Combining a Nanowire SERRS Sensor and a Target Recycling Reaction for Ultrasensitive and Multiplex Identification of Pathogenic Fungi

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***D**evelopment of a rapid, sensitive, and multiplex pathogen DNA sensor enables early diagnosis and, subsequently, the proper treatment of infectious diseases, increasing the possibility to save the lives of infected patients. Here, the development of an ultrasensitive and multiplex pathogen DNA detection method that combines a patterned Au nanowire (NW)-on-film surface-enhanced resonance Raman scattering (SERRS) sensor with an exonuclease III-assisted target DNA recycling reaction is reported. Multiple probe DNAs are added to the target DNA solution, and among them, only the complementary probe DNA is selectively digested by exonuclease III, resulting in the decrease in its concentration. The digestion process is repeated by recycling of target DNAs. The decrease of the complementary probe DNA concentration is detected by SERRS. Combining the high sensitivity of the NW-on-film sensor and the target recycling reaction significantly improves DNA detection performance, resulting in the detection limit of 100 fM corresponding to 3 amole. By positioning Au NWs at specific addresses, multiple pathogen DNAs can be identified in a single step. Clinical sample tests with multiple genomic DNAs of pathogens show the potential of this sensor for practical diagnosis of infectious diseases.*

1. Introduction

Because infectious disease is caused by pathogenic microbial agents, rapid and accurate identification of pathogens from patients is vital for its early diagnosis, treatment, and prevention. Although culture-based assays have traditionally been used to identify pathogens, these methods are inherently time consuming and labor intensive. Furthermore, the wider spectrum of pathogenic fungi and their higher resistances of antifungal agents markedly reduce the accuracy and sensitivity of these methods.[1] To overcome these limitations, culture-based assays have recently been complemented by molecular diagnostic methods that allow detection and analysis of biomarkers such as DNA, RNA, and proteins.[2]

Several groups have recently suggested highly sensitive DNA detection methods that employ target recycling reactions, which allow repeated use of a single target DNA for hybridization with probe DNAs.[3] In these reactions, the enzyme selectively digests only the probe DNAs hybridized with the target DNAs. The intact target DNAs released after the digestion hybridize again with the probe DNAs repeatedly, thereby achieving improved sensitivity. To date, various sensing methods based on fluorescence,[3a,b,j] electrochemical signal,[3d,i] surface plasmon resonance (SPR),[3e–g] and color change[3c] have employed target recycling reactions to improve the sensitivity compared with the conventional 1:1 hybridization-based approach. Surface-enhanced

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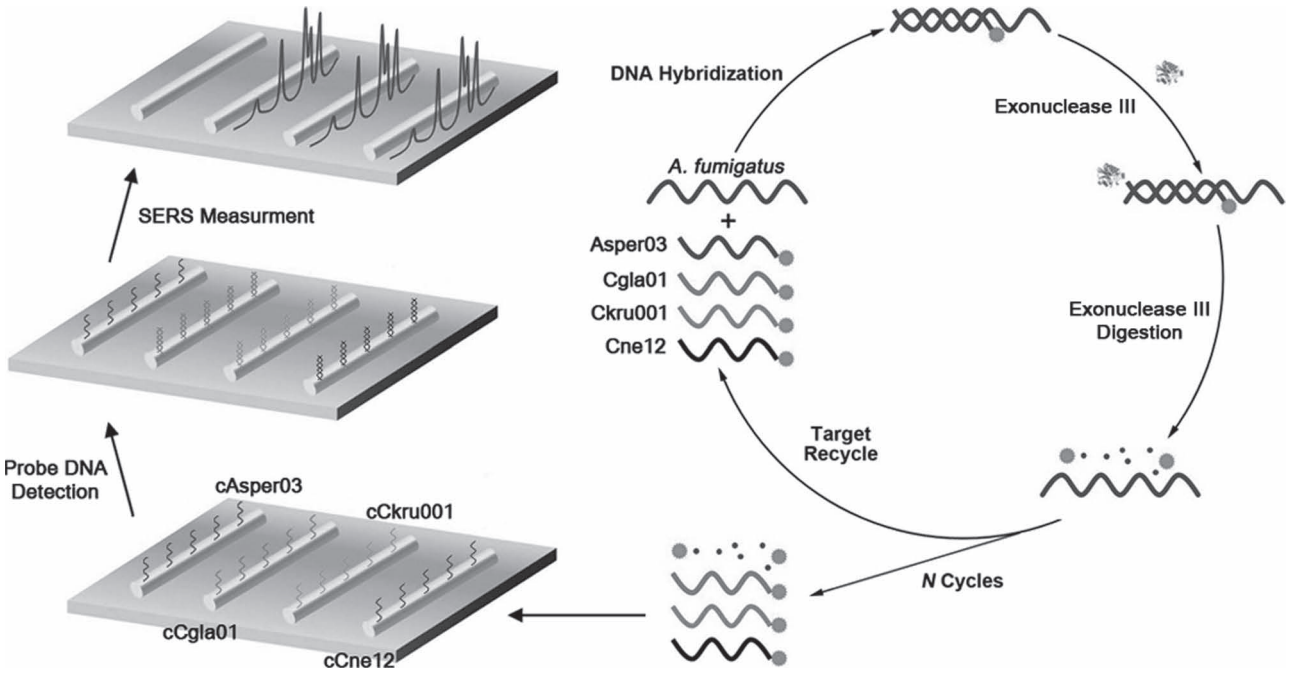

