (in a human plasma)	Antibody	[10]
2	Antibody	Amperometry	1 ng/mL	Antibody	[17]
3	Antibody	CV/EIS	0.2 ng/mL	Label-free	[18]
4	Antibody /GNP	EIS	0.2 ng/mL	Label-free	[19]
5	Antibody/AuNP	ECL	0.06 ng/mL	Label-free	[16]
6	Aptamer/AuNP	chronoamperometry	24 pg/mL (in a serum solution)	hydrazine	[12]
7	Aptamer/ Fc-SiNPs	SWV	24 pg/mL	Label-free	[21]
8	Antibody/AuNP	SAW	6.2 pg/mL	AuNP	[23]
9	antibody	EIS	0.94 pg/mL (in a human serum)	Label-free	[24]

10	**DNA 3WJ/AuNS**	**CV**	**24 pg/mL (1pM) (in a dilluted serum)**	**Label-free**	**Present work**