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Response to Cardiac Markers in Human Serum Analyzed by Guided-Mode Resonance Biosensor

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Cardiac markers in human serum with concentrations less than 0.1 ng/mL were analyzed by use of a guided-mode resonance (GMR) biosensor. Cardiac troponin I (cTnI), creatine kinase MB (CK-MB), and myoglobin (MYO) were monitored in the serum of both patients and healthy controls. Dose−response curves ranging from 0.05 to 10 ng/mL for *c*TnI, from 0.1 to 10 ng/mL for CK-MB, and from 0.03 to 1.7 *μ*g/mL for MYO were obtained. The limits of detection (LOD) for *c*TnI, CK-MB, and MYO were less than 0.05, 0.1, and 35 ng/mL, respectively. Analysis time was 30 min, which is short enough to meet clinical requirements. Antibody immobilization and the hydrophilic properties of the guided-mode resonance filter (GMRF) surface were investigated by X-ray photoelectron spectroscopy (XPS) and by monitoring the peak wavelength shift and water contact angle (CA). Both assays used to evaluate the surface density of the immobilized antibodies, a sandwich enzyme-linked immunosorbent assay (ELISA) and a sandwich immunogold assay, showed that the antibodies were successfully immobilized and sufficiently aligned to detect the low concentration of biomarkers. Our results show that the GMR biosensor will be very useful in developing low-cost portable biosensors that can screen for cardiac diseases.

Cardiac markers such as cardiac troponin I (cTnI), creatine kinase MB (CK-MB), and myoglobin (MYO) are useful tools for diagnosing acute myocardial infarction (AMI).[1,2] For example, cTnI is a preferred marker for diagnosing myocardial injury because it is a highly sensitive and specific biochemical marker for myocardial cell damage.[3,4] Therefore, serial measurement of cTnI has become an important tool for risk stratification of patients presenting with acute coronary syndrome. CK-MB and MYO are also valuable diagnostic markers for the majority of patients with suspected myocardial damage. They have traditionally been used as the standard biochemical markers for the diagnosis of AMI.[5]

Currently, hospital laboratories use immunoassays with a labeling technology that an enzyme-linked immunosorbent assay (ELISA)[6−8] to detect cardiac makers such as cTnI, CK-MB, and MYO. However, labeling technologies need more time than label-free detection of the direct interaction between antigen and antibody without the detection (secondary) antibody. There are various label-free detection methods reported that use optical technologies[9−15] and microstructured devices[16,17] for quantitatively measuring biological molecule concentrations. An optical biosensor is attractive because it can detect low concentrations of biomarkers. Various optical biosensors have been reported, including surface plasmon resonance (SPR),[9,10] waveguide-type biosensor, interferometer-based biosensor, disk resonator biosensor,[11] and guided-mode resonance (GMR) biosensors.[12−15]

Guided-mode resonance filters (GMRF) are powerful instruments that monitor reflection (or transmission) processes at the interface in different chemical and biological research areas.[15] Their label-free detection of proteins has become an important topic in biological analysis. It has inherent merits of simple operational procedures and avoidance of disturbances from conjugated labels such as fluorescent dye or gold nanoparticles

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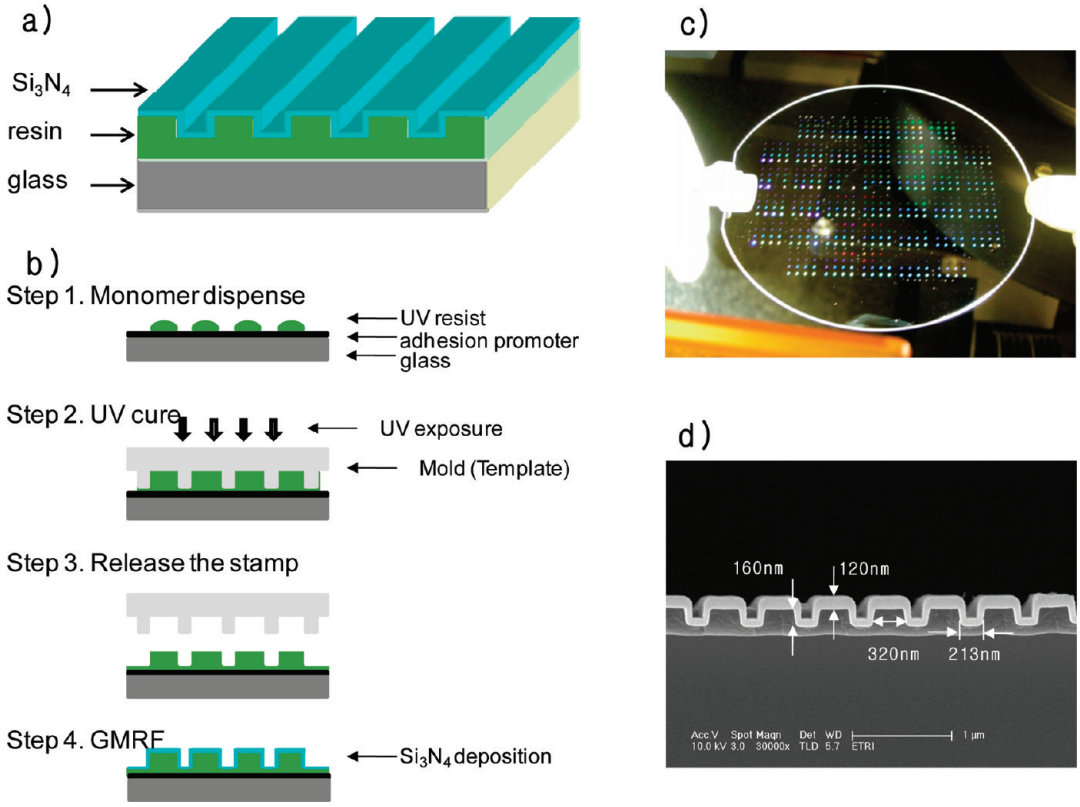

