



## Detection of Hepatitis B Virus (HBV) DNA at femtomolar concentrations using a silica nanoparticle-enhanced microcantilever sensor

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### ABSTRACT

We report Hepatitis B Virus (HBV) DNA detection using a silica nanoparticle-enhanced dynamic microcantilever biosensor. A 243-mer nucleotide of HBV DNA precore/core region was used as the target DNA. For this assay, the capture probe on the microcantilever surface and the detection probe conjugated with silica nanoparticles were designed specifically for the target DNA. For efficient detection of the HBV target DNA using silica nanoparticle-enhanced DNA assay, the size of silica nanoparticles and the dimension of microcantilever were optimized by directly binding the silica nanoparticles through DNA hybridization. In addition, the correlation between the applied nanoparticle concentrations and the resonant frequency shifts of the microcantilever was discussed clearly to validate the quantitative relationship between mass loading and resonant frequency shift.

HBV target DNAs of 23.1 fM to 2.31 nM which were obtained from the PCR product were detected using a silica nanoparticle-enhanced microcantilever. The HBV target DNA of 243-mer was detected up to the picomolar (pM) level without nanoparticle enhancement and up to the femtomolar (fM) level using a nanoparticle-based signal amplification process. In the above two cases, the resonant frequency shifts were found to be linearly correlated with the concentrations of HBV target DNAs. We believe that this linearity originated mainly from an increase in mass that resulted from binding between the probe DNA and HBV PCR product, and between HBV PCR product and silica nanoparticles for the signal enhancement, even though there is another potential factor such as the spring constant change that may have influenced on the resonant frequency of the microcantilever.

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## 1. Introduction

Microcantilevers have emerged as viable biosensors demonstrating the prominent performance (Waggoner and Craighead, 2007; Fritz, 2008). Recently, the structures and materials of microcantilever have been improved due to advances in the micro/nano electromechanical system (MEMS/NEMS), and the applications of microcantilevers have been significantly expanded due to their utilization in the nano/biotechnology field. Microcantilevers have outstanding features such as high sensitivity and label-free detection, and have been used successfully for DNA hybridization (Wu et al., 2001a,b), immunoassays (Wu et al., 2001a,b) and particle detection including virus, bacteria and cell (Ilic et al., 2004). In par-

ticular, the static-mode microcantilevers have played a significant role in detecting DNA hybridization, and this mode of detection has been actively used for specific hybridization and single nucleotide polymorphisms (SNPs) detection (Hansen et al., 2001). Recently, dynamic-mode millimeter-sized cantilevers have been used for label-free detection of oligonucleotides at extremely low concentrations (Rijal and Mutharasan, 2007). In addition, there was a report on the sensitive DNA detection which used nanoparticle probe and silver enhancement to amplify the signal (Su et al., 2003).

Hepatitis B virus (HBV) infection is one of the most severe viral infectious diseases worldwide, with an estimated 400 million people chronically infected (Mao et al., 2006; Kim et al., 2007). Approximately, 70% of hepatocellular carcinoma cases are developed from the chronic hepatitis type B. HBV also has a capacity to escape immune surveillance by mutations of the structural genes, which encode epitopes that are recognized by the immune system, resulting in a quasi-species population. Because of the

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clinical reasons described above, it is very important to diagnose HBV at the early stage before chronicity and fatal mutations occur.

In this paper, we report Hepatitis B Virus (HBV) DNA detection using a silica nanoparticle-enhanced dynamic microcantilever biosensor. A 243-mer nucleotide of HBV DNA precore/core region was used as the target DNA. For this assay, the capture probe on the microcantilever surface and the detection probe conjugated with silica nanoparticles were designed specific for the target DNA. To efficiently detect the HBV target DNA using silica nanoparticle-enhanced DNA assay, the size of silica nanoparticles and the dimension of microcantilever were optimized by directly binding the silica nanoparticles through DNA hybridization. Finally, the correlation between the applied nanoparticle concentrations and the resonant frequency shifts of the microcantilever was discussed in order to validate the quantitative relationship between mass loading and resonant frequency shift.

## 2. Experimental

### 2.1. Device fabrication and resonant frequency measurement

The piezoelectric actuating layer (PZT) embedded microcantilevers were fabricated through a micro-machining process. PZT-microcantilevers do not require external actuators because of their direct signal transmission, which means that they have higher sensitivity than mono-layered microcantilevers that require an external actuator. Our microcantilevers consist of multi-layers with the following structure: Ta/Pt/PZT/Pt/SiO<sub>2</sub> on a SiN<sub>x</sub> supporting layer. A process for the PZT microcantilever fabrication is reported earlier (Hwang et al., 2006, 2004; Lee et al., 2004, 2005). Through the micro-fabricated process, rectangular shaped microcantilevers with two different dimensions were fabricated. Their dimensions were 50 μm × 150 μm × 1.75 μm and 30 μm × 90 μm × 1.75 μm (width × length × thickness), respectively. To minimize the nonspecific binding of DNA, the microcantilevers were treated with PEG-Si (2-[methoxy(polyethyleneoxy)propyl]trimethoxysilane, Gelest, Inc., USA). The microcantilevers were dipped in a 100 mM PEG-Si solution (in 99.5% EtOH) for 6 h, and then they were cured for 3 min at 110 °C to firm the PEG layer on the microcantilever surface (Backmann et al., 2005).

The reference resonant frequency of the microcantilever was measured before and after hybridization using an impedance analyzer (4294A, Agilent, USA) in a temperature-controlled chamber with a relative humidity controlled environment of 20%. The resonant frequency of the microcantilevers was measured by monitoring the phase angle of the impedance (Hwang et al., 2007).

### 2.2. Preparation of HBV target DNA probes

The clinical serum, which was stored at −70 °C before using, was collected in Korea, and the HBV-positive serum was confirmed using a nested polymerase chain reaction (PCR). The DNA was extracted from 140 μL serum with QIAamp DNA Mini-Kit (Qiagen, Germany), as recommended by the manufacturer. The HBV target probe used for hybridizing the HBV pre C, C region was generated by nested PCR using the outer primer (sense: GGCATGGACATTGACCC(G/T)TATAA; anti-sense: CTAATTCCTGGATGCTGG(G/A)TCT, 256 bp) and inner primer (sense: CATTGACCC(G/T)ATAAAGAATT; anti-sense: TCCTGGATGCTGG(G/A)TCTTCCAAA, size: 243 bp). The 20 μL reaction mixture contained 0.5 U of Taq polymerase (Supertaq, England) and 10× PCR reaction buffer with 1.5 mM MgCl<sub>2</sub>, 200 μM dNTP and 10 pmol of each primer. A 4.5 μL DNA sample was added to the PCR mix-

**Table 1**  
DNA sequences for the experiments.

|                         |                                                                                                                                                                                                                                                                                                                                           |
|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| cDNA1 (37mer)           | 5'-HS-T <sub>10</sub> CTT TCC TTC TAT TCG AGA TCT CCT CGA-3'                                                                                                                                                                                                                                                                              |
| dDNA1 (37mer)           | 3'-GAA AGG AAG ATA AGC TCT AGA GGA GCT T <sub>10</sub> -NH <sub>2</sub> -5'                                                                                                                                                                                                                                                               |
| cDNA2 (35mer)           | 5'-HS-T <sub>10</sub> TGG AGC TTC CGT GGA GTT ACT CTC T-3'                                                                                                                                                                                                                                                                                |
| HBV Target DNA (243mer) | 5'-TCC CTG GAT GCT GGG TCT TCC AAA TTA CTT CCC ACC CAG GTG GCC AGA TTC ATC AAC TCA CCC CAA CAC AGA ATA GCT TGC CTG AGT GCT GTA TGG TGA GGT GAA CAA TGT TCC GGA GAC TCT AAG GCC TCC CGA TAC AAA GCA GAG GCG GTG TCG AGG AGA TCT CGA ATA GAA GGA AAG AAG TCA GAA GGC AAA AAA GAG AGT AAC TCC ACG GAA GCT CCA AAT TCT TTA TAC GGG TCA ATG-3' |
| dDNA2 (35mer)           | 5'-ATC TGG CCA CCT GGG TGG GAA GTA A T <sub>10</sub> -NH <sub>2</sub> -3'                                                                                                                                                                                                                                                                 |

All DNA probes are HPLC grade and over 1 OD.

ture. The PCR conditions were 5 min at 94 °C followed by 35 cycles of 45 s at 94 °C, 45 s at 56 °C, and 45 s at 72 °C in a 9600 thermal cycler (PerkinElmer, USA). A final extension step of 72 °C for 5 min was included. The first PCR product of 1.5 μL was amplified using nested PCR primers (sense: CATTGACCC(G/T)ATAAAGAATT; anti-sense: TCCTGGATGCTGG(G/A)TCTTCCAAA, size: 243 bp) for an additional 25 cycles of 45 s at 94 °C, 45 s at 57 °C, and 45 s at 72 °C. The PCR product was purified using a QIAquick PCR purification kit (Qiagen, Germany), as recommended by the manufacturer.

### 2.3. Design of capture and detection DNA probes

All of the HPLC grade single-stranded DNA (ssDNA) probes were purchased from Bioneer (Korea). The details are shown in Table 1. To covalently immobilize the cDNA on the cantilever surface, the 5'-ends of two cDNAs (cDNA1 and cDNA2) were modified into thiol groups, and TTTTTTTTTT (T<sub>10</sub>) was inserted between the thiol termini and cDNA sequence to increase the hybridization efficiency by creating space between the complementary DNA of cDNA and the surface (Herne and Tarlov, 1997). 35mer cDNA2 and 35mer dDNA2 were selected for hybridization with the HBV target DNA as presented in Table 1. Also, dDNA1 was designed as a complementary sequence of cDNA1. The 5'-end of two dDNAs (dDNA1 and dDNA2) were modified into amine groups for conjugation to the SiNPs.

### 2.4. Immobilization of capture probes

The following procedure was used to immobilize the thiolated cDNA. Cr/Au layers (10 nm/50 nm) were deposited on the bottom side of the microcantilever using an e-beam evaporator. The Cr layer was used as an adhesive layer between the SiN<sub>x</sub> layer and the Au layer. Freshly Au-coated microcantilevers were immersed in 1 μM cDNA solution (TE buffer (Tris-EDTA buffer, pH 8.0)) for 3 h at RT, followed by spacer (5 mM HSC<sub>11</sub>-EG<sub>3</sub>-OH, Cos Biotech, Korea) backfilling for 90 min, which helps the formation of the self-assembled monolayer (SAM).

### 2.5. Preparation of silica nanoparticles–DNA conjugate

The detection probes were conjugated with 50 nm and 140 nm sized-SiNPs containing RITC (rhodamine B isothiocyanate) in their matrices. The prepared SiNPs were conjugated with the detection probes (called as dDNA-SiNPs). A 1 mg of SiNPs was dispersed in 1 mL of a MES buffer (2-(N-morpholino)ethanesulfonic acid) solution (pH 6.0). A 50 μL of 50 mg/mL EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) and 50 μL of 50 mg/mL NHS (N-hydroxysuccinimide) were then added to the

solution. The mixture was shaken for 1 h at room temperature. The activated SiNPs were rinsed once with a MES buffer solution, and then redispersed in 1 mL of the MES buffer solution. A 50  $\mu\text{L}$  (5 nmol) aliquot of the 100  $\mu\text{M}$  dDNA solution was added to the dispersion and shaken for 2 h at room temperature. The resulting dDNA-SiNPs were rinsed sequentially with PBST (PBS buffer with 1% of Tween-20) and PBS, and stored at 4 °C in the dark.

## 2.6. Direct hybridization of DNA-conjugated silica nanoparticles

For direct hybridization, the cDNA1 immobilized cantilevers were treated with dDNA1-SiNPs at 65 °C at a fixed concentration of 10  $\mu\text{g/mL}$  (TE buffer). For the quantitative hybridization, the cDNA1-immobilized microcantilevers were hybridized with different dDNA1-SiNPs concentrations of 1  $\mu\text{g/mL}$ , 10  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$ .

## 2.7. Silica nanoparticles-enhanced HBV DNA detection

The HBV DNA assay was performed on the cDNA-immobilized microcantilevers. They were incubated in the HBV target DNA solution at a concentration that ranged from 23.1 fM to 2.3 nM in TE buffer solution (0.1 mL) for 1 h at 65 °C. Thereafter, the microcantilevers were rinsed with PBST, PBS and deionized water. In the signal amplification experiments using the SiNPs, the HBV target DNA-captured microcantilevers were incubated with 10  $\mu\text{g/mL}$  of the dDNA2-SiNPs in TE buffer (1 mL) for 1 h at 65 °C. After the sandwich DNA assay, the microcantilevers were rinsed with PBST, PBS and deionized water.

## 3. Results and discussion

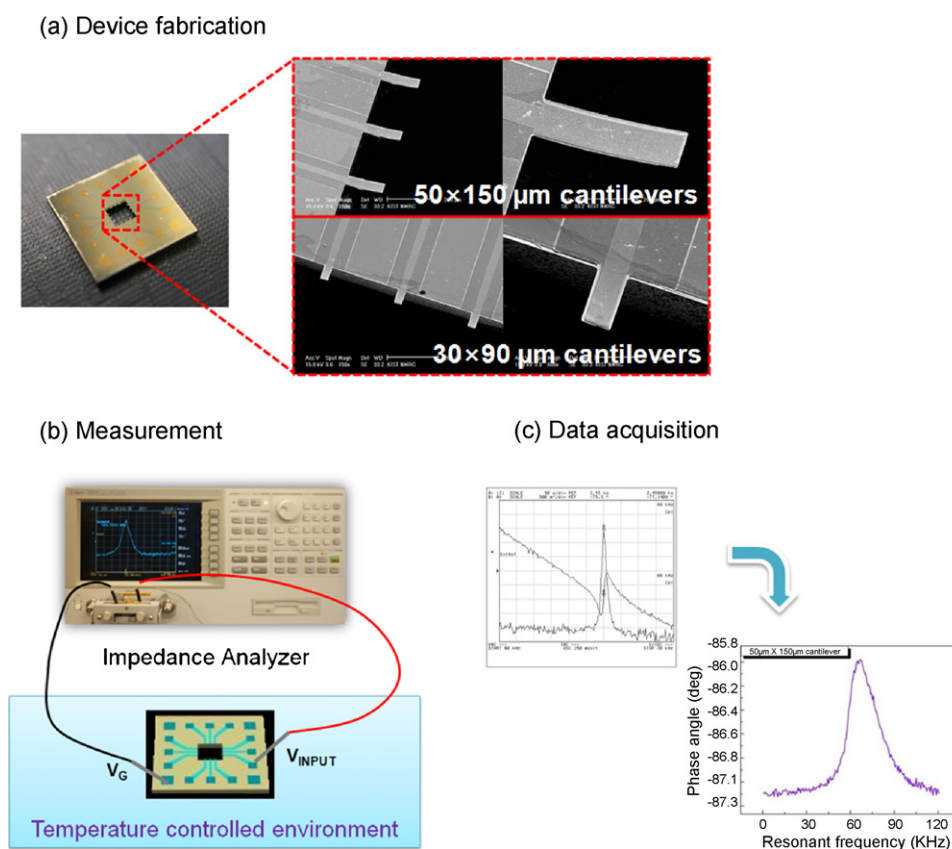
### 3.1. Surface functionalization of cantilever

The PZT-based dynamic microcantilevers were fabricated as previously described (Fig. 1a, 50  $\mu\text{m} \times 150 \mu\text{m} \times 1.75 \mu\text{m}$  and 30  $\mu\text{m} \times 90 \mu\text{m} \times 1.75 \mu\text{m}$  (width  $\times$  length  $\times$  thickness)) (Chun et al., 2007). For the SiNPs-enhanced DNA assays, the surface of the microcantilever was modified with PEG-Si prior to Au film deposition and subsequent DNA assay steps to minimize the nonspecific binding of biomolecules as previously reported (Backmann et al., 2005).

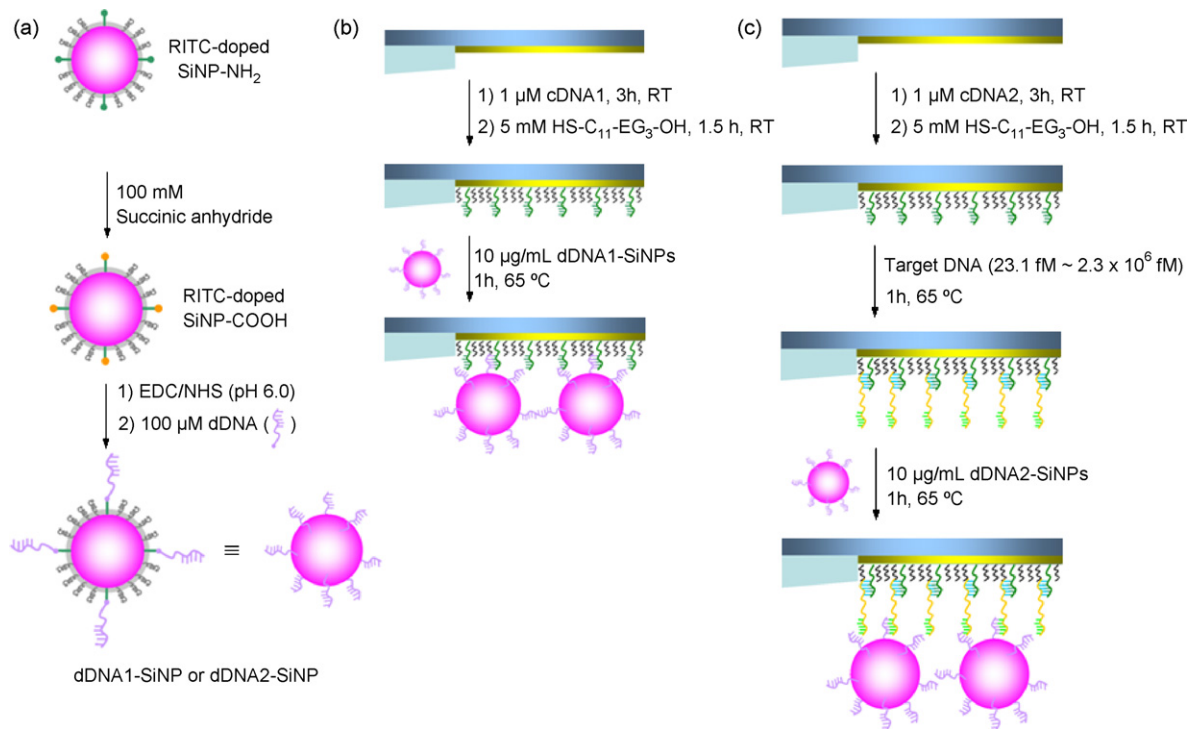
The Au film, which was used as the recognition layer, was placed on the bottom side of the microcantilever. Thiolated cDNA (1  $\mu\text{M}$ ) were immobilized on the Au film, followed by spacer backfilling. A spacer containing a hydrocarbon chain and ethylene glycol units was chosen to prevent nonspecific binding of biomolecules in the media and to optimize the arrangement of cDNA. Moreover, the length of the hydrocarbon linker between the oligonucleotide and thiol group was designed similar to that of backfilling spacer to facilitate the binding of complementary DNA (especially, HBV target DNA).

### 3.2. Preparation of DNA-conjugated silica nanoparticles

RITC-doped SiNPs (50 nm and 140 nm) were synthesized as previously reported (Wang and Tan, 2005; Yu et al., 2007), and the surface of the SiNPs was coated with the PEG (mw:  $\sim 600$ ) to reduce nonspecific binding to the microcantilever surface. The conjugation between carboxy-modified SiNPs and the dDNA was carried out as



**Fig. 1.** Dynamic microcantilevers and the data acquisition process for measuring the resonant frequency: (a) photograph of the single device with twelve microcantilevers and SEM images of two types of microcantilevers, (b) schematic diagram of the equipment used to measure the resonant frequency from the dynamic microcantilevers, which utilizes an impedance analyzer, and (c) resonant frequency data obtained from the impedance signal.



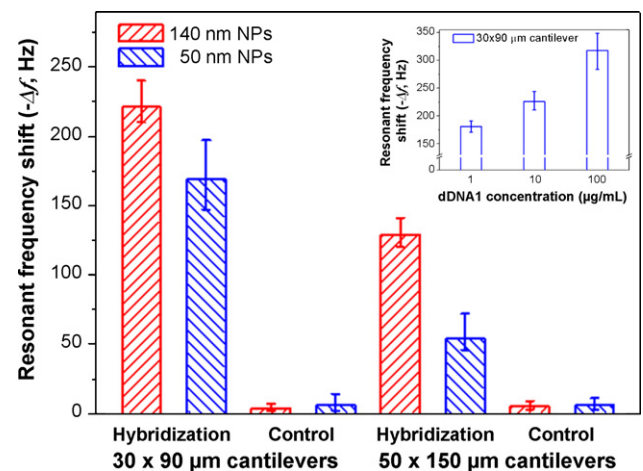
**Fig. 2.** Experimental procedures of the DNA assay using the SiNPs-enhanced microcantilever: (a) preparation of the dDNA-SiNPs, (b) an assay protocol for the direct hybridization between dDNA-SiNPs and cDNA on the microcantilever, and (c) HBV target DNA sandwich assay protocol based on SiNPs-enhanced microcantilevers.

shown in Fig. 2(a). Aminated DNA was coupled with the carboxy group on the SiNPs via EDC/NHS activation.

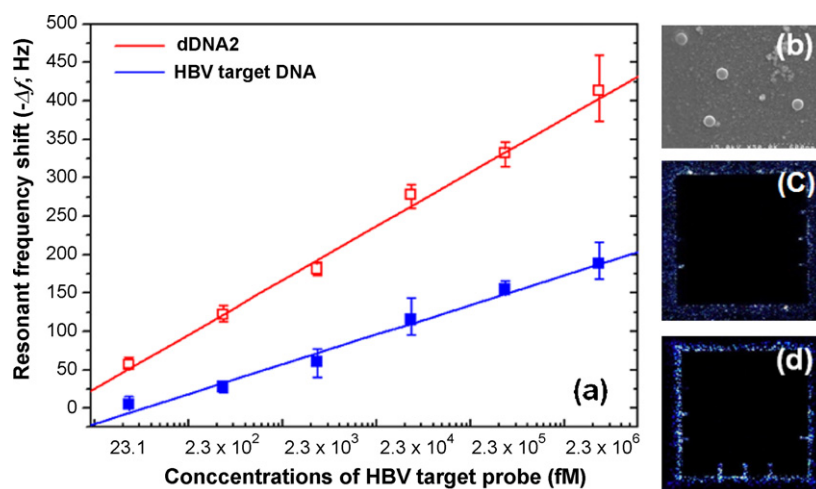
### 3.3. Optimization of SiNPs-enhanced DNA detection

The influence of SiNPs binding on the nanomechanical response of the microcantilever was investigated before the SiNPs-enhanced HBV target DNA detection was conducted. The frequency shifts were measured according to the size of the dDNA-SiNPs and the dimension of the microcantilever. First, we evaluated two types of SiNPs that differed in diameter as 50 nm and 140 nm respectively, and two types of microcantilevers that differed in dimension as  $30\ \mu\text{m} \times 90\ \mu\text{m}$  and  $50\ \mu\text{m} \times 150\ \mu\text{m}$  respectively (same thickness as  $0.7\ \mu\text{m}$ ). As shown in Fig. 2(b), the dDNA1-SiNPs were bound to the cDNA1-immobilized microcantilever through DNA hybridization. The concentration of dDNA1-SiNPs was fixed at  $10\ \mu\text{g/mL}$  for all these experiments. When the microcantilevers were smaller and the SiNPs were larger, the change in the resonant frequency was greater (Fig. 3). The microcantilever with dimension of  $30\ \mu\text{m} \times 90\ \mu\text{m}$  had bigger response to the 140 nm-sized SiNPs. In respect to microcantilever dimension, these results comply with the fact that the cantilevers are more sensitive as their dimensions become smaller (Chun et al., 2007). On the other hand, the larger-sized SiNPs may have more trouble interacting with the microcantilever surface due to the spatial instability of the large SiNPs ( $>200\ \text{nm}$ ), and small-sized SiNPs may not result in sufficient mass-induced signals ( $<50\ \text{nm}$ ). Based on this premise, a comparative study on the mass-inducing effect of both 50-nm and 140-nm-sized SiNPs was performed. The SiNPs with 140 nm diameter were more efficient in nanoparticle-based signal generation than the SiNPs with 50 nm diameter. Therefore, in all subsequent experiments SiNPs of 140 nm diameter and  $30\ \mu\text{m} \times 90\ \mu\text{m}$  dimension microcantilevers were used. As a final optimization step of the nanoparticle-based sandwich assay, we evaluated the resonant frequency shifts according to the concentrations of the applied SiNPs. The dif-

ferent dDNA1-SiNPs concentrations used in these experiments were  $1\ \mu\text{g/mL}$ ,  $10\ \mu\text{g/mL}$  and  $100\ \mu\text{g/mL}$ , which approximately correspond to  $3.5 \times 10^8$  particles/mL,  $3.5 \times 10^9$  particles/mL and  $3.5 \times 10^{10}$  particles/mL. This range covers much larger span than the maximum number of SiNPs which can completely cover the microcantilever surface as monolayer ( $\sim 1.6 \times 10^5$  particles). Actually, the binding efficiency of heterogeneous DNA hybridization between the solid nanoparticles and sensor surface is typically much lower than that of DNA hybridizations in solution. To overcome this limitation and obtain reliable results, an excess amount of SiNPs had to be applied to the nanoparticle-based sandwich assay. As shown in Fig. 4, the resonant frequency shift increased ( $-181\ \text{Hz}$  to  $-308\ \text{Hz}$ ) as the concentration of dDNA1-SiNPs increased ( $1$ – $100\ \mu\text{g/mL}$ ).



**Fig. 3.** The resonant frequency shifts measured according to the size of captured SiNPs and the dimension of the microcantilever. The microcantilever with dimension of  $30\ \mu\text{m} \times 90\ \mu\text{m}$  had the largest response to the 140 nm-sized SiNPs. The inset graph displays the resonant frequency shifts of the microcantilever ( $30\ \mu\text{m} \times 90\ \mu\text{m}$ ) according to the concentrations of the applied SiNPs (140 nm).



**Fig. 4.** The results from the HBV DNA assay using the SiNPs-enhanced dynamic microcantilevers: (a) plots of the resonant frequency shifts acquired from the HBV DNA assay and the silica nanoparticle-enhanced HBV DNA assay in the concentration range from 23.1 fM to 2.3 nM, (b) SEM image of the microcantilever surface with the captured SiNPs at 2.3 pM of the HBV target DNA, and the fluorescent images of the microcantilevers, (c) top side and (d) bottom side at 2.3 pM.

The binding assay for 1  $\mu\text{g/mL}$  of dDNA1-SiNPs generated the least resonant frequency shift. However, the deviation was also the lowest. In contrary, the reverse trend was observed when 100  $\mu\text{g/mL}$  of dDNA1-SiNPs was applied. As a result, the concentration of 10  $\mu\text{g/mL}$  was determined to be the optimal concentration of dDNA1-SiNPs, which was used for all subsequent experiments (HBV target DNA detection).

#### 3.4. SiNPs-enhanced HBV target DNA detection

Based on the optimized assay conditions the HBV target DNA (243 bp, PCR product) by using the SiNPs-enhanced dynamic microcantilever was first detected. The nanoparticle-based sandwich assay is a common method used for enhancing the sensitivity and amplifying the signal of biosensors. Many commercial diagnostic instruments (Englebienne et al., 2000) as well as analytical tools (Penn et al., 2003) have employed this strategy for performance improvement. In addition, this enhancement method has been used in dynamic microcantilever biosensors to detect a short nucleic acid (Su et al., 2003) and the prion protein (Varshney et al., 2008). However, these assay systems are less practical for DNA diagnosis because the measurement apparatus is complex or it requires additional staining step. In order to obtain the reliable signals from practical target DNA, the DNA-modified SiNPs (140 nm) for signal enhancement were employed, and the resonant frequency shifts were measured under controlled humid air conditions. There may be a change of spring constant induced by the DNA hybridization and silica particle binding, which can change the resonant frequency of microcantilever. However, we did not consider the effect of spring constant on the resonant frequency shift because the resonant frequency shifts induced by mass of HBV DNA (243 bp) as a target molecule or silica nanoparticles as a detection probe are dominant.

As shown in Table 1, the HBV target DNA of 243 bp which was obtained from PCR had two binding regions (underlines). The first underlined sequence indicates the hybridization site of cDNA2 (first underline) to the microcantilever surface and the second underlined sequence represents hybridization site of the dDNA2-SiNP. These are the first and the fifth candidates among the five HBV-specific sequences inducing HBV infection. The two terminal candidate regions were chosen to increase the step hybridization efficiency of both the HBV target DNA against the capture probe and the nanoparticle-conjugated detection probe against HBV target DNA. The cDNA2 was immobilized onto the Au surface of the

microcantilever, and the aminated dDNA2 was conjugated to the SiNPs as shown in Fig. 2(a and c).

The nanoparticle-enhanced HBV DNA assay is presented in Fig. 2(c). The cDNA2-immobilized microcantilevers were incubated with HBV target DNA samples at various concentrations (23.1 fM–2.3 nM), followed by the binding of 10  $\mu\text{g/mL}$  dDNA2-SiNPs. The resonant frequency of the microcantilever was measured at every step, and at least 10 microcantilevers per concentration were evaluated. When the concentrations were ranged between 23.1 fM and 2.3 nM, the resonant frequency shifts of the microcantilever ranged from  $-4$  Hz to  $-187$  Hz as a result of the capture of the HBV target DNA, and from  $-56$  Hz to  $-413$  Hz due to the enhancements by the dDNA2-SiNPs (Fig. 4(a)). The resonant frequency shifts of negative control (25-mer noncomplimentary DNA) were  $\sim -7$  Hz at the DNA binding step and  $\sim -24$  Hz at the nanoparticle enhancement step respectively (not presented in plots). After fitting the results, the resonant frequency shifts were found to be linearly correlated with the HBV target DNA concentrations in both cases. As a result of SiNPs enhancement, the sensitivity at each concentration improved by at least 2 times, and the limit of detection (LOD) was lowered by  $\sim 100$  times (LOD can be estimated around 2.3 fM via extrapolation although LOD of SiNP-enhanced HBV target DNA assay was not measured here). In addition, the SEM image of Fig. 4(b) exhibits that the SiNPs specifically bound to the microcantilever surface at 2.3 pM of HBV DNA, and the two images shown in Fig. 4(c and d) are fluorescent images of the top side and the bottom side of the microcantilever after the SiNP-enhanced HBV target DNA detection was conducted (2.3 pM). A 2.3 fM HBV target DNA (1 mL) corresponds to 1,300,000 copies/mL of HBV. The LOD levels of commercial devices are  $<5000$  copies/mL in the signal amplification techniques and  $<50$  copies in the target amplification techniques (Hatzakis et al., 2006). In this regard, the biosensor reported in this paper was not capable of reaching the sensitivity of commercial products. However, these commercial techniques have multiple and complex amplification steps compared with the microcantilever system described in this study. Thus, the simplicity of this novel system has significant and important benefits over commercially available system, and further study to improve the sensitivity by 3–4 orders of magnitude is in progress.

#### 4. Conclusions

We report the HBV DNA detection using a SiNPs-enhanced dynamic microcantilever biosensor. The HBV target DNA of 243 bp

was detected up to the picomolar (pM) level without using nanoparticle enhancement and up to the femtomolar (fM) level with using a signal amplification process. There were almost 2–3 orders of magnitude increase in sensitivity, and the microcantilever was found to be a highly sensitive and reliable diagnostic tool for DNA detection. The resonant frequency shifts were linearly correlated with the concentration of HBV target DNA in the above two cases. We believe that this linearity mainly originated from an increase in mass that resulted from binding between the probe DNA and HBV PCR product, and between HBV PCR product and SiNPs for the signal enhancement, even though there is another potential factor such as the spring constant change that may have influenced on the resonant frequency of the microcantilever.

According to these efforts, the microcantilever-based DNA assays for applications to PCR-free detection with extremely high sensitivity and the full sequence virus detection with extremely high selectivity could be advanced. Moreover, various methods are being developed to improve the sensitivity and selectivity. The microcantilever is expected to be a versatile biosensor for detection with high sensitivity such as DNA hybridization, immunoassay, and counting cells or virus particles as well as for detecting single defects in biomolecules and DNA mutations.

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### References

- Backmann, N., Zahnd, C., Huber, F., Bietsch, A., Pluckthun, A., Lang, H.P., Guntherodt, H.J., Hegner, M., Gerber, C., 2005. *Proc. Natl. Acad. Sci. U.S.A.* 102, 14587–14592.
- Chun, D.W., Hwang, K.S., Eom, K., Lee, J.H., Cha, B.H., Lee, W.Y., Yoon, D.S., Kim, T.S., 2007. *Sens. Actuators B* 135, 857–862.
- Englebienne, P., Van Hoonacker, A., Valsamis, J., 2000. *Clin. Chem.* 46, 2000–2003.
- Fritz, J., 2008. *Analyst* 133, 855–863.
- Hansen, K.M., Ji, H.F., Wu, G.H., Datar, R., Cote, R., Majumdar, A., Thundat, T., 2001. *Anal. Chem.* 73, 1567–1571.
- Hatzakis, A., Magiorkinis, E., Haida, C., 2006. *J. Hepatol.* 44, S71–S76.
- Herne, T.M., Tarlov, M.J., 1997. *J. Am. Chem. Soc.* 119, 8916–8920.
- Hwang, K.S., Lee, S.M., Eom, K., Lee, J.H., Lee, Y.S., Park, J.H., Yoon, D.S., Kim, T.S., 2007. *Biosens. Bioelectron.* 23, 459–465.
- Hwang, K.S., Eom, K., Lee, J.H., Chun, D.W., Cha, B.H., Yoon, D.S., Kim, T.S., Park, J.H., 2006. *Appl. Phys. Lett.* 89, 173905.
- Hwang, K.S., Lee, J.H., Park, J., Yoon, D.S., Park, J.H., Kim, T.S., 2004. *Lab Chip* 4, 547–552.
- Ilic, B., Yang, Y., Craighead, H.G., 2004. *Appl. Phys. Lett.* 85, 2604–2606.
- Kim, J., Han, K.-C., Yu, Y.G., Yang, E.G., 2007. *Biochip J.* 1, 134–139.
- Lee, J.H., Hwang, K.S., Park, J., Yoon, K.H., Yoon, D.S., Kim, T.S., 2005. *Biosens. Bioelectron.* 20, 2157–2162.
- Lee, J.H., Yoon, K.H., Hwang, K.S., Park, J., Ahn, S., Kim, T.S., 2004. *Biosens. Bioelectron.* 20, 269–275.
- Mao, H.L., Wang, H.M., Zhang, D.L., Mao, H.J., Zhao, J.L., Shi, J., Cui, Z.C., 2006. *Clin. Biochem.* 39, 67–73.
- Penn, S.G., He, L., Natan, M.J., 2003. *Curr. Opin. Chem. Biol.* 7, 609–615.
- Rijal, K., Mutharasan, R., 2007. *Anal. Chem.* 79, 7392–7400.
- Su, M., Li, S.U., Dravid, V.P., 2003. *Appl. Phys. Lett.* 82, 3562–3564.
- Varshney, M., Waggoner, P.S., Tan, C.P., Aubin, K., Montagna, R.A., Craighead, H.G., 2008. *Anal. Chem.* 80, 2141–2148.
- Waggoner, P.S., Craighead, H.G., 2007. *Lab Chip* 7, 1238–1255.
- Wang, L., Tan, W., 2005. *Nano Lett.* 6, 84–88.
- Wu, G.H., Ji, H.F., Hansen, K., Thundat, T., Datar, R., Cote, R., Hagan, M.F., Chakraborty, A.K., Majumdar, A., 2001a. *Proc. Natl. Acad. Sci. U.S.A.* 98, 1560–1564.
- Wu, G.H., Datar, R.H., Hansen, K.M., Thundat, T., Cote, R.J., Majumdar, A., 2001b. *Nat. Biotechnol.* 19, 856–860.
- Yu, K.N., Lee, S.M., Han, J.Y., Park, H., Woo, M.A., Noh, M.S., Hwang, S.K., Kwon, J.T., Jin, H., Kim, Y.K., Hergenrother, P.J., Jeong, D.H., Lee, Y.S., Cho, M.H., 2007. *Bioconjugate Chem.* 18, 1155–1162.