Figure 1. Schematic representation for the identification of pathogenic fungal DNAs by a patterned NW-on-film SERRS sensor coupled with an exonuclease III-assisted target recycling reaction.

Raman scattering (SERS) is a phenomenon that enormously increases Raman signals of molecules near the noble metal nanostructures.[4] It has been proven to be an attractive method for sensitive detection of DNAs because strong SERS signals can be observed from only a few molecules at localized nanoscale gaps, namely hot spots.[5] However, there still remains a challenging task to develop a practical SERS sensor that can detect multiple target DNAs simultaneously while retaining high sensitivities.

Here, we report the development of an ultrasensitive and multiplex DNA detection method that utilizes both a single nanowire (NW) surface-enhanced resonance Raman scattering (SERRS) sensor and target recycling reaction. This sensor allowed detection of pathogenic target DNA with a detection limit of 100 fM, which is ≈2 orders of magnitude more sensitive than that achieved with the previously reported SERS sensors that do not use enzymatic amplification.[6] Also, multiplex detection of target DNAs could be verified by testing with DNAs of various lengths and sequences. The disease diagnostic capability of this sensor could be further demonstrated by direct identification of the pathogenic fungal DNAs from clinical samples.

2. Results and Discussion

The schematic representation of pathogen DNA identification using a target recycling reaction coupled NW-on-film SERRS sensor is shown in **Figure 1**. Four pathogenic fungal DNAs, *Aspergillus fumigatus* (*A. fumigatus*), *Candida glabrata* (*C. glabrata*), *Candida krusei* (*C. krusei*), and *Cryptococcus neoformans* (*C. neoformans*), were chosen as targets. They account for more than 10% of septicaemia related pathogens and cause serious opportunistic infections in immunocompromised patients (e.g., transplant patients, AIDS sufferers, and cancer patients).[7] To identify these pathogenic fungal DNAs, four kinds of probe DNAs (Asper03, Cgla01, Ckru001, and Cne12; **Table 1**) labeled with Cy5 were prepared. The probe DNAs were designed so that they can selectively hybridize with their target DNAs but do not interact with each other. Cy5 as a Raman dye has an absorption maximum of 647 nm, allowing the incoming light with a wavelength of 633 nm to excite resonant vibration of molecule.[8a] SERRS typically enhances Raman signals by $\approx 10^2$ to 10^3 times more compared with nonresonant SERS.[8a] When *A. fumigatus* DNA was added as a target to a mixture of probe DNAs, the complementary Asper03 DNA selectively hybridized with this target DNA, forming a double-stranded DNA (dsDNA) with a blunt end. When exonuclease III, which specifically recognizes hybridized DNAs with blunt end and catalyzes stepwise removal of mononucleotides from 3′-hydroxyl termini of dsDNA,[3a] was added to the reaction tube, the enzyme digested Asper03 DNA and released *A. fumigatus* DNA from the DNA duplex. The released *A. fumigatus* DNA hybridized with another Asper03 DNA and a new cycle started. After all Asper03 DNA were hydrolyzed, only the probe DNAs (Cgla01, Ckru001, and Cne12) that were non-complementary to *A. fumigatus* DNA remained in the sample solution. These probe DNAs were then identified by the patterned NW-on-film SERRS sensor. The Au NW-on-film structure is a well-defined SERS active platform, providing a line of SERS hot spots at the gap between the NW and the film upon optical excitation.[8] The NW sensor was fabricated by placing four Au NWs, each attached with four different capture DNAs (cAsper03, cCgla01, cCkru001, and cCne12; Table 1) complementary to

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Table 1. DNA sequences used in the experiments.