Figure 1. (a) Structure of a GMRF, (b) schematic illustration of the fabrication procedure, (c) photograph of the GMRF array fabricated on a 6-in. wafer, and (d) cross-section FE-SEM image of the fabricated GMRF.

(Au NPs).[18,19] A GMRF as an optical sensor has a simple structure with fewer layers and superior spectral properties (i.e., high efficiency, narrow bandwidth, and angular sensitivity).[20−23] GMRF biosensor chips enable mass production and reduced fabrication costs by using nanoimprinting technology.[24,25]

In this paper, we report the analysis of cardiac markers in human serum samples by using a GMR biosensor. Good linearity was observed for cardiac markers (cTnI, CK-MB, and MYO) with coefficients of variation (CV) under 20%. The GMRFs used for this CV test were fabricated by an imprinting method with the antibodies immobilized on the surface. To test the immobilization status of the antibodies, we measured the surface using the water contacting angle (CA) and X-ray photoelectron spectroscopy (XPS). Using a GMRF, we observed the real-time peak wavelength shift during a chemical reaction between the aldehyde-linked (−CHO) Si_3N_4 surface and the amine ($-NH_2$) group of lysine residues in the antibodies. We analyzed the surface density of the immobilized antibodies using a sandwich ELISA and sandwich-type immunogold assay, obtaining sufficient sensitivity to detect low concentrations of biomarkers.

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EXPERIMENTAL SECTION

Structure and Fabrication of Guided-Mode Resonance Filters. Figure 1a depicts the structure of a GMRF with a subwavelength periodic structure. The grating layer has a periodic modulated structure on top of a substrate. Si_3N_4 with a high refractive index of 2.0 was coated onto the grating layer to create the guided mode in the filter. The filter reflects light when the wavelength of the incident light is matched to the resonant condition of the guided mode.

The GMRF with high refractive index materials was fabricated by using flash imprint lithography (S-FIL) and plasma-enhanced chemical vapor deposition (PECVD) (Figure 1b). The transparent glass substrate was rinsed with acetone, ethanol, and deionzed (DI) water and then dried with N_2 gas. The adhesive polymer resin (monomer) used as an adhesion promoter was spin-coated onto the substrate at 3000 rpm for 60 s, resulting in a thickness of 60 nm. It was baked on a hot plate at 120 °C for 60 s to evaporate the solvent, and a UV resist was dispensed on top.

In the next step, the UV resist was filled in a quartz master mold with a period of 533 nm and cured for approximately 1 min under a UV lamp. The master mold then was released from the substrate. After imprinting, Si_3N_4, the high refractive index material, was deposited onto the nanometer-scale grating structure by the PECVD technique to create the guided mode. The refractive index of Si_3N_4 was measured with an ellipsom-

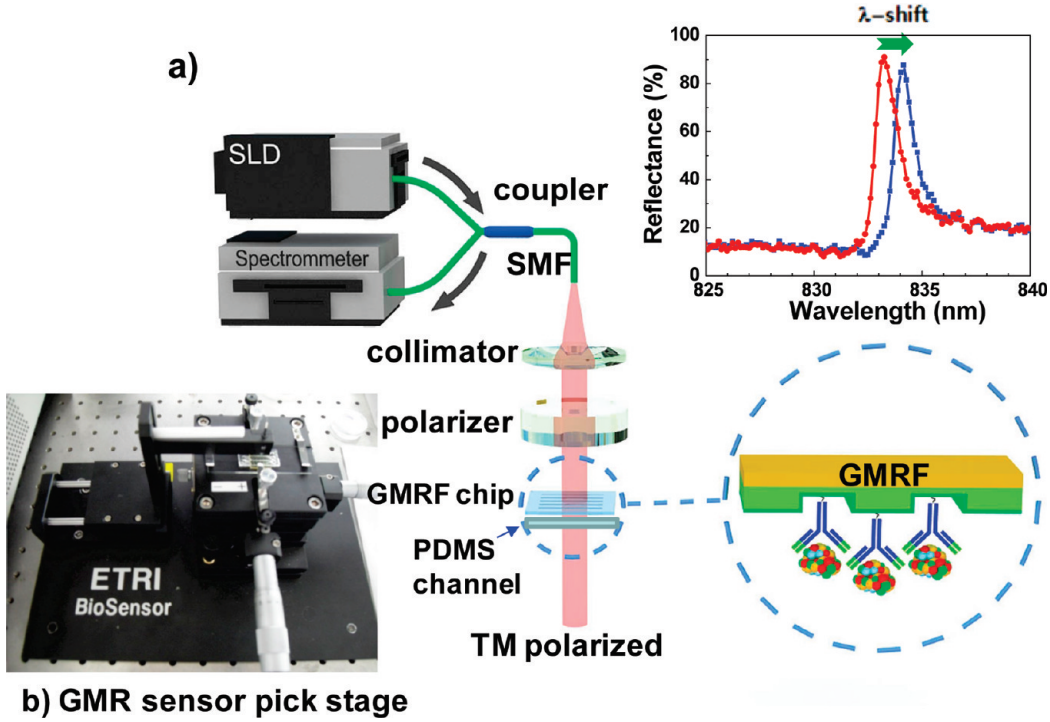

Figure 2. (a) Schematic diagram of the experimental setup for measuring the peak wavelength of a GMRF and (b) GMR sensor stage (SLD, super luminescent diode; SMF, single-mode fiber; GMRF, guided-mode resonance filter).

eter to be ~2.0 at a wavelength of 633 nm. The PECVD instrument was operated at 13.56 MHz and 600 W, with a process pressure of 0.9 Torr. The Si_3N_4 films were deposited at 150 °C with a SiH_4/N_2 gas flux ratio of 117/900 sccm. The deposited Si_3N_4 layer was treated with oxygen plasma to render the surfaces hydrophilic, which is necessary for effective biomolecule immobilization. The Si_3N_4 coating thickness was 120 nm.

Figure 1c depicts a GMRF array fabricated onto a glass wafer. The grating area of the fabricated GMRF was 0.5 × 0.5 mm^2. Figure 1d shows the cross-section SEM image of the fabricated GMRF. The height (h_s) of the grating step was 160 nm, and the widths of the grating step and groove were 320 and 213 nm, respectively.

Setup for Measuring Peak Wavelength. As the cardiac markers are captured by the antibody-immobilized surface of the GMRF, the reflection peak wavelength of the GMRF is shifted due to an increase in the thickness of the waveguide layer. The concentration of the cardiac markers can thus be measured by observing the shift in peak wavelength. Figure 2 shows a schematic diagram of the experimental setup for measuring the GMRF peak wavelength. A super luminescent diode (SLD) was used as a broadband light source with the following settings: output power of 1 mW, a 3 dB spectral line width, and central wavelengths of 30 and 799 nm. The light output was split and transmitted to a collimation lens through a 50:50 fiber coupler and a single-mode fiber (SMF) at a wavelength of 780 nm. The transverse magnetic (TM) -polarized collimated beam illuminated onto the GMRF through a linear polarizer; that is, the polarization axis was perpendicular to the GMRF line, because the fabricated GMRF has a narrower resonance line width for the TM-polarized light compared to transverse electric (TE) -polarized light. The grating surface of the GMRF was set to face downward (see Figure 2, inset circular diagram) to remove the deposition effect of proteins. The reflection light from the GMRF was retransmitted through the SMF and 50:50 fiber coupler and then inserted into an optical spectrometer (Andor, Shamrock SR-500i) with a wavelength resolution of 0.13 nm. For precise measurement of the GMRF reflection peak wavelength, we fit the curve to the second-degree polynomial from the reflection spectrum.

Antibody Immobilization. The immobilization of an antibody or receptor is required to induce the specific binding of a target biomarker. The deposited Si_3N_4 layer was treated by oxygen plasma to form hydroxyl groups (−OH) for chemical conjugation between the antibodies and the Si_3N_4 surface. Antibodies were immobilized onto the GMRF surface through self-assembled monolayers (SAMs), surface aldehyde formation, and antibody attachment sequentially (Figure 3a). This is the initial step in fabricating a chemically active GMRF surface to immobilize the cTnI, CK-MB, and MYO antibodies. In step 1, the GMRF surface was treated with oxygen plasma for 300 s (30 pa, 100 mL/min oxygen, and 100 W). The hydroxylated surface was then silanized for 30 min in ethanol containing 1 wt % 3-aminopropyl triethoxysilane (APTES, Sigma-Aldrich) to form surface amino functional groups (step 2). After the surface was rinsed with ethanol, the GMRF was baked at 120 °C for 15 min. Reactive amino groups were then introduced to the surface. The amine-modified surface was treated with glutaric aldehyde (GA, Sigma−Aldrich) as a bifunctional cross-linking agent that converts amino groups into reactive aldehyde (CHO) groups that can be coupled with anti-cTnI (step 3). GA treatment was performed by dipping the amino-silanized surface of the GMRF into 10 mL of GA solution containing 25 wt % GA and 0.1% sodium cyanoborohydride ($NaBH_3CN$, Sigma−Aldrich) in DI water for 4 h at room temperature. The GMRF chips were then rinsed with DI water and dried under nitrogen. For antibody coupling to the CHO

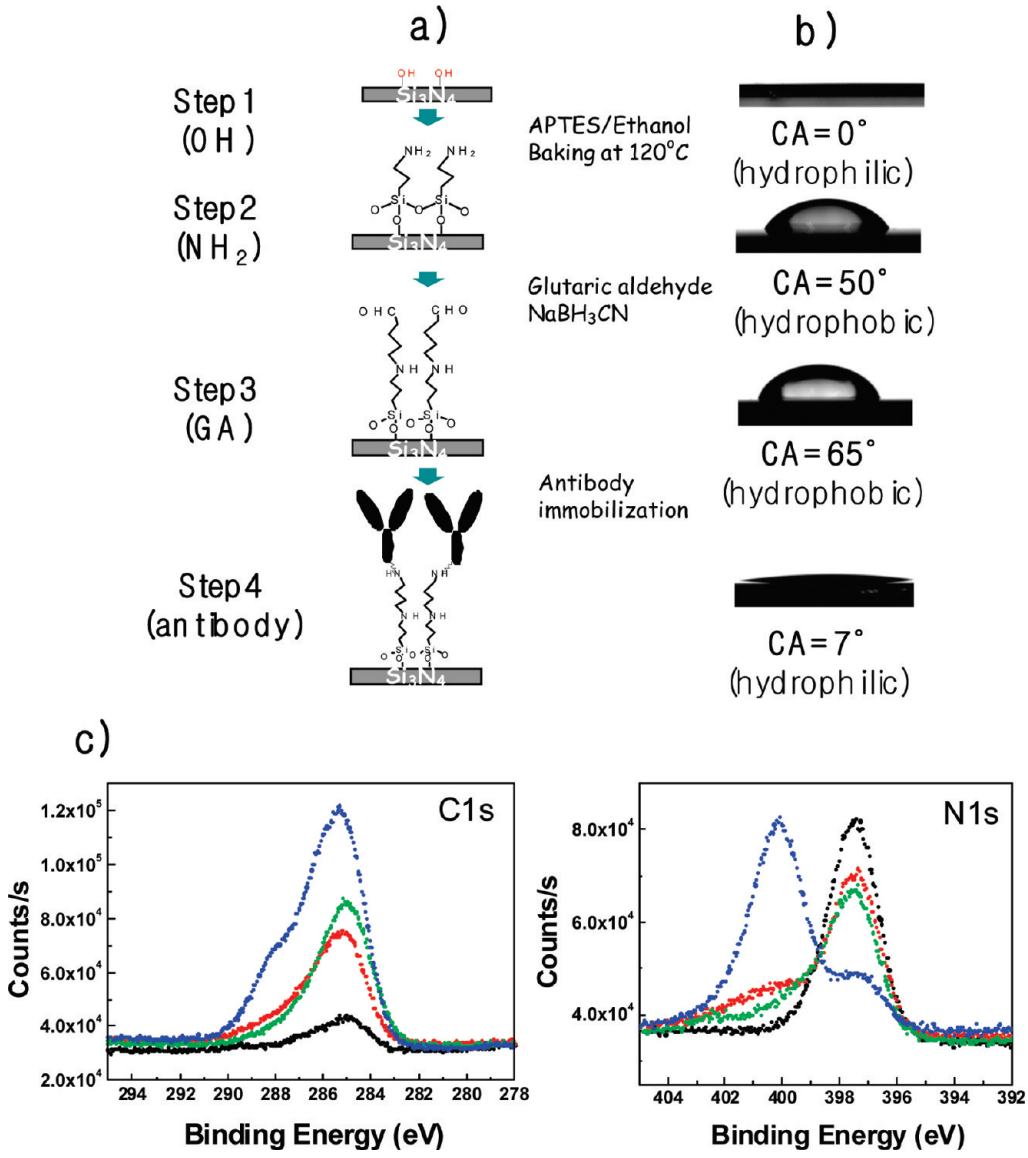

Figure 3. (a) Schematic representation of the antibody-immobilization process on the GMRF surface and (b) water contact angle measured at each antibody immobilization process. (c) X-ray photoelectron spectra of C1s and N1s for functionalized Si_3N_4 surfaces after plasma (black), APTES (red), glutaraldehyde (green), and antibody (blue) treatments.

groups, the GMRF chips were incubated at 4 °C for 2 h in each of the three cardiac antibody solutions (Standard Diagnostics, Inc.) that had antibody concentrations of 0.2 mg/mL (10 mM PBS buffer, pH 7.4). The antibody-immobilized chip was soaked in 1× PBS buffer with 3% bovine serum albumin (BSA, Pierce) at 4 °C for 30 min to block any residual CHO groups and then rinsed with PBS solution and DI water (step 4).

Human Serum Separation. Human serum samples with a wide range in cardiac marker concentrations were provided by Seoul National University Bundang Hospital (SNUBH). Peripheral blood was collected in serum separation tubes. After fibrinogen or other clotting factors were removed by centrifugation for 7 min at 3000 rpm, the sera were separated. After the turbidity of the sera was inspected, turbid samples were centrifuged and aspirated again to remove any remaining insoluble materials. The clear sera were transferred to clean specimen tubes by use of Pasteur pipets. cTnI, CK-MB, and MYO concentrations in the separated sera were measured by a cardiac test tool (Dimension RxL Max integrated chemistry system, Siemens). The concentrations prepared here were as follows: cTnI at 0, 0.05, 0.1, 1, 5, and 10 ng/mL; CK-MB at 0.1, 2, 4.4, 7, and 10 ng/mL; and MYO at 35, 75, 200, 500, and 1702 ng/mL.

RESULTS AND DISCUSSION

Since the GMRF surface should be hydrophilic to improve binding efficiency with biomarkers, we investigated immobilization processes using the water contact angle (CA) measurement.[26] Measurements were taken after each reaction step under atmospheric laboratory conditions (25 °C and 32% humidity). Results and images measured are depicted in Figure 3b. The water CAs of the hydroxylated surface (step 1) and antibody-modified surface (step 4) were 0° and 7°, respectively. The silanized surface (step 2) and the aldehyde surface (step 3) measured 50° and 65°, respectively. The change in the value of water CA reveals the hydrophobic/hydrophilic property of the surface, which can be

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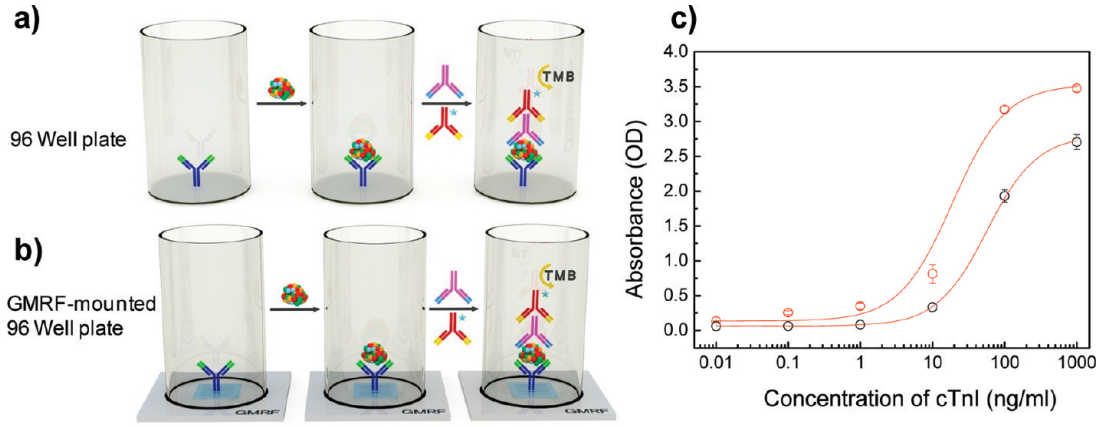

Figure 4. Activity test for the surface density of the immobilized antibodies using a typical sandwich ELISA: Schematic diagrams of (a) a typical sandwich ELISA using a 96-well plate, (b) a GMRF-mounted 96-well plate, and (c) the measured absorbance of the 96-well plate (black circles) and the GMRF-mounted 96-well plate (red circles) as a function of the antigen concentration measured by the ELISA test at 450 nm wavelength. Conditions were as follows: coating buffer (1× PBS) was incubated for 2 h at 20 °C; blocking buffer was composed of 0.5 μL of 1% casein-PBS incubated at 20 °C for 30 min; washing buffer was composed of PBS in 0.05% Tween-20; target antigen (cTnI) at concentrations of 0, 0.01, 0.1, 1, 10, 100, and 1000 ng/mL was incubated at 20 °C for 30 min.; 50 μL of the specific polyclonal antibody (0.5 μg/mL) for 1 h at 20 °C; TMB color developing time of 10 min (blue); stopped solution 20 min later with 50 μL of 1 N HCl (yellow).

related to silane SAM formation and the immobilization of antibodies. Proteins are relatively hydrophilic due to an abundance of amino acids, carboxylic acid groups, and peptide bonds. Therefore, the water CA decreases from 65° in the aldehyde surface to 7° after antibody immobilization, indicating that immobilization on the GMRF surface was complete.

To verify successful surface functionalization and antibody immobilization, we also monitored the GMRF surface after each reaction step using X-ray photoelectron spectroscopy. Figure 3c shows the XPS data for the functionalization of Si_3N_4 with linker molecules (APTES and glutaraldehyde) and antibodies. The C1s spectra show an increase in peaks at 285.0 eV after each reaction step, associated with aliphatic carbons (C−H), and confirm the successful attachment of the linker molecules (APTES and glutaraldehyde) and antibodies. Following the reaction of the oxidized Si_3N_4 surface with APTES, an increase of the C1s peak at 286.5 eV (red) is observed, assigned to carbons linked to free and protonated amino groups.[27] The N1s spectrum (red) shows the appearance of a broad peak around 400 eV. This is associated with the convolution of two peaks at 399.7 eV (Si_3N_4) and 401.9 eV, assigned to the free (NH_2) and protonated (NH_3^+) amines of APTES, respectively.[26−28] Furthermore, the Si_3N_4 peak at 397.5 eV decreases due to the densely packed APTES, which allows fewer photoelectrons to be detected. After glutaraldehyde treatment, the C1s peak intensity (green) at 286.5 eV increases, and the N1s spectrum (green) exhibits a decrease in peaks at 399.7 eV (amines) and 401.9 eV (Si_3N_4). These results confirm the reaction of amino groups with glutaraldehyde. After the immobilization of antibodies on the aldehyde-modified Si_3N_4 surface, the C1s spectrum (blue) showed a peak increase at 286.4 eV associated with peptide-bonded carbons.[27,28] The XPS N1s spectrum (blue) of the antibody-immobilized surface shows a considerable increase in the peak at 400.1 eV, primarily assigned to peptide bond nitrogens (O=C−N). A further decrease in the Si_3N_4 peak at 397.5 eV is observed after antibody immobilization (size = 14 nm),[16] since the electron escape depth is 10 nm at most.[29] Taken together, the XPS data confirm the successful attachment of linker molecules and antibodies.[30]

Antibody immobilization was also verified by use of a GMR biosensor measurement system. We first monitored the binding interaction between the aldehyde-linked Si_3N_4 surface and the amine group of lysine contained in antibody in real time. Figure 2 shows the formation of imines (Schiff bases) during the reaction between amines and either an aldehyde or a ketone, proceeding through a carbinolamine intermediate. The peak wavelength of the GMRF was red-shifted after addition of antibodies onto the aldehyde-activated surface (Figure S1a in Supporting Information). Figure S1b shows the increasing peak wavelength shift as a function of antibody immobilization time. The shift (λ-shift) in peak wavelength (nanometers) indicates a change in the quantity of antibodies immobilized onto the GMRF surface, which was saturated 20 min after the antibody was added at room temperature.

It is also important to verify the surface density of the immobilized antibodies because the sensitivity of a biosensor to detecting antibody−antigen interactions corresponds with antibody activity. Before measurement of biomarker concentrations in human serum, the surface density of the immobilized anti-cTnI on the GMRF chips was investigated by two assays: a sandwich ELISA and a sandwich-type immunogold assay that used gold colloids conjugated with detection antibodies.

The activity of the immobilized antibodies was verified by a typical sandwich ELISA with a standard 96-well plate and a GMRF-mounted 96-well plate. The GMRF-mounted 96-well plate was assembled by replacing the bottom of a standard 96-well plate (Standard Lids, Nunc version) with the aldehyde-reactivated GMRF plate. Figures 4 panels a and b show the sandwich ELISA process for the typical 96-well plate and the GMRF-mounted 96-

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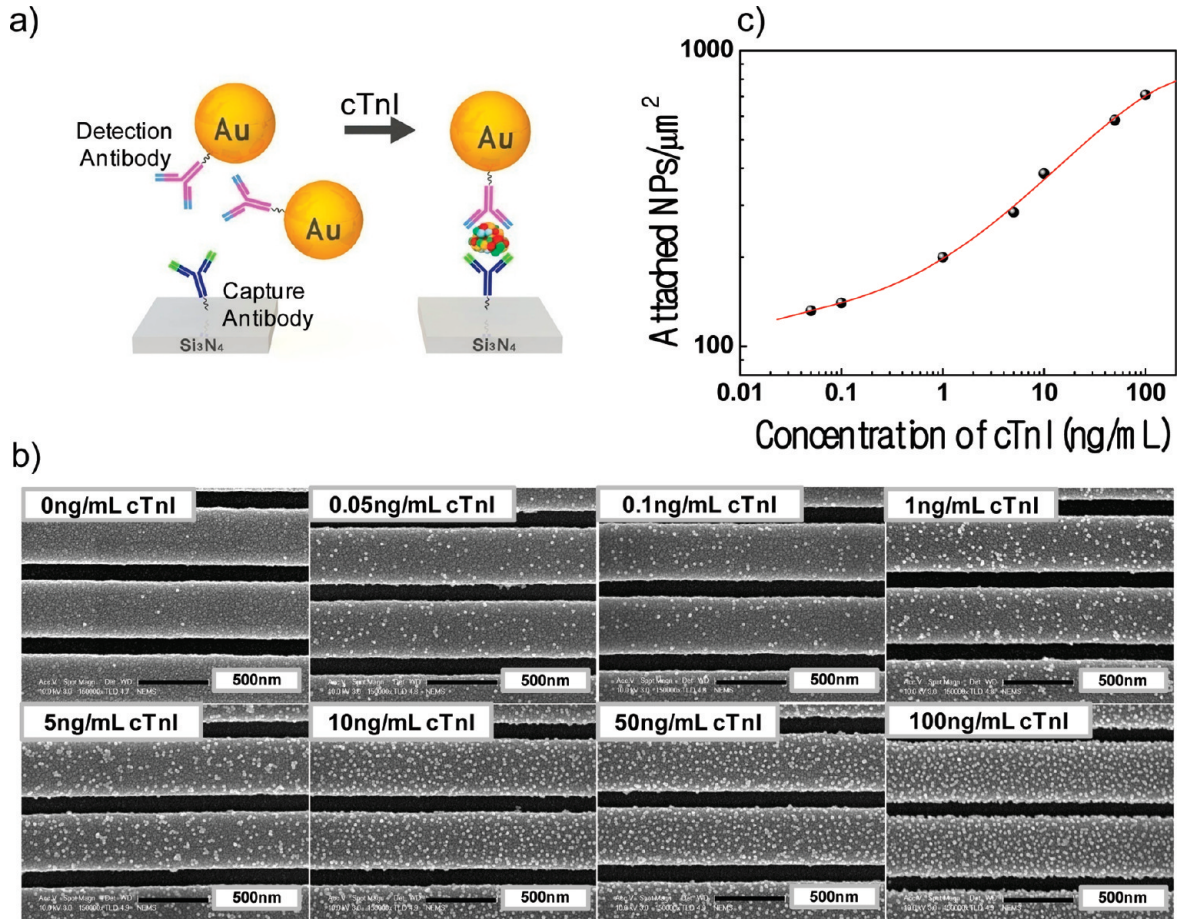

Figure 5. Activity test for the surface density of the immobilized antibodies by an immunogold assay. (a) Schematic diagram of the detection principle; (b) FE-SEM images of anti-cTnI immobilization efficiency examined by a sandwich-type polyclonal anti-cTnI Au NPs conjugate immunoassay; (c) quantitative relationship between the immobilization efficiency and concentration of cTnI tested by the immunogold assembly. The surfaces immobilized with anti-cTnI were immersed in a solution of 0, 0.05, 0.1, 1, 5, 10, 50, and 100 ng/mL cTnI antigen (1× PBS, pH 7.4) for 20 min and then immersed in the polyclonal cTnI-conjugated Au NPs (0.4 nM) for 60 min.

well plate, respectively. Monoclonal anti-goat cTnI (Standard Diagnostics, Inc.) was immobilized onto the bottom surface of both 96-well plates. Target cTnI antigen (HyTest Ltd.) solutions at 0, 0.01, 0.1, 1, 10, 100, and 100 ng/mL were added separately to investigate immobilized antibody activity. After incubation, polyclonal anti-mouse cTnI (Standard Diagnostics, Inc.), a secondary antibody, and anti-mouse IgG (Fc-specific) horseradish peroxidase (HRP, Sigma) were sequentially added into the 96-well plates. The plates were then treated with 3,3′, 5,5′-tetramethylbenzidine (TMB, Sigma) and quenched by 1 N HCl. The black circles in Figure 4c show the absorbance at 450 nm for the various concentrations of cTnI observed in typical 96-well plates. The red circles in Figure 4c depict the absorbance of the GMRF-mounted 96-well plate. The absorbance of the GMRF-mounted 96-well plate is slightly higher than that of the typical 96-well plate, indicating that the aldehyde-modified GMRF has higher surface density of immobilized antibodies for specific binding to cardiac markers. This can be attributed to the difference in antibody immobilization via covalent bonding to the Si_3N_4 surface of the GMRF-mounted 96-well plate and physical adsorption to the polystyrene surface of a typical 96-well plate.

To further investigate the surface density of the immobilized antibodies, a sandwich immunogold assay was performed. Gold nanoparticles (Au NPs) were conjugated to detection antibodies (polyclonal anti-cTnI) as shown in Figure S2 (Supporting Information). These polyclonal anti-cTnI-conjugated Au NPs would attach to the GMRF surface only when the target antigen was captured by the immobilized antibody. Therefore, the surface density of the immobilized antibodies on the GMRF chip can be investigated by measuring the amount of conjugated Au NPs. Au NPs were synthesized according to literature procedures.[31] The diameter of Au NPs used here was 13 nm, and the Au NPs solution was fixed at pH ~ 9 with K_2CO_3 (Sigma−Aldrich). The polyclonal anti-cTnI (Standard Diagnostics, Inc.) was added to the solution. The addition of 1 M NaCl (Sigma−Aldrich) to the conjugated Au NPs solution confirmed successful antibody conjugation. Antibody-labeled Au NPs can be attached to the surface to capture the target antigen (cTnI), as shown in Figure 5a. To capture cTnI, the antibody-immobilized GMRF chips were incubated with the target antigen at concentrations of 0, 0.05, 0.1, 1, 5, 10, 50, and 100 ng/mL. After incubation, each GMRF surface was treated with the antibody-labeled Au NPs. Figure 5b shows the field emission scanning electron microscopy (FE-SEM) images

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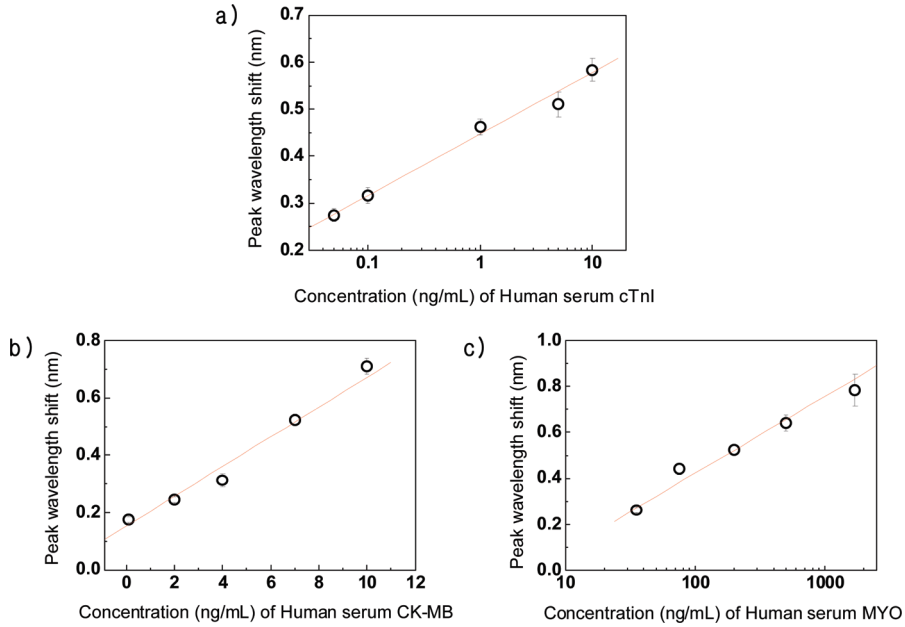

Figure 6. Dose−response curves: (a) cTnI between 0.05 and 10 ng/mL (healthy people have 0−0.05 ng/mL cTnI), (b) CK-MB between 0.1 and 10 ng/mL (healthy people have 0−3.6 ng/mL CK-MB), and (c) MYO between 0.03 and 1.7 μg/mL (healthy people have 10−92 ng/mL MYO).

of the immunogold NP-attached GMRF surfaces. The number of immunogold NPs attached to the GMRF surface increased when the concentration of the cTnI antigen increased. Figure 5c shows the quantitative relationship between the number of attached immunogold NPs and the concentration of the cTnI antigen. The numbers of NPs per square micrometer bound onto the GMRF surface for each concentration of cTnI were 708 f for 100 ng/mL, 582 for 50 ng/mL, 384 for 10 ng/mL, 284 for 5 ng/mL, 200 for 1 ng/mL, 140 for 0.1 ng/mL, 132 for 0.05 ng/mL, and 4 for 0 ng/mL. The number of NPs was proportional to cTnI concentration, indicating that anti-cTnI was efficiently immobilized onto the GMRF chip and confirming that some of the antibodies are able to recognize the cognate antigen. Furthermore, the plot of number of particles versus concentration exhibits a slightly sigmoidal behavior, similar to the immunoassay data in Figure 4c.

To insert the prepared human serum into the GMRF chip, a PDMS [poly(dimethylsiloxane)] fluidic channel was attached to the GMRF chip surface, as illustrated in Figure 2. We monitored the peak reflection wavelength of the GMRF chip in real time as the human serum was inserted. Specific binding between the immobilized antibodies and antigens in the human serum occurred as the serum was inserted. The saturation time of specific binding was approximately 30 min. Therefore, we measured the relative shift between the peak wavelength initially and after 30 min for the various concentrations of antigens. Results for cTnI, CK-MB, and MYO are depicted in Figure 6 panels a−c, respectively.

Peak wavelength shift increased with increasing antigen concentration. Linear dose−response relationships ranged from 0.05 to 10 ng/mL for cTnI, from 0.1 to 10 ng/mL for CK-MB, and from 0.03 to 1.7 μg/mL for MYO, respectively. The lowest concentrations detected in human serum were 50 pg/mL for cTnI, 100 pg/mL for CK-MB, and 35 ng/mL for MYO. Because the peak shift is much larger than the resolution (~0.1 nm) of an optical spectrometer, concentrations less than 50 pg/mL can be detected. These results are promising for AMI screening, as the boundary concentrations between a healthy person and a patient for cTnI, CK-MB, and MYO are 0.05, 3.6, and 92 ng/mL, respectively.[31] Coefficients of variation (CV) from the mean and standard deviation (SD) of 14, 10, and 10 human serum samples for cTnI, CK-MB, and MYO, respectively, were within 20% (see error bars in Figure 6).

To test nonspecific binding, CK-MB antigens (10 ng/mL) were added to the serum with 0.05 ng/mL cTnI, and the peak wavelength shift was observed on the anti-cTnI antibody immobilized sensor surface. The shifted wavelengths are similar to the cTnI serum without CK-MB antigens (Figure S7 in Supporting Information), indicating that the anti-cTnI antibody immobilized sensor surface is specific for cTnI antigens in human serum.

CONCLUSION

In summary, GMRF chips were evaluated for the analysis of cTnI, CK-MB, and MYO concentrations in human serum to diagnose cardiac disease. The GMRF chip was fabricated by UV-nano imprint lithography (UV-NIL) and plasma-enhanced chemical vapor deposition (PECVD) techniques that produce inexpensive, portable biosensors. In addition, we investigated antibody immobilization characteristics, such as the surface density and hydrophilicity of the immobilized antibody. The GMRF chip surface was characterized at all stages of the immobilization process by monitoring the GMRF peak wavelength shift (i.e., surface density) and measuring the CA (i.e., hydrophilicity). Antibody immobilization and surface density of the immobilized antibodies were confirmed by two methods: a typical sandwich ELISA with the enzyme-mediated reaction between HRP and the TMB substrate, and an immunogold conjugate with gold colloids. Results confirmed that the surface density of the immobilized antibodies was well aligned and immobilized to detect low concentrations of biomarkers.

Dose−response curves ranging from 0.05 to 10 ng/mL for cTnI, from 0.1 to 10 ng/mL for CK-MB, and from 0.03 to 1.7 μg/

mL for MYO were obtained. The limits of detection (LOD) of cTnI, CK-MB, and MYO were estimated to be 0.05, 0.1, and 35 ng/mL, respectively. We are able to distinguish between normal and elevated levels of these antigens in human serum samples because the LOD are less than the boundary concentrations between a healthy person and a patient (i.e., 0.05 ng/mL for cTnI, 3.6 ng/mL for CK-MB, and 92 ng/mL for MYO).[32] GMR sensor technology will be useful in developing low-cost and portable biosensors that can screen for cardiac diseases.

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SUPPORTING INFORMATION AVAILABLE

Eight figures showing reflection spectra of the GMRF chip and measured peak wavelength shift; one-step fabrication of polyclonal anti-cTnI-conjugated Au NPs; AFM images of GMRF chip surface after each reaction step; coefficients of variation for cTnI, CK-MB, and MYO measured in human serum; nonspecific binding of anti-cTnI immobilized sensor surface; and ellipsometry thickness measurement. This material is available free of charge via the Internet at http://pubs.acs.org.

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