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## Reusable surface plasmon resonance biosensor chip for the detection of H1N1 influenza virus

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### ABSTRACT

We developed a reusable magnetic surface plasmon resonance (SPR) sensor chip for detecting various target molecules repeatedly in a conventional SPR system. Here, ferromagnetic patterns on a SPR sensor chip were utilized to trap a layer of magnetic particles, and they were utilized as a solid substrate for SPR sensing in a conventional SPR system. After a sensing experiment, the used magnetic particles were removed by external magnetic fields, and a new layer of magnetic particles was immobilized to the SPR sensor chip for additional sensing measurements. Since magnetic particles were trapped on the ferromagnetic patterns, we could use our reusable SPR chip for SPR sensing measurements in a traditional SPR system without any applied magnetic fields. Significantly, ferromagnetic patterns on the sensor chip surface deflected the strong external fields, so that the large aggregation of magnetic particles on the sensor surface was reduced. We demonstrated using a single reusable SPR sensor chip to measure the nucleoprotein (NP) of H1N1 influenza virus solution ranging repeatedly for more than 7 times without significant signal degradation. Also, different target molecules could be repeatedly measured in a single SPR chip. Since our reusable SPR sensor chip can be repeatedly used in a conventional SPR system without any chemical processes for refreshment, the cost for SPR sensing should be significantly reduced. In this case, our reusable SPR sensor chip can be a major breakthrough and can be used for versatile practical applications of SPR sensors.

### 1. Introduction

A surface plasmon resonance (SPR) based sensing method is a powerful tool for the real-time and label-free monitoring of biomolecular interactions (Krishnamoorthy et al., 2010; Mandenius et al., 2008; Wang et al., 2013; Wong and Olivo, 2014; Zeng et al., 2014). In SPR-based biosensors, the selective bindings of target molecules to receptors on the SPR sensor surface induced its refractive index changes, which can be measured for the label-free detection of versatile target analytes in real time (Wang et al., 2013; Wong and Olivo, 2014). Also, various SPR-based measurement systems have been used extensively for the analyses of interaction kinetics between different biomolecules such as protein-ligand, protein-protein, or DNA-protein (Jecklin et al., 2009; Karlsson and Falt, 1997; Patching, 2014; Pollet et al., 2009; Wegner et al., 2003). However, most of SPR biosensor chips are rather expensive compared with common commercial biosen-

sors such as strip-type sensors. Also, it is usually very difficult to reuse a used SPR sensor chip. Such a limitation has been one of the major stumbling blocks holding back its diverse commercial applications.

Recently, several groups have developed methods for reusable SPR sensors (Choi and Chae, 2009; Hsu et al., 2015; Lee et al., 2011; Makaraviciute et al., 2015; Roche et al., 2011; Zhao et al., 2014). In one method, a used SPR sensor chip was treated chemically to remove the used receptors, and new receptor molecules were applied for addition sensing measurements (Choi and Chae, 2009; Makaraviciute et al., 2015; Zhao et al., 2014). However, such a chemical treatment often contaminated sensor surfaces and degraded the sensor performance. Previous works show that chemical treatments can damage bioreceptors, causing losses of the sensor signals (Goode et al., 2015). In another work, receptor-modified magnetic particles were assembled on a SPR sensor surface via external magnetic fields and used for SPR sensing measurements (Lee et al., 2011). After a sens-

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ing, magnetic fields with an opposite direction were applied to remove the magnetic particles, leaving a fresh SPR sensor surface ready for additional sensing. This method based on magnetic fields can minimize the chemical contamination. However, the strong external magnetic fields may result in the aggregation of magnetic particles and leave residues on the SPR sensor surface, which may reduce the reliability of the sensor. Furthermore, in this method, an external magnetic field should be maintained during the SPR sensing process, and, thus, a conventional SPR sensing system should be modified to include an electromagnetic device for sensing measurements. Until now, it is very difficult to build a SPR-based biosensor which can be reused repeatedly in a conventional SPR systems without degrading its signals. Another powerful strategy for cost-effective SPR biosensor can be optical fiber-based SPR sensors. The use of optical fibers for SPR sensing allowed one to develop low cost and even miniaturized SPR biosensors. However, such optical fiber-based SPR biosensors usually require specifically-configured equipment and cannot be utilized in conventional SPR systems (Chiavaioli et al., 2018; Guo, 2017; Loyez et al., 2019; Shushama et al., 2017; Zhao et al., 2019).

Herein, we report a reusable magnetic SPR sensor chip which allows us to repeatedly detect target analytes such as H1N1 virus in a conventional SPR system. In this strategy, ferromagnetic patterns on a SPR chip trapped a layer of magnetic particles with a fresh surface, and they were used as a solid substrate for SPR sensing in a conventional SPR system. Then, the used magnetic particles were removed via external magnetic fields, and a new layer of fresh magnetic particles was trapped on the SPR chip for additional sensing measurements. Since the ferromagnetic patterns were holding magnetic particles, our reusable SPR chip could be used for SPR sensing measurements in a conventional SPR system without any external magnetic fields. Furthermore, the strong external magnetic fields were deflected by the ferromagnetic patterns on the sensor surface, which avoided the large aggregation of magnetic particles on the surface and enabled rather large sensing signals comparable to those of conventional SPR chips. Using a single reusable SPR sensor chip, we could repeatedly detect the nucleoprotein (NP) of H1N1 influenza virus with the protein concentration ranging from 30 ng/ml to 10  $\mu$ g/ml. Importantly, the sensing signals did not degrade even after more than 7 times of repeated SPR measurements. We also demonstrated the detection of different target molecules using a single SPR chip. Since the reusable SPR chip can be used repeatedly in a conventional SPR system and does not require any complicated chemical processes for refreshment, our method should dramatically reduce the cost for sensing processes and open up versatile practical applications of SPR sensors such as a molecular recognition study, a drug screening, a blood test, and a disease diagnosis.

## 2. Materials and methods

### 2.1. Materials

A superparamagnetic nanoparticle (nano-screenMAG-CMX) solution was purchased from Chemicell GmbH (Germany). The hydrodynamic size of a magnetic particle was about 100 nm, and the surface of the magnetic particle was covered with carboxymethyl-dextran (CMX) having a carboxyl group. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), and Ethanolamine hydrochloride-NaOH were purchased from GE Healthcare (Sweden). SIA KIT Au containing a SPR support, double-sided adhesive tape, and a protective sheath were also purchased from GE Healthcare. BioNano Health Guard Research Center (H-GUARD) prepared nucleoprotein (NP) of influenza type A H1N1 and its corresponding monoclonal antibody. 1x PBS (pH 7.4, 154 mM NaCl, 5.6 mM  $\text{Na}_2\text{HPO}_4$ , 1.1 mM  $\text{KH}_2\text{PO}_4$ ) was purchased from Lonza (USA).

### 2.2. Fabrication of nickel patterned reusable SPR sensor chip

4-inch glass wafers were purchased from Namkang Hi-Tech co. Ltd (Korea). For the fabrication of a reusable SPR chip, the Cr/Au film (45 nm Au on 5 nm Cr) was first prepared on a glass wafer by a thermal evaporation process. Then, Ni/Au patterns (10 nm Au on 50 nm Ni,  $5 \mu\text{m} \times 10 \mu\text{m}$ ) were fabricated by the photolithography and thermal evaporation.

### 2.3. Conventional SPR sensing using a reusable SPR sensor chip

The 0.1 mg/ml solution of protein G functionalized with cysteine was first flowed for 350 s. Since the cysteine groups formed sulfide or disulfide bonding with a gold surface, the protein G was attached to the gold surface of a sensor chip (Lee et al., 2007). Afterwards, buffer solution (PBS) was flowed to remove the non-specifically bound protein G and stabilize a signal. The corresponding C-reactive protein (CRP) antibody solution of 0.1 mg/ml was flowed for 350 s to immobilize the antibody molecules on the protein G molecules. The buffer solution was flowed for 500 s to remove the non-specifically bound antibody molecules and stabilize a signal. Then, a CRP antigen solution of 5  $\mu$ g/ml was flowed for 350 s, and the buffer solution was flowed. Here, the signal of CRP antigen is determined by a difference between the signal before and after the passage.

### 2.4. Repetitive trap and release of magnetic particles over a Ni patterned chip

First, the solution of fluorescence labeled magnetic particles was prepared. For trapping the magnetic particles on a Ni patterned chip, the solution was placed on the chip and a magnetic field of 150 mT was applied to the chip for 1 min. Then, it was washed lightly with PBS solution. For releasing the trapped magnetic particles, a magnetic field of 35 mT was applied for 30 s in the opposite direction to the previous magnetic field, while the chip was washed with DI water.

### 2.5. Repetitive sensing process using a reusable SPR sensor chip

A reusable SPR sensor chip was attached to a conventional SPR chip support by a piece of double-sided adhesive tape. 100  $\mu$ l solution of magnetic particles (0.25 mg/ml) was introduced on the sensor chip, and an external magnetic field of 150 mT was applied for 1 min by a neodymium magnet. Then, the sensor chip was gently washed with PBS solution. The remaining solution was removed by a science wiper (Yuhan-Kimberly, model Kimtech Science Wiper). The sensor chip was installed on a protective sheath. Then, it was inserted to a conventional SPR detection system (Biacore T100, GE Healthcare). In the system, buffer solution (PBS) flow was first used for a few minutes to stabilize sensor signals. After stabilization of sensor signals, antibodies were immobilized on the magnetic particles following an amine coupling manual (Fischer, 2010). Mixed solution (0.1 M EDC and 0.4 M NHS) was introduced for 5 min to the sensor chip for activating the carboxyl group of magnetic particles. Then, antibody solution of 50  $\mu$ l/ml was introduced for 5 min to the sensor chip. Within 5 min, SPR signals were saturated, indicating that antibody molecules were fully immobilized on the magnetic particles (Fig. S1 in Supplementary Material). However, it is worth mentioning that some antibody molecules can be adsorbed in a wrong orientation due to the non-selective bindings of linker molecules and they may not function properly, which can be improved by utilizing other more selective linkers such as aptamers or affimers (Centi et al., 2007; Li et al., 2016; Xie et al., 2017). To block the remaining activated carboxyl groups of the magnetic particles, 1 M ethanolamine solution was injected to the sensor chip

for 1 min. For the detection of target molecules, target molecules were introduced to the sensor chip for 300 s. Then, buffer solution was introduced to the sensor chip for 200 s to remove target molecules bound non-specifically to the sensor chip. We repeated the detection process with target solution having different concentrations of target molecules. After sensing, we took out the sensor chip from the SPR detection system. Then we applied a magnetic field of 35 mT in an opposite direction to the previous magnetic field. As a result, the used magnetic particles were removed from the chip surface. Then, the sensor chip was washed with DI water for 1 min and dried. In order to repeat the sensing process, the process described above was repeated.

### 3. Results and discussion

#### 3.1. Repeated sensing processes of a reusable SPR sensor chip

Fig. 1 shows a schematic diagram depicting repeated sensing processes of a reusable SPR sensor chip. The detailed fabrication process of a reusable SPR chip is presented in the Materials and methods section. In brief, a metal film (45 nm Au on 5 nm Cr) was first deposited on a glass substrate. Then, Ni/Au pattern arrays (10 nm Au on 50 nm Ni,  $5 \mu\text{m} \times 10 \mu\text{m}$ ) were fabricated on the substrate (Fig. 1A). Here, the Au layer was used to prevent the oxidation of Ni and to provide stable bindings of biochemical molecules for SPR sensing experiments. The substrate was attached to a sample holder of an SPR measurement system (Biacore, GE Healthcare) using a double-sided adhesive tape (center of Fig. 1). As a first step of sensing, the solution of magnetic particles functionalized with carboxylic groups was first introduced on the chip. Then, an external magnetic field (H-field, 150 mT) was applied on the substrate with perpendicular direction to the chip (Fig. 1B). Here, the external magnetic field magnetized the ferromagnetic Ni patterns (red arrow) and made magnetic fields near the patterns stronger than other regions. As a result, the magnetic particles were attracted toward the Ni patterns and trapped on them. The chip with trapped magnetic particles was washed with PBS to leave only the magnetic particles bound strongly on the magnetized Ni patterns. Note that the ferromagnetic Ni patterns can hold the trapped magnetic particles even without external magnetic fields. The substrate was inserted into a SPR detection system. In the SPR detection system, PBS solution flowed through a microfluidic channel to stabilize a sensor signal. Then, antibodies were immobilized on the magnetic particles by using EDC and NHS (Fig. 1C). After that, a target molecule solution was introduced for a specific sensing (Fig. 1D). In the sensing process, refractive index changes near the sensor chip surface were measured in a unit of RU (resonance unit). After the sensing, the sensor chip was taken out from the SPR detection system. For the removal of used magnetic particles, an external magnetic field with an opposite direction to the previous applied magnetic field was applied to the sensor chip (Fig. 1E). In this case, the external magnetic field was not strong enough to change the direction of magnetization of the Ni patterns. Thus, total magnetic field above the Ni patterns became weaker than those of other regions. Due to the magnetic field gradient, the magnetic particles were washed out from the sensor chip with DI water. As a result, we could recover a fresh surface of the sensor chip without magnetic particles for the next sensing measurements. In our method, magnetic fields were used to remove the used magnetic particles without any chemical treatment, which reduced chemical contamination on the SPR sensor surface. Furthermore, an external magnetic field doesn't have to be maintained during the SPR sensing process so that our reusable SPR sensor chip can be used in a conventional SPR sensing system.

#### 3.2. Characterization of a SPR sensor chip with ferromagnetic Ni patterns

Fig. 2A shows the optical image of a reusable SPR sensor chip. A dark square region in Fig. 2A (i) is a ferromagnetic nickel pattern ar-

ray. Fig. 2A (ii) is the magnified image of Ni patterns. White squares are Ni patterns whose size are about  $10 \mu\text{m} \times 5 \mu\text{m}$ . These patterns were used for the trapping and releasing of magnetic particles. These results indicate that the sizes of Ni patterns and distances between them are uniformly controlled. The uniformly controlled Ni patterns evenly deflect an applied external magnetic field. As a result, they can hold magnetic particles without a large aggregation (Yoo et al., 2016).

To confirm that the fabricated reusable SPR sensor chip shows similar SPR sensing signals as a conventional SPR sensor chip, we performed a conventional SPR sensing process using a reusable SPR sensor chip (Fig. 2B). The experimental procedure is in the Methods section. The SPR signal image shows increased SPR signals when protein G, C-reactive protein (CRP) antibody, and CRP antigen solutions were introduced on the sensor chip. These results indicate that the measured SPR signals from our reusable SPR sensor chip were similar to measured SPR signals from a conventional SPR sensor chip, even with a Ni pattern array fabricated on our reusable SPR sensor chip.

#### 3.3. Magnetic trapping and releasing of magnetic particles with a reusable SPR sensor chip

Fig. 3A shows the simulation result of magnetic field distributions according to the applied magnetic fields at 1 mm above Ni patterns and the schematic diagrams of a simulation situation. The brighter regions represent those with a stronger magnetic field. Fig. 3A (i) shows a magnetic field distribution when a strong magnetic field was applied to ferromagnetic Ni patterns. The magnetic fields of the regions above the Ni patterns are stronger than those of the surrounding regions. Since magnetic fields caused by the magnetization of Ni patterns were added to the external magnetic field, the total magnetic fields near the patterns became relatively stronger than those of other regions. This magnetic field gradient generated a magnetic force that attracts paramagnetic materials such as magnetic particles. On the other hand, Fig. 3A (ii) shows a result when a rather weak magnetic field in the opposite direction to the previous applied magnetic field is applied to the Ni patterns. The magnetic field of the region above the Ni pattern is weaker than that of the surrounding portion. Since ferromagnetic materials have hysteresis characteristics for magnetization, they maintain the magnetization direction. Therefore, the magnetic field near the nickel pattern is reduced and becomes weaker than that of the surrounding area. This forces the magnetic particles on the nickel pattern to come out of the pattern. This simulation results show that we can control the trap and release of magnetic particles on ferromagnetic patterns via external magnetic fields (Yoo et al., 2016).

Fig. 3B shows the fluorescence images of repeated trap and release of magnetic particles over a Ni patterned chip. The experimental procedure is in the Methods section. The above trap and release processes were repeated twice. The bright regions represent the trapped magnetic particles on Ni patterns. The above results show that the magnetic particles can be efficiently trapped and released repeatedly.

Fig. 3C shows SEM images taken after trapping magnetic particles on a Ni pattern (i), and releasing them (ii). The trapped magnetic particles are clearly shown in Fig. 3C (i) (marked by red arrows). Fig. 3C (i) shows that the magnetic particles were selectively trapped in the Ni pattern. Also, the magnetic particles were somewhat evenly distributed on the Ni pattern without large aggregations. As a result, our reusable SPR sensor chip with trapped magnetic particles can have optical properties similar to those of a bare Au film deposited on a glass substrate. It also should be mentioned that the coverage of nanoparticles on a single Ni pattern may not be uniform presumably due to some variations of individual patterns. However, since there are 375,000 of such Ni patterns on our sensor chip, we could still obtain uniform coverage of nanoparticles over the entire sensor surface (Yoo et al., 2016). On

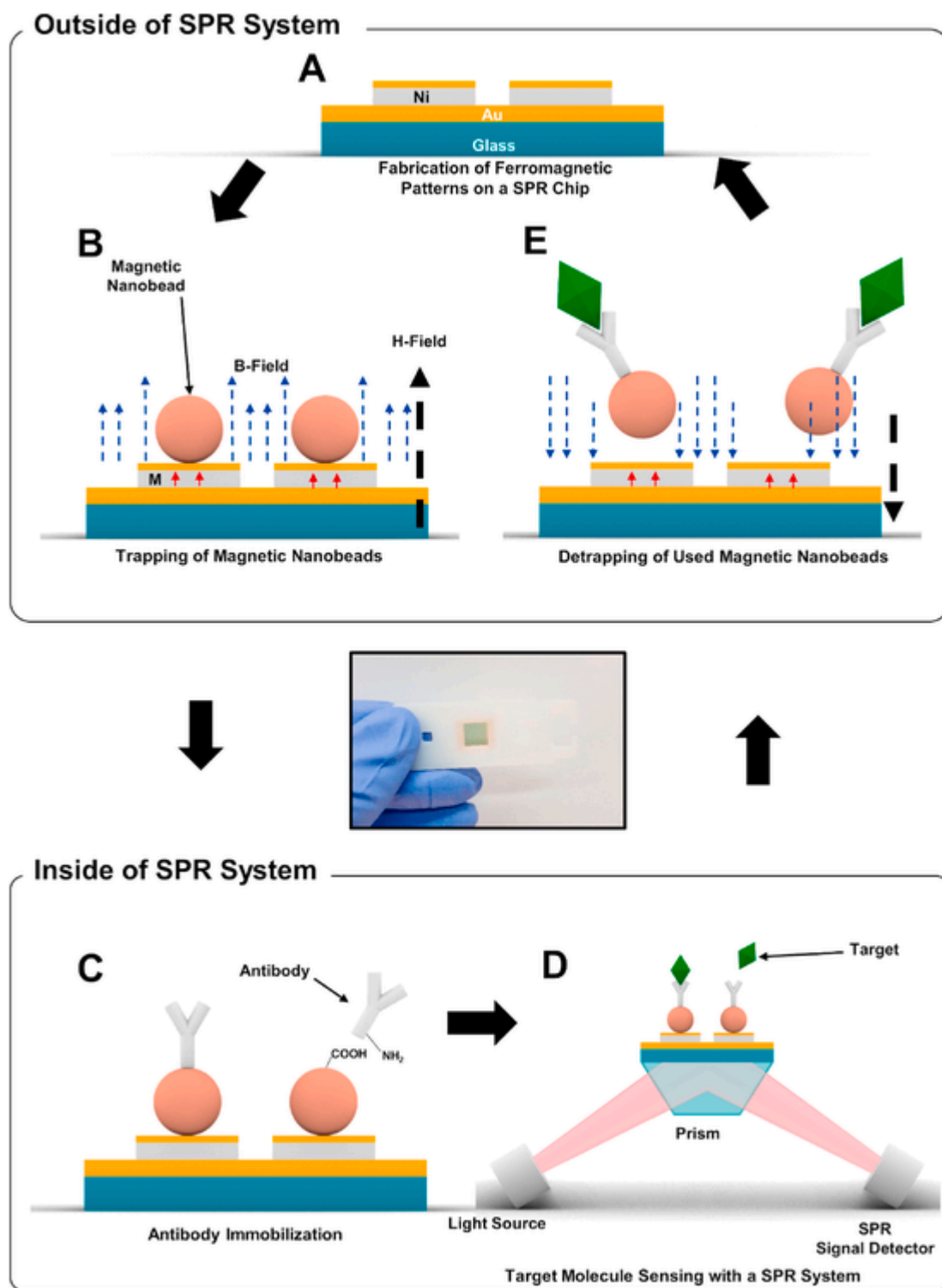


Fig. 1. Schematic diagram depicting the cyclic process for the repeated sensing measurements using our reusable SPR biosensor chip. (A) A reusable SPR chip including ferromagnetic nickel patterns on a conventional SPR chip structure. (B) Trapping of magnetic particles on the reusable SPR chip through an external magnetic field. (C) Immobilization of antibodies on magnetic particles using EDC-NHS coupling in a conventional SPR system. (D) Detection of target molecules. (E) Removal of magnetic particles by an external magnetic field in an opposite direction to that for trapping.

the other hand, in Fig. 3C (ii) the magnetic particles have clearly removed, indicating that our reusable SPR sensor chip can be reused for additional sensing processes.

Fig. 3D shows UV-VIS transmission spectra of an Au film, a Ni patterned Au film and a Ni patterned Au film with trapped magnetic particles. All the Au films were deposited on a glass substrate. Note that

the transmittance intensity decreased after Ni patterning and magnetic particle trapping because the deposited Ni films and the trapped magnetic particles somewhat blocked lights (Srivastava et al., 2012; Su et al., 2005). However, the peak wavelength of the transmissions remained as ~520 nm, which is similar to the previous results (Axelevitch et al., 2013; Siegel et al., 2011). Also, the shape of the transmittance

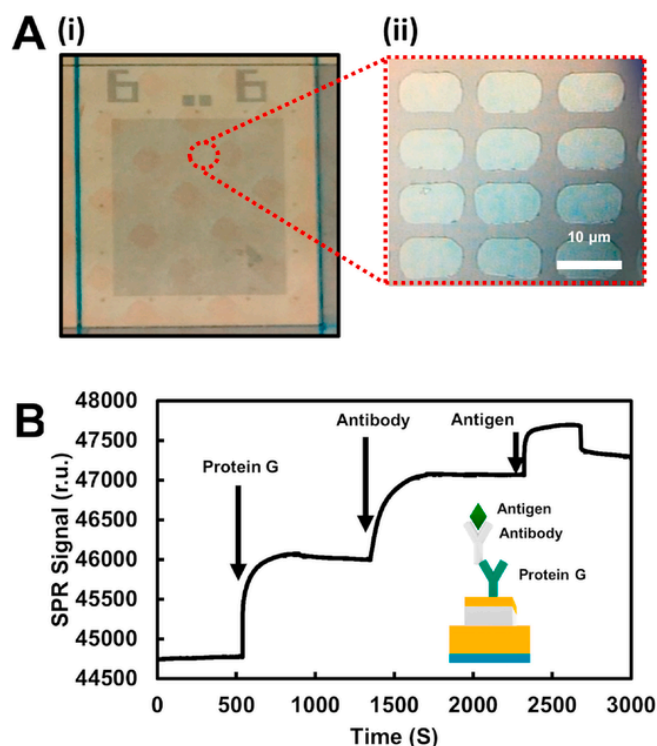


Fig. 2. Characteristics of a reusable SPR sensor chip. (A) Optical image (i) of a reusable SPR sensor chip and magnified optical image (ii) showing Ni patterns. The patterns are uniformly fabricated. (B) Real-time sensing of CRP antigen using a reusable SPR sensor chip in a conventional SPR sensing process.

tance curves did not change much even after the deposition of Ni layers and magnetic particles. These results show that the basic optical properties of Au film on a glass substrate were maintained even after Ni patterns were fabricated on Au films or magnetic particles were trapped on the Ni patterns on Au film. In this case, we can expect that SPR signals were not significantly affected because they are based on the change of *evanescent waves* rather than light transmission (Ekgasit et al., 2005). Therefore, for a sensing process using our reusable SPR sensor chip, we can obtain SPR signals similar to those of a conventional SPR sensing process.

### 3.4. Repeated SPR sensing measurements of NP and IL-13

Fig. 4A shows a real-time graph of a sensing process using a reusable SPR sensor chip. Target proteins were nucleoprotein (NP) of H1N1 viruses. The detailed experimental procedure is shown in the Materials and methods section. Here, after the stabilization of the sensor signal, the solution of NP with different concentrations from 300 ng/ml to 10 μg/ml was sequentially injected to the sensor chip. Note that the SPR signal immediately increases when the NP solution was injected to the sensor chip (Chung et al., 2005; Nanduri et al., 2007). Furthermore, the injection of the NP solution with a larger concentration resulted in a larger increase in the SPR signal. The slight decrease of SPR signals at a high antigen concentration is presumably due to the dissociation of NP and antibodies as reported previously (Scarano et al., 2010). This result shows that our reusable SPR sensor chip could be used to measure the concentration of target molecules in real-time.

Fig. 4B shows SPR sensing results by 7 repeated sensing measurements using a single reusable SPR chip. The detailed experimental procedure is in the Methods section. A  $\Delta$ SPR sensing signal was calculated from the difference of SPR signals before NP solution was injected

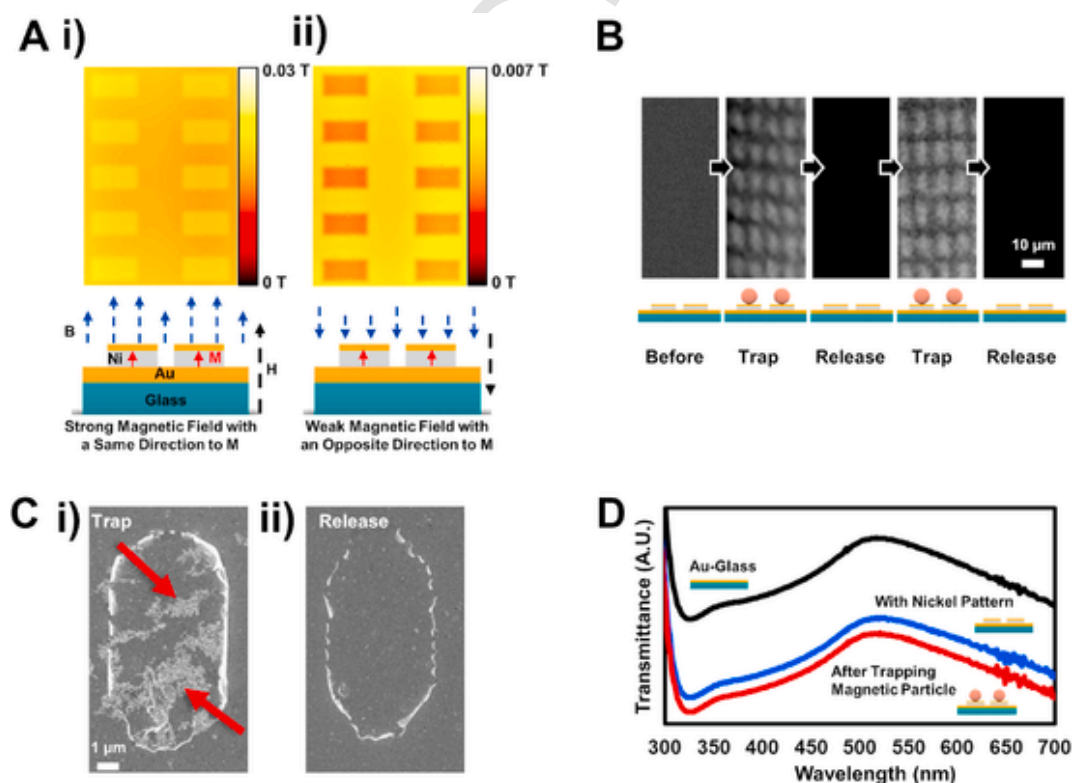


Fig. 3. Trapping and releasing of magnetic particles on reusable SPR sensor chips. (A) Simulation results showing magnetic field strength around Ni patterns during (i) trapping and (ii) releasing steps. (B) Fluorescence images of sequential trap and release of magnetic particles on Ni patterns. (C) SEM images after trapping magnetic particles (i) and releasing (ii) on a Ni pattern. (D) UV-vis spectra of Au film on glass substrate (black), Ni patterned Au film on glass substrate (blue), and magnetic particles trapped Ni patterned substrate (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



to the SPR sensor chip. Note that, as the concentration of NP was increased,  $\Delta$ SPR signals increased like a conventional SPR sensor chip (Homola et al., 2002; Nanduri et al., 2007). The average coefficient of variation (CV) was  $\sim 12\%$  until the 5th repeated sensing measurements, which is in an acceptable range (Jearanaikoon et al., 2016; Wang et al., 2018). In 6th and 7th measurements, the sensor exhibited some fluctuations at a rather low concentration condition with the total average CV of  $\sim 21\%$ , presumably due to some contaminations on the substrate and small signals for low concentration samples. However, at a rather high concentration condition, we could still achieve high accuracy with 9% of average CV value for all 7 repeated sensing measurements.

Fig. 4C shows the schematic diagram (i) and the sensing results (ii) of different target molecules using a single reusable sensor chip. Here, we repeated three different sensing measurements using a single SPR chip (Fig. 4C (i)). For the first measurement, the trapped magnetic particles were functionalized with Interleukin 13 (IL-13) antibody and IL-13 antigen solution ranging from 1  $\mu\text{g}/\text{ml}$  to 30  $\text{ng}/\text{ml}$  was sequentially measured. In the second measurement, NP solution of 10  $\mu\text{g}/\text{ml}$  was measured without any magnetic particle and antibody. For the third measurement, NP solution ranging from 1  $\mu\text{g}/\text{ml}$  to 10  $\mu\text{g}/\text{ml}$  was sequentially measured with NP antibody. After each measurement was completed, the used magnetic particles were removed via an external magnetic field for an additional sensing measurement. Fig. 4C (ii) shows the normalized  $\Delta$ SPR signals from our reusable SPR sensor chip during the repeated measurements of different target molecules with different concentrations. The normalized signals were calculated by normalizing  $\Delta$ SPR signals with respect to their maximal  $\Delta$ SPR signal values at high concentration conditions. The results of the first measurement show that an increase in concentrations of IL-13 resulted in an increase of a normalized  $\Delta$ SPR signal. By functionalizing the magnetic particles with the IL-13 antibody, we could successfully measure the IL-13 solution with our reusable SPR sensor chip. For the second measurement, the normalized  $\Delta$ SPR signal is negligibly small. This result indicates that the SPR signal increases shown in Fig. 4A

was caused by the specific reactions of NP molecules and their antibodies instead of non-specific bindings of NP molecules and Ni patterns. The results of the third measurement are similar to those of the measurements in Fig. 4B. Note that our reusable SPR sensor chip was used to measure two different target molecules (IL-13 in the first measurement and NP in the third measurement), which allowed one to overcome the limitation of a conventional SPR sensor chip. Additionally, although we conducted the first and second measurement before the third measurement, our reusable SPR sensor chip could be used to measure NP solution with reliable results. These results clearly show that our reusable sensor chip can be repeatedly used for sensing various target molecules without the degradation of signals.

#### 4. Conclusions

In conclusion, we developed a reusable SPR sensor chip which can be used to repeatedly detect different target molecules in a conventional SPR system. Here, we used ferromagnetic patterns fabricated on a SPR sensor chip to trap a layer of magnetic particles, and those with trapped magnetic particles were used as a substrate for a SPR sensing in a conventional SPR system. Note that the Ni patterns and trapped magnetic particles did not affect the SPR characteristics of our sensor chip. After the sensing was completed, we applied external magnetic fields to the patterns for removing the used magnetic particles. The magnetic particles were completely removed so that we could trap a new layer of fresh magnetic particles on the SPR chip for additional sensing measurements. With only a single reusable SPR sensor chip, we successfully repeated sensing measurements to detect the nucleoprotein (NP) of H1N1 influenza virus for 7 times with high accuracy. With only a single reusable SPR sensor chip, we successfully repeated sensing measurements to detect the nucleoprotein (NP) of H1N1 influenza virus for more than 7 times without significant degradation of sensing signals. Also, we demonstrated that a single SPR chip could be utilized to detect different target molecules. Our method is a powerful strategy which allows one to reuse rather expensive SPR chips in a conventional SPR system and bring them for versatile practical applications such as

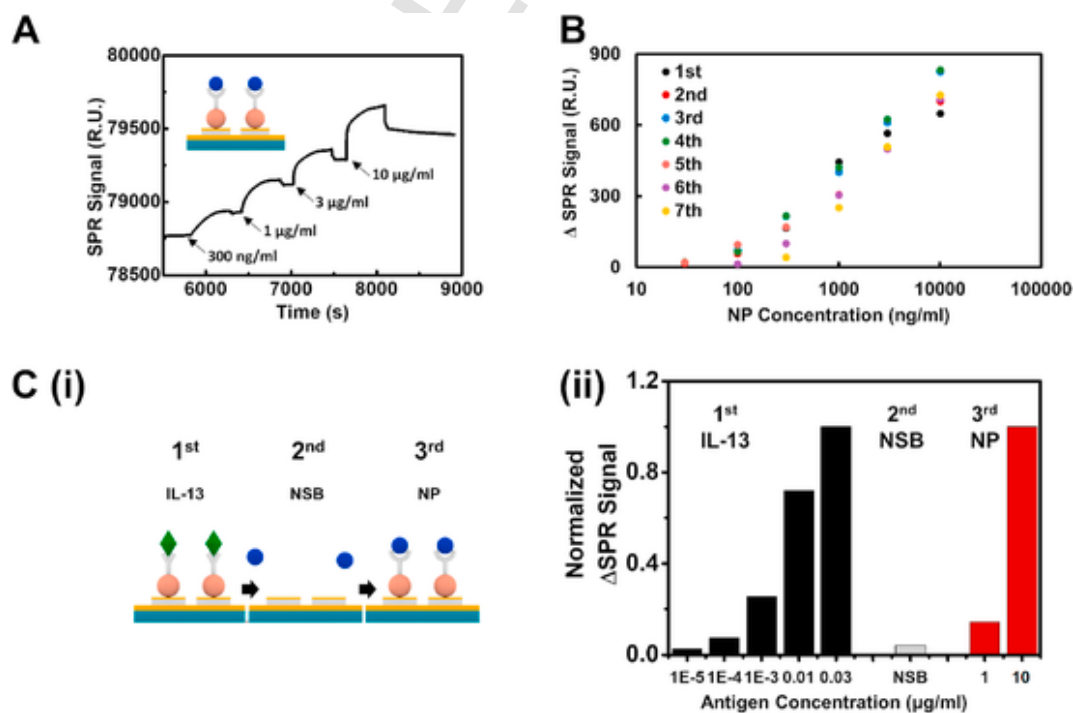


Fig. 4. Repeated SPR sensing measurements with reusable SPR sensor chips. (A) Real-time sensor responses after the sequential introduction of NP solution to a reusable SPR sensor chip. (B) Dose-dependence response of 7 repeated SPR sensing measurements with a single reusable SPR sensor chip. (C) Repeated SPR sensing measurement of two different target molecules (IL-13, NP) with a single reusable SPR sensor chip. (i) Schematic diagram of the repeated sensing measurement. (ii) Dose-dependence response of the measurement.

a molecular recognition study, a drug screening, a blood test, and a disease diagnosis.

### CRedit authorship contribution statement

**Haneul Yoo:** Methodology, Investigation, Writing - original draft, Visualization. **Junghyun Shin:** Methodology, Investigation, Writing - original draft, Visualization. **Jieun Sim:** Investigation, Visualization. **Hyunmin Cho:** Investigation, Visualization. **Seunghun Hong:** Conceptualization, Supervision, Project administration, Funding acquisition.

### CRedit authorship contribution statement

**Haneul Yoo:** Methodology, Investigation, Writing - original draft, Visualization. **Junghyun Shin:** Methodology, Investigation, Writing - original draft, Visualization. **Jieun Sim:** Investigation, Visualization. **Hyunmin Cho:** Investigation, Visualization. **Seunghun Hong:** Conceptualization, Supervision, Project administration, Funding acquisition.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bios.2020.112561>.

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