Classification	Name	Sequence (5′ → 3′)
Probe DNA	Asper03 Cgla01 Ckru001 Cne12	Cy5-CATGGCCTTCACTGGCTGT Cy5-CTGGAATGCACCCGG Cy5-CTGGGCGGACGGTCT Cy5-TCCTGTATGCTCTTTACTGGGTGTGCAG
Capture DNA	cAsper03 cCgla01 cCkru001 cCne12	SH-$(CH_2)_6$-ACAGCCAGTGAAGGCCATG SH-$(CH_2)_6$-CCGGGTGCATTCCAG SH-$(CH_2)_6$-AGACCGTCCGCCCAG SH-$(CH_2)_6$-CTGCACACCCAGTAAAGAGCATACAGGA
Target DNA	tAsp tCgla tCkru tCneo	ACAGCCAGTGAAGGCCATGTAGTCTATGGCAGCATC CCGGGTGCATTCCAGTAGTCTATGGCAGCATC AGACCGTCCGCCCAGTAGTCTATGGCAGCATC CTGCACACCCAGTAAAGAGCATACAGGATAGTCTATGGCAGCATC

the corresponding probe DNAs, on a Au film. By measuring SERRS spectra from all four Au NWs after incubation of a sample solution on the NW-on-film sensor, the missing probe DNA could be detected from the positional address of the Au NW that did not show SERRS signal. If the cAsper03-attached NW shows no SERRS signal of Cy5, it means that Asper03 DNA is missing and that the target DNA is *A. fumigatus* DNA complementary to Asper03 DNA.

While nicking endonucleases, ribonuclease H (RNase H), and deoxyribozymes (DNAzymes) have been used to detect DNA by a similar scheme,[3c,e,f,j] exonuclease III was chosen in this study because it is easy to handle and works well on DNAs of various lengths and sequences.[3a] In the current method, employing patterned Au NWs as a SERS platform is critical for multiplex detection of pathogen DNAs. Conventional fluorescence, SPR, and color perception methods have used the positional encoding methods represented by microarray.[3e,g] The size, complex fabrication process, and lack of biocompatibility, however, still limit their broad usage.[9] Since Au NWs are biocompatible and very small in size (a few micrometers), they can be a superb material for making small sensor chips for multiplex detection.[6,10]

In order to improve the detection limit of this sensor, the probe DNA concentration, enzyme concentration, and incubation time were optimized for two kinds of probe DNAs (Asper03 and Cgla01) and synthetic target DNA (tAsp;Table 1) complementary to Asper03 DNA. First, the effect of concentration of probe DNA was investigated. The 0.1 μM target DNA were incubated with different concentrations of probe DNA (0.5, 1, 2.5, 5, 10, 20, and 30 μM) in solution containing exonuclease III (100 U per assay) at 37 °C for 1 h and the optimal probe DNA concentration was determined to be 5 μM (**Figure 2**a). Next, the reaction conditions for exonuclease III were optimized. It is important to determine the enzymatic reaction conditions because the exonuclease III digestion is the rate-limiting step of the target recycling reaction.[3d] The solution containing 5 μM probe DNA and 0.1 μM target DNA were treated with various concentrations of exonuclease III (50, 100, 150, and 200 U per assay) at 37 °C for 1 h (Figure 2b). In order to determine the optimal time of enzyme reaction, the mixture of 5 μM probe DNA, 0.1 μM target DNA, and 100 U per assay exonuclease III was incubated at 37 °C for varied incubation times (10, 20, 30, 40, 50, and 60 min) (Figure 2c). Figure 2b,c show that an enzyme concentration of 100 U per assay and a reaction time of 40 min are optimal. Thus, all subsequent experiments were performed for 40 min using 5 μM probe DNA and 100 U per assay exonuclease III. In Figure 2d, dark gray spectrum of cAsper03 was obtained after full digestion with the enzyme, while the gray spectrum of cCgla01 remained intact after digestion. Both spectra were obtained under optimized conditions. The weak SERRS signals of cAsper03 NW can be attributed to the non-specific binding of Cy5. The distinct difference of the two 1580 cm^{-1} band intensities clearly showed the identification of the target DNA. The entire identification process required only a few hours.

Figure 3 shows a plot of the SERRS intensities versus target DNA concentrations in ≈10^{-15} to 10^{-9} M. The SERRS intensity increased linearly with decreasing target DNA concentration between 10^{-12} and 10^{-14} M. From the three times standard deviation rule, the detection limit of this method was estimated to be 100 fM corresponding to 3 amol (1.8×10^6 molecules), when a typical assay volume of 30 μL is used. This sensitivity is about two to three orders of magnitude better than that obtained with the previously reported SERS-based detection methods.[6,11]

Next, samples prepared by mixing four target DNAs (tAsp, tCgla, tCkru, and tCneo; Table 1) were tested to see the possibility of multiplex detection. The concentration of only tAsp was varied while concentrations of the other target DNAs were fixed at 100 nM. The same detection limit of 100 fM could be achieved (Supporting Information, Figure S2a), indicating that the NW SERRS sensor can identify pathogen DNA even from a mixture of various DNAs at high concentrations without cross reactivity. Detection of pathogenic fungus was next performed using this sensor system. DNAs were extracted from the reference pathogenic strain and a concentration of 100 nM was used. Pathogenic fungal DNA and exonuclease III were added to a mixture of four different probe DNAs (Asper03, Cgla01, Ckru001, and Cne12). After incubation at 37 °C for 40 min, the resultant DNA solutions were incubated on the NW-on-film sensor consisting of four Au NWs modified by four capture DNAs (cAsper03, cCgla01, cCkru001, and cCne12), respectively. **Figure 4** shows that the SERRS intensity decreased only at the NW-on-film structure modified by capture DNA corresponding to the pathogenic fungal DNA. This result verifies

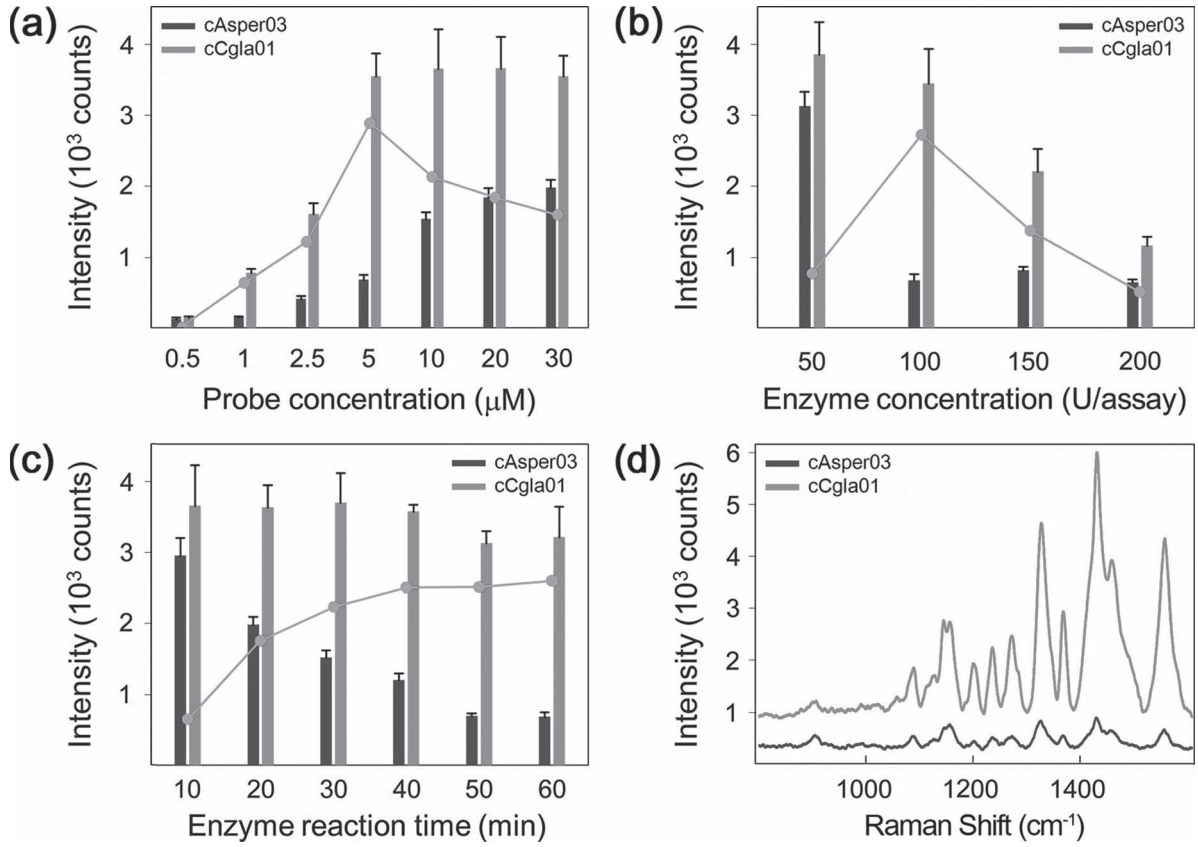

Figure 2. Determination of the optimal conditions for exonuclease III-assisted target recycling reaction. The dark gray and gray bars represent the SERRS intensities of 1580 cm^{-1} band measured from cAsper03 and cCgla01-modified NW-on-film structures, respectively. The line represents the difference of two SERRS intensities. a) Effect of probe DNA concentration on SERRS intensity. Target DNA (tAsp) was incubated with different concentration of probe DNAs (Asper03 and Cgla01; 0.5, 1, 2.5, 5, 10, 20, and 30 μM) in solution containing exonuclease III. b) Effect of enzyme concentration on SERRS intensity. The probe (Asper03 and Cgla01) and target (tAsp) DNAs were incubated with various concentrations of exonuclease III (50, 100, 150, and 200 U per assay). c) Effect of reaction time on SERRS intensity. The probe (Asper03 and Cgla01) and target (tAsp) DNAs were incubated with exonuclease III for different reaction times (10, 20, 30, 40, 50, and 60 min). d) SERRS spectra of Cy5 obtained from cAsper03 and cCgla01-modified Au NW-on-film sensors under optimized conditions.

that multiplex detection of real pathogen DNAs is possible. More importantly, the detection limit of this sensor was not affected by the length of DNA. The SERRS results for long *A. fumigatus* DNA (307 bp) showed a detection limit of 100 fM (Supporting Information, Figure S2b). Considering that one of the major challenges for SERS-based assays lies in the detection of long single-stranded DNA sequences, this result is quite promising.

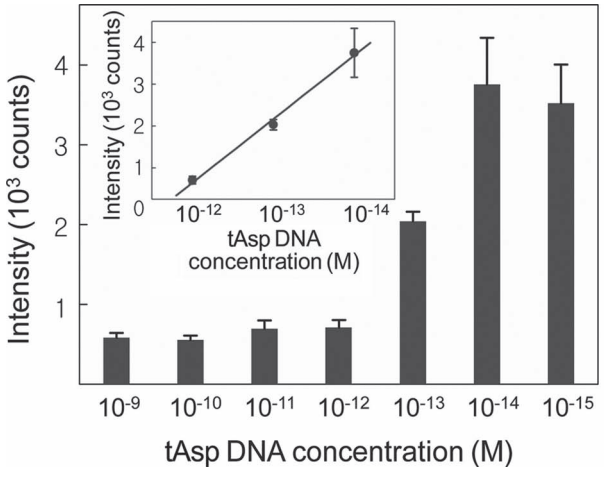

Figure 3. Plot of SERRS intensities of 1580 cm^{-1} band versus concentrations of target DNA (tAsp). The inset shows a dynamic range and linear fit line.

Finally, diagnosis of real clinical samples was performed using this sensor. The target DNAs were extracted from positive blood culture samples and dropped onto the NW-on-film sensor in a blind manner. Among the eight clinical samples, five samples showed decreased SERRS signals on the NWs attached with cAsper03 DNA and three samples at the NWs with cCgla01 DNA (Supporting Information, Figure S3). The diagnostic results from the NW sensor exactly agreed with those obtained by conventional culture-based assays.

Practical pathogen DNA assay should be simple, sensitive and fast, and allow detection of multiple pathogenic agents in one reaction.[2a] The sensing method presented in this study is extremely sensitive, rapid, and allows multiplex detection of pathogens simultaneously after a reaction in a single tube. All these advantages are attained by combining a patterned Au NW-on-film sensor with a target recycling reaction.

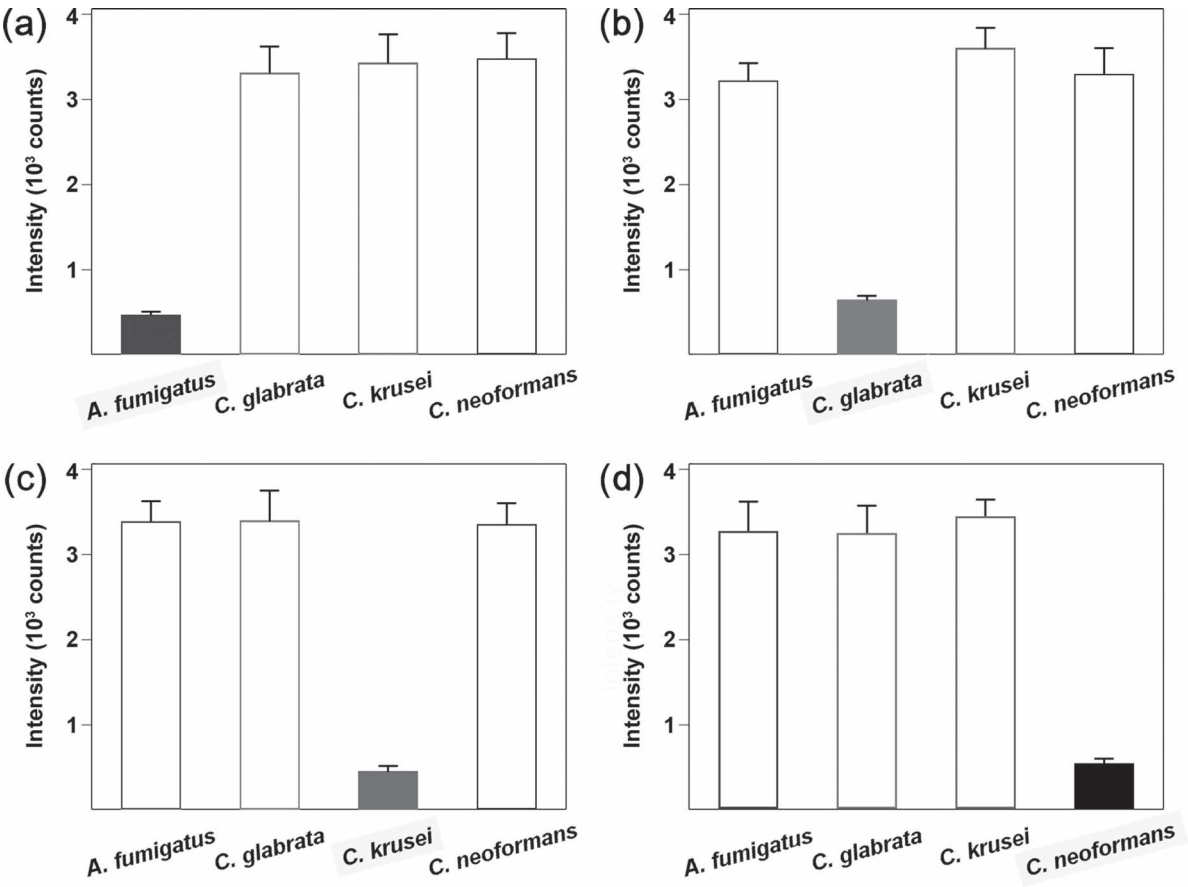

Figure 4. a–d) Identification of pathogenic fungal DNAs by a patterned NW-on-film sensor coupled with a target recycling reaction. The bars represent the SERRS intensities of the 1580 cm^{-1} band obtained from each NW-on-film sensor. When the sample contains fungal target DNAs, only the corresponding capture DNA-modified NW-on-film sensor shows decreased SERRS signals.

3. Conclusion

We have developed a highly sensitive and multiplex DNA detection assay system using a patterned Au NW-on-film SERRS sensor combined with a target recycling reaction employing exonuclease III. This sensor system allowed detection of target DNAs with a detection limit of as low as 100 fM and was used to successfully identify target pathogen DNAs from clinical samples, showing its potential for practical diagnosis of infectious diseases.

4. Experimental Section

Materials: All oligonucleotides were synthesized and purified by Genotech, Daejeon, Korea. Thiol-functionalized capture DNAs were treated with 1 M dithiothreitol (DTT; Sigma-Aldrich) and purified using a NAP-5 column (GE healthcare Co.). Genomic DNA from clinical isolates were kindly provided by the Yonsei Severance Hospital (Seoul, Korea).

Preparation of the NW-on-Film SERRS Sