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Environmental Stability of Plasmonic Biosensors Based on Natural versus Artificial Antibody

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Abstract

Plasmonic biosensors based on the refractive index sensitivity of localized surface plasmon resonance (LSPR) are considered to be highly promising for on-chip and point-of-care biodiagnostics. However, most of the current plasmonic biosensors employ natural antibodies as biorecognition elements, which can easily lose their biorecognition ability upon exposure to environmental stressors (e.g., temperature and humidity). Plasmonic biosensors relying on molecular imprints as recognition elements (artificial antibodies) are hypothesized to be an attractive alternative for applications in resource-limited settings due to their excellent thermal, chemical, and environmental stability. In this work, we provide a comprehensive comparison of the stability of plasmonic biosensors based on natural and artificial antibodies. Although the natural antibody-based plasmonic biosensors exhibit superior sensitivity, their stability (temporal, thermal, and chemical) was found to be vastly inferior to those based on artificial antibodies. Our results convincingly demonstrate that these novel classes of artificial antibody-based plasmonic biosensors are highly attractive for point-of-care and resource-limited conditions where tight control over transport, storage, and handling conditions is not possible.

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ASSOCIATED CONTENT

Supporting Information

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LSPR shift along the molecular imprinting process, stability of the MIP-based biosensor, and concentration-dependent LSPR shift of freshly prepared and 1-weekstored plasmonic biosensors based on natural antibody (PDF)

Graphical Abstract

Accessible and affordable health care needs in developing countries have motivated the development of budiagnostic devices that can be deployed in resource-limited settings. Owing to the label-free detection, high sensitivity, simple operation, and optical read-out, plasmonic biosensors based on the refractive index sensitivity of localized surface plasmon resonance (LSPR) of metal nanoparticles are considered to be highly attractive for point-of-care (POC) devices.^{1–3} LSPR involves the collective oscillation of dielectrically confined conduction electrons, which results in a number of unique optical properties such as large absorption and scattering cross sections and large enhancement of electromagnetic field surrounding metal nanostructures.¹ The LSPR wavelength of noble metal nanostructures is extremely sensitive to the refractive index of the surrounding medium and is therefore able to transduce a biomolecular binding event into a measurable shift in the LSPR wavelength.^{4–8}

While there have been phenomenal advances in the design and implementation of plasmonic biosensors, such as rational design of highly sensitive plasmonic nanotransducers and the development of hand-held read-out devices, the real-world translation of this class of biosensors to resource-limited settings is still hindered by the poor thermal, chemical, and environmental stability of the natural antibodies, which are the most commonly employed biorecognition elements.^{9–11} Apart from the high cost associated with natural antibodies, biosensors relying on antibodies require stringent control of environmental conditions to maintain the structure and function (biorecognition) of the antibodies employed as biorecognition elements. Cold chain, a temperature-controlled supply chain, is typically employed to transport and store the biochips and budiagnostic reagents. However, such expensive logistics (i.e., cold chain transport and refrigerated storage are not feasible in remote areas with very limited infrastructure such as electricity and fresh water) pose a lethal challenge to the real-world deployment of biosensors relying on natural antibodies. Thus, there is an urgent need for materials and technologies that can facilitate the wide deployment of biosensors in resource-constrained areas.^{9,12,13}

The development of molecularly imprinted polymer (MIP) has raised promising perspectives in the design and development of sensing and separation systems that utilize artificial antibodies as recognition elements instead of natural antibodies.^{14–18} MIP involves the polymerization of functional monomers in the presence of template species, typically the target analytes. After removing the templates, the polymer is left with binding pockets that

possess complementary shape and chemical functionality to template species. This binding pocket is expected to serve as an artificial antibody by providing covalent and noncovalent interactions (e.g., electrostatic, hydrogen bonding, van der Waals, and hydrophobic interactions) for specific binding of the target biomolecule. Liu et al. have investigated the stability of molecularly imprinted microprobe and showed that it exhibited excellent stability (binding capability) even after 2 months of storage under ambient conditions.¹⁷ We have demonstrated the implementation of MIP on plasmonic nanostructures such as gold nanorods and nanocages for the label-free detection of various bioanalytes.^{19,20} More recently, we have demonstrated methods (PEGylation of noncavity regions and rationale choice of functional monomers) to improve the sensitivity and selectivity of the plasmonic biosensors based on these artificial antibodies.^{19–21} While all of these earlier efforts establish plasmonic biosensors based on artificial antibodies as a viable platform for POC and resource-limited settings, there have been no reports that systematically investigate the stability of artificial antibodies in comparison to their natural counterparts.

In this study, we compare the stability of artificial and natural antibodies using plasmonic nanostructures as model transducers. We demonstrate that artificial antibody-based plasmonic biosensors exhibit prolonged shelf life and excellent thermal and pH stability compared to their natural counterparts. The remarkable stability of artificial antibodies eliminates the need for cold chain transportation and refrigerated storage and handling of the biosensors. The clinical application of artificial antibody-based biosensors subjected to extreme environmental conditions is demonstrated using kidney injury molecule-1 (KIM-1), a representative urinary biomarker for acute kidney injury (AKI) and chronic kidney disease (CKD).

Compared with solid plasmonic nanostructures, hollow structures such as gold nanocages, nanoshells, nanoframes, and nanorattles exhibit a much higher refractive index sensitivity. The higher refractive index sensitivity makes them excellent candidates as plasmonic nanotransducers.²² Here, gold nanorattle (AuNRT), a hollow core–shell nanostructure made of a gold nanosphere core and porous gold shell, was used as plasmonic nanotransducer.²³ AuNRT synthesis involves the galvanic replacement of silver with gold in Au@Ag core–shell nanocubes.^{21,23} The LSPR wavelength of AuNRT was highly tunable and could be controlled by the amount of Au precursor (HAuCl₄) added to the Au@Ag nanocube suspension. Transmission electron microscopy (TEM) images of AuNRT revealed the yolk–shell nanocube structures with sharp corners (Figure 1A). The edge length and side wall thickness of AuNRT were measured to be 38.4 ± 1.7 nm ($n > 30$) and 3.8 ± 0.4 nm ($n > 30$), respectively, which signify the narrow size distribution of the AuNRT. UV–vis extinction spectrum of an aqueous suspension of the AuNRT exhibited an LSPR wavelength of ~650 nm (Figure 1B).

EXPERIMENTAL SECTION

Apparatus

Transmission electron microscopy micrographs were recorded on a JEM-2100F (JEOL) field emission instrument operating at an accelerating voltage of 200 kV (JEOL U.S.A., Inc.). The TEM sample was prepared by drying 2 μ L of twice-centrifuged AuNRT solution

on a carbon-coated TEM grid. A Shimadzu 1800 spectrophotometer (Shimadzu, Kyoto, Japan) was employed to record the vis-NIR extinction spectrum of gold nanorattles during the molecular imprinting process and the binding of the target biomarkers.

Reagents

Cetyltrimethylammonium bromide (CTAB), MPTES ((3-mercaptopropyl) triethoxysilane), hydrochloric acid (HCl, 1 M), ascorbic acid, silver nitrate (purity >99.99%), cetyltrimethylammonium chloride (CTAC), chloroauric acid (HAuCl₄), sodium borohydride (NaBH₄), 4-aminothiophenol (pATP), (3-aminopropyl)trimethoxysilane (APTMS), glutaraldehyde (GA), trimethoxy(propyl)silane (TMPS), hemoglobin (MW = 64.5 kDa), and polyclonal hemoglobin antibody were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). KIM-1 was purchased from Sino Biological (Beijing, China). 1-Ethyl-3-(3-(dimethylamino)propyl)-carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Thermo scientific (Waltham, MA, U.S.A.). SH-PEG-COOH (MW = 5000 g/mol) and SH-PEG-methoxy (MW = 5000 g/mol) were purchased from Jenkem Technology (Plano, TX, U.S.A.).

Synthesis of Gold Nanorattles

The synthesis of gold nanorattles begins with the synthesis of gold nanospheres with a diameter of ~8 nm, which serve as the cores in the Au nanorattles.²³ Au nanospheres were made by the seed-mediated method.^{23–25} To synthesize gold seeds, freshly prepared icecold sodium borohydride solution (10 mM, 0.6 mL) was quickly injected to a mixture of chloroauric acid (10 mM, 0.25 mL) and CTAB (0.1 M, 9.75 mL) under vigorous stirring. The solution was further stirred for 10 min, and then left undisturbed for 1 h at room temperature. The growth solution was made by adding 4.5 mL of ascorbic acid (100 mM) into a mixture of 6 mL of chloroauric acid (0.5 mM) and 6 mL of CTAC (0.2 M) under stirring. Next, the gold seed (0.3 mL) was quickly injected into the growth solution under vigorous stirring. The mixture was further stirred for 5 min, and then kept undisturbed for 20 min at room temperature to form 8 nm gold nanospheres. The gold nanosphere solution was then centrifuged at 12 000 rpm for 1 h and redispersed into a certain volume of Nanopure water to achieve a final extinction of 1.2.

As-synthesized gold nanospheres were then employed as the cores to direct the formation of silver nanocubes. This growth reaction was carried out at 60 °C in a water bath. First, 0.2 mL of gold nanosphere solution (extinction ~1.2) was mixed with 4.8 mL of CTAC (20 mM). The mixture was then heated to 60 °C in a water bath under stirring. After 20 min, 5 mL of silver nitrate (2 mM), 2.5 mL of CTAC (80 mM), and 2.5 mL of ascorbic acid (100 mM) were injected into the mixture in sequence. The solution was kept under stirring in a dark environment at 60 °C for 4 h. After the reaction was complete, Au@Ag nanostructures were collected by centrifugation (10 000 rpm) and redispersed to 15 mL of CTAC (50 mM).

Au@Ag suspension (15 mL) was heated on a hot plate with temperature set to 90 °C. Chloroauric acid (0.5 mM) was then injected (using a syringe pump) into the Au@Ag suspension at a rate of 0.5 mL/min under vigorous stirring. The injection of chloroauric acid was stopped when the localized surface plasmon resonance wavelength of the resulting

nanostructures reached around 660 nm. The color of the suspension changed from yellow to dark blue, which indicated the formation of gold nanorattles. Finally, gold nanorattles were collected by centrifugation at 10 000 rpm and redispersed into 8 mL of Nanopure water. The entire synthesis Au nanorattles was performed on the same day.

Absorption of Gold Nanorattles on Glass Surfaces

Glass substrates ($\sim 1\text{ cm} \times 2\text{ cm}$) were cleaned by immersing them into Piranha solution (150 mL of concentrated sulfuric acid and 50 mL of 30% hydrogen peroxide solution) for 40 min. (Caution: Piranha solution is extremely dangerous, and proper care needs to be executed in handling and disposal.) The substrates were then rinsed thoroughly using a copious amount of Nanopure water and dried with nitrogen gas. Piranha-cleaned glass substrates were incubated in freshly prepared MPTES ethanolic solution (1%) for 1 h. After 1 h, the substrates were washed extensively with ethanol followed by thorough rinsing with Nanopure water. The MPTES-modified glass substrates were dried with nitrogen gas and used within 1 week. Before absorption of the AuNRT on glass, the AuNRT solution was centrifuged again to remove the excess amount of CTAC. The pH of the AuNRT solution was adjusted to 3.0 using 1 M HCl. The MPTES-modified glass substrate was incubated with pH-adjusted AuNRT solution overnight to allow the absorption of AuNRT. The substrate was then rinsed with Nanopure water and dried with nitrogen.

Molecular Imprinting of Hemoglobin

The glass substrate absorbed with AuNRT was rinsed with 100 mM aqueous solution of NaBH_4 for 10 min to remove CTAC from the gold surface. The substrate was then thoroughly rinsed with Nanopure water and dried with nitrogen gas. Amounts of 4 μL of pATP ethanolic solution (4 mM) and 4 μL of glutaraldehyde (25%) were mixed with 2 mL of 20 mM phosphate buffer (pH = 8.0). The glass substrate with AuNRT was incubated in the mixture for 30 s, and then rinsed twice with phosphate buffer. The substrate was subsequently incubated with 25 $\mu\text{g/mL}$ hemoglobin (in 20 mM phosphate buffer, pH = 8.0) at 4 $^\circ\text{C}$ for 2.5 h, followed by washing twice with phosphate buffer. To prepare silane-based solution for polymerization, 3 μL of TMPS and 3 μL of APTMS were added in sequence to 3 mL of phosphate-buffered saline (PBS, 1 $\times$), and the solution was thoroughly mixed by inversion. The glass substrate was incubated with the TMPS and APTMS solution for 3 min to allow the formation of the siloxane copolymer on the AuNRT, followed by rinsing twice with 1 $\times$ PBS. Finally, the substrate was exposed to 2% aqueous sodium dodecyl sulfate (SDS) solution containing 10 mM oxalic acid (pH $\sim$ 2.3) under gentle shaking for 1 h to remove the template protein hemoglobin from the surface of AuNRT.

Molecular Imprinting of KIM-1

The process for KIM-1 imprinting is similar to that of hemoglobin (Hb) with small modifications. Briefly, after pATP and GA modification, the substrate was immersed into 5 $\mu\text{g/mL}$ KIM-1 solution (in 20 mM phosphate buffer, pH = 8.0), followed by washing twice with phosphate buffer. In the polymerization step, 1.5 μL of TMPS and 1.5 μL of APTMS were added to 3 mL of 1 $\times$ PBS, and the polymerization time was adjusted to 1 min.

AuNRT–Anti-Hemoglobin Antibody (Natural Antibody) Conjugates Preparation

To a solution of SH–PEG–COOH in water (37.5 μL , 20 μM), EDC and NHS with the same molar ratio as that of SH–PEG–COOH were added followed by shaking for 1 h. The pH of the above reaction mixture was adjusted to 7.4 by adding 10 $\times$ concentrated phosphate-buffered saline (PBS), followed by the addition of anti-hemoglobin antibody (10 μL , 75 μM). The reaction mixture was incubated for 2 h, and then filtered to remove any byproduct during the reaction using a centrifuge tube with a 50 kDa filter. The final SH–PEG–antibody conjugates solution (0.75 μM) was obtained after washing with PBS buffer (pH 7.4) twice. The AuNRT–antibody conjugates solution was prepared by adding 50 μL of SH–PEG–antibody conjugates solution to 1 mL of AuNRT solution followed by incubation for 1 h. The AuNRT–anti-hemoglobin antibody conjugates were finally immobilized onto MPTES-modified glass.

RESULTS AND DISCUSSION

Molecular imprinting on the surface of the AuNRTs involves multiple steps as illustrated in Figure 2A.¹⁹ As a proof of concept, we first employ Hb as the model template protein. AuNRTs are first modified with *p*-aminothiophenol (pATP) and GA, which serve as molecular linkers to bind protein template Hb to the surface of the AuNRT by forming a reversible imine bond. Subsequently, organosilane monomers (TMPS and APTMS) are copolymerized around the AuNRT conjugated with Hb. We have employed silane-based monomers as they offer distinct advantages: (i) a wide variety of organosilanes with different functional groups are readily available providing a variety of noncovalent interactions between the template and the functional monomer, (ii) the polymerization is relatively simple and can be well-controlled to realize either surface or bulk imprinting, (iii) owing to the noncovalent (weak) interactions of the polymer network and the template molecules, the removal of the biomolecules after the polymerization is relatively easy, (iv) the polymerization can be performed in aqueous medium, preserving the native state of the biomolecules being employed as templates during the polymerization, and (v) more importantly, the imprints in polysiloxanes are stable compared to those in soft gels such as polyacrylamide that are prone to swelling and loss of recognition ability.²⁶

During the copolymerization process, the methoxy groups of TMPS and APTMS undergo a rapid hydrolysis and subsequent condensation to form a thin layer of polysiloxane network connected by the siloxane bonds, leaving functional end groups ($-\text{NH}_3^+$, $-\text{OH}$, $-\text{CH}_3$) interacting with hemoglobin through electrostatic, hydrogen bonding, and hydrophobic interactions. The template hemoglobin is subsequently removed from the polymer matrix by exposure to SDS and oxalic acid. Finally, AuNRT is left with a thin layer of siloxane copolymer with molecular cavities (imprints), which possess complementary shape and chemical functionality to hemoglobin (template and target). Each step is monitored by obtaining the UV–vis spectrum of the AuNRT. We noticed a progressive red shift in the LSPR wavelength (~ 18 nm in total) of the AuNRT along the imprinting process (formation of molecular linker layer, immobilization of Hb, and polymerization) and a subsequent blue shift (~ 5.5 nm) after the template release (Figure 2B and Figure S1). The sensitivity of the artificial antibody-based biosensor was monitored by measuring the LSPR shift after

exposing the biochip to analyte solution containing different concentrations of Hb. The LSPR shift of the artificial antibody-based biosensor exhibited a monotonic increase with increase in Hb concentration. The LSPR shift essentially plateaued at concentrations higher than 1 $\mu\text{g/mL}$ indicating that the recognition elements have been essentially saturated with the target analytes (Figure 2C). We employed the saturation concentration of hemoglobin (1 $\mu\text{g/mL}$) for the quantification of the bioactivity of the artificial antibody in the following experiments.

As mentioned above, we compared the stability of plasmonic biosensor based on artificial antibodies with that of natural antibodies. In the case of natural antibody-based LSPR biosensor, commercially available polyclonal anti-hemoglobin antibody was employed as the biorecognition element. The anti-hemoglobin antibody was first conjugated with thiol-PEG-COOH (MW = 5000) using carbodiimide coupling chemistry, and then covalently conjugated to the AuNRT surface through the SH-Au linkage (Figure 3A).²⁷ The successful conjugation of anti-Hb onto the AuNRT was evidenced by an ~ 6 nm red shift in the LSPR wavelength (Figure 3B). The bioconjugated AuNRTs were immobilized on a glass substrate and exposed to different concentrations of Hb. Notably, the natural antibody-based biosensor (Figure 3C) exhibited a significantly higher LSPR shift and a lower limit of detection (1 ng/mL) compared to its artificial counterpart (Figure 2C). The higher sensitivity of the natural antibody biosensor could be attributed to the higher binding affinity of the natural antibody to the antigen compared to that of the artificial antibody.²⁸ Considering the highest LSPR shift for natural antibody-based biosensor occurred at a concentration of 5 $\mu\text{g/mL}$, we have employed this concentration to quantify its retained activity in the subsequent experiments.

Next, we set out to test the stability of the natural and artificial antibodies after subjecting them to harsh storage conditions. To advance plasmonic biosensors to the real world, especially resource-limited settings, several environmental conditions must be considered. To understand the temporal stability (shelf life) of the plasmonic biosensors under nonrefrigerated storage conditions, we stored the plasmonic biochips (with natural and artificial recognition elements) for various time periods at room temperature (~ 25 °C) in the dry state. Subsequently, the biosensors were exposed to analyte solution and the LSPR shifts were measured. As noted above, Hb concentrations were 1 $\mu\text{g/mL}$ for artificial antibody and 5 $\mu\text{g/mL}$ for natural antibody (concentrations resulting in highest LSPR shift) based biosensors. The retained recognition capability was calculated as the percentage of the LSPR red shift upon binding of target protein to recognition element on the biochip after being challenged with various storage conditions (different storage period, temperature, and pH) compared with the red shift obtained from the same batch of freshly made substrate. Therefore, the retained biorecognition activity of biochip after subjecting to certain storage condition is expected to be linearly related to the observed LSPR shift. Different time intervals (1 day, 3 days, 1 week, and 2 weeks) were selected to test the performance of artificial and natural antibody biosensors. The retained recognition ability for natural antibody-based biosensor quickly dropped to 40% after storage for 24 h and further decreased to less than 10% after storage for 7 days, implying a significant and rapid loss in the structure and recognition ability of the natural antibody under ambient storage conditions (Figure 4A). On the contrary, artificial antibody-based biosensors exhibited a stable LSPR

response with fully retained recognition ability over 2 weeks of storage (Figure 4A). Notably, even after 3 months of storage, the artificial antibody-based biosensor exhibited nearly 85% recognition capability, signifying the remarkable stability of artificial antibodies compared to their natural counterparts (Figure S2).

We further tested the sensitivity of the biosensors based on natural and artificial antibodies stored for 7 days under different concentrations of Hb. For a wide range of Hb concentrations, the artificial antibody-based biosensor exhibited LSPR shifts closely matching those of a freshly prepared biosensor, indicating excellent preservation of the sensitivity (Figure 4B). In stark contrast, the LSPR shift of the natural antibody-based biosensor at 5 ng/mL hemoglobin is almost completely (97%) lost after 1 week of storage (Figure S3). For lower concentrations, the natural antibody biosensor exhibited slight blue shifts, which might be due to the partial dissociation of antibody from the AuNRT surface. On the basis of these results, we conclude that, although the plasmonic biosensors based on artificial antibodies exhibit inferior sensitivity compared to those based on natural antibodies, the biorecognition ability of the artificial antibody-based biosensor after prolonged storage is far superior, making them better candidates for resource-limited settings.

To further investigate the stability of the antibodies, we stored the biosensors at various temperatures (from 4 to 40 °C). As is common with most proteins, antibodies are expected to denature upon exposure to high temperature and thus lose their biorecognition capability due to the disruption of the secondary and tertiary structure. Both natural and artificial antibody based biosensors were sealed and stored at different temperatures for 14 h. Subsequently, the biosensors were exposed to analyte (Hb) solutions (1 μ g/mL for artificial antibody, 5 μ g/mL for natural antibody) and LSPR shifts were measured. Retained recognition abilities were calculated in the same way as mentioned above. The artificial antibody-based plasmonic biochip showed a remarkable thermal stability with ~100% recognition ability for all temperatures tested (Figure 4C). The slightly higher LSPR shift (~10%) observed after storing the artificial antibodies at 30 and 40 °C is due possibly to the annealing of the siloxane copolymer, which improves the binding affinity. Conversely, the natural antibody lost nearly 50% of its biorecognition ability after storage at 10–40 °C for 14 h. Even storage under refrigerated condition (4 °C) resulted in only 85% of the biorecognition ability compared to nearly 100% retained sensitivity of the biosensor based on artificial antibodies (Figure 4C). Overall, these results demonstrate the poor shelf life stability of plasmonic biosensors based on natural antibody and highlight the advantages of the artificial recognition elements for plasmonic biosensors in resource-limited settings.

Besides the excellent thermal stability, artificial antibody-based biosensors also exhibited excellent stability against a broad range of pH conditions. In resource-limited settings, the pH of water used in the assay procedure is usually difficult to regulate compared to laboratory settings where deionized water or buffers with stabilized pH are employed for performing the bioassays. Furthermore, pH of the biofluids from patients, especially urine, is known to exhibit large variation (pH 4.5–8.5) depending on the diet and pathological conditions of the subjects. For investigating the stability of the biosensors against pH variations, the biosensors were exposed to solutions with different pH for 30 min (typical

time period for washing steps) followed by exposure to the analyte solution. The artificial antibody exhibited nearly 100% recognition capability within the range from pH 3 to pH 7. Notably, natural antibody also exhibited nearly 95% retained activity after exposure to pH 4–7 (Figure 4D). A significantly lower (~20%) activity was observed for the natural antibody-based biosensors exposed to pH 3, while the artificial antibody still showed nearly 100% bioactivity under this condition.

Finally, we investigated the applicability of the artificial antibody as a biorecognition element for the detection of KIM-1, a clinically relevant disease biomarker for AKI and CKD.^{29,30} AKI is characterized as a rapid and intensive decline of renal function, associated with high morbidity and mortality.³¹ Nearly two million people die of AKI annually worldwide, and survivors have an enhanced risk of chronic kidney disease.³² Early diagnosis and intervention in AKI could significantly improve the prognosis.³³ The current standard biomarker for AKI is serum creatinine, which peaks days after injury occurs. Among various urinary protein biomarkers for AKI, growing evidence shows that KIM-1 is superior for early detection of AKI, increasing within 24 h of injury, well before serum creatinine, thereby enabling detection and potential intervention at a very early stage.^{33,34}

The fabrication of the KIM-1 artificial antibody-based biosensor is similar to that of the Hb one as described above except that KIM-1, instead of Hb, is used as the template protein in the molecular imprinting process. Due to the lower molecular weight of KIM-1 (MW ~ 30 kDa) compared with Hb (MW ~ 64 kDa), polymerization conditions were slightly modified to form a thinner layer of siloxane copolymer around the KIM-1 template, which subsequently ensured efficient release of the template proteins. The thickness of the polymer was controlled by the amount of monomer (APTMS and TMPS) added to the polymerization solution. UV–vis extinction spectra obtained after each step during the imprinting of KIM-1 revealed a continuous red shift of the LSPR wavelength (Figure 5A). Subsequently, template was released using a mixture of SDS and oxalic acid, resulting in a blue shift in the LSPR wavelength (steps 3–4 in Figure 5B). The artificial antibody-based biochips exhibited a monotonic increase in the LSPR shift with increasing concentrations of KIM-1 (Figure 5C). The detection limit was found to be 10 ng/mL (Figure 5C). We chose the saturation concentration (500 ng/mL) to investigate the stability of the plasmonic biochip. The biochip exhibited nearly 100% recognition ability after storage for 3 weeks at room temperature (Figure 5D). No significant loss of bioactivity was noted upon exposure to temperatures ranging from –20 to 40 °C for 14 h (Figure 5E). The biosensor also exhibited remarkable stability against exposure to wide range of pH conditions (Figure 5F). Apart from the generality of the artificial antibody as the recognition element for various protein biomarkers, these results also demonstrate the excellent stability of a clinically relevant artificial antibody-based biosensor for applications in point-of-care and resource-limited settings.

CONCLUSION

In summary, we have demonstrated the excellent thermal, temporal, and chemical stability of the artificial antibody-based plasmonic biosensors. Compared to its natural counterpart, the artificial antibody-based biochip offers significantly superior recognition ability after being

challenged with elevated temperatures, prolonged storage periods, and a wide range of pH conditions (more than 90% retained activity). The superior stability of this class of sensors makes them excellent candidates in resource-limited settings such as at-home care, rural clinics, developing countries with low and moderate incomes, and the battlefield, where refrigeration and tight regulation of environmental conditions is not always possible. Furthermore, artificial antibodies for a new target analyte can be developed and tested more rapidly compared to natural antibodies, which is critical for rapidly responding to public health emergencies (e.g., infectious diseases) in resource-limited settings. Overall, we expect the high stability of the artificial antibody to greatly facilitate the application of plasmonic biosensors as simple, portable, sensitive, and stable on-chip biondiagnostics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) TEM image showing the core-shell structure of Au nanorattles (AuNRT) employed as the nanotransducers in plasmonic biosensors. (B) Vis-NIR extinction spectrum of aqueous suspension of AuNRT.

Figure 2.

(A) Schematic illustration showing the concept of a plasmonic biosensor based on artificial antibodies as biorecognition elements. The artificial antibody is realized on the AuNRT by molecular imprinting using siloxane copolymer. (B) Vis–NIR extinction spectra of the AuNRT along the molecular imprinting process (the inset shows zoom-in spectra highlighting the LSPR shift). (C) Plot showing the LSPR shift of the AuNRT with artificial antibody (artificial anti-hemoglobin) after exposure to various concentrations of hemoglobin. Results are the mean $\pm$ SD ($n = 3$).

Figure 3.

(A) Schematic illustration representing the fabrication of a plasmonic biosensor based on natural antibodies as recognition elements. (B) Vis–NIR extinction spectra showing the LSPR shift of AuNRT after conjugation with natural anti-hemoglobin antibody (the inset reveals the red shift in the LSPR wavelength to be 6 nm). (C) LSPR shift of the plasmonic biosensor based on natural antibodies (anti-hemoglobin antibody) upon exposure to various concentrations of hemoglobin. Results are the mean $\pm$ SD ($n = 3$).

Figure 4.

(A) Retained recognition ability of natural and artificial antibody-based biosensors stored at room temperature for different durations. (B) Retained recognition of the artificial antibody-based biosensor, as determined by exposure to various concentrations of the analyte (hemoglobin) solution, after storage at room temperature for 1 week. (C) Retained recognition ability of natural and artificial antibody-based biosensors stored for 1 day at various temperatures. (D) Retained recognition ability of natural and artificial antibody-based biosensors after exposure to a wide range of pH conditions. Results are the mean $\pm$ SD ($n = 3$).

Figure 5.

(A) Vis–NIR extinction spectra of the AuNRT exhibiting a progressive red shift in the LSPR wavelength upon modification with pATP and GA, immobilization of KIM-1, and copolymerization of the silane monomers. (B) LSPR shift of the AuNRT after each step along the formation of the artificial antibodies, template removal, and template rebinding. (C) LSPR shift of KIM-1-imprinted AuNRT upon exposure to various concentrations of KIM-1 solutions. (D) Retained recognition ability of the KIM-1-imprinted artificial antibody biosensor after storage at room temperature for different durations, (E) at various temperatures for 1 day, and (F) after exposure to a wide range of pH conditions. Results are the mean $\pm$ SD ($n = 3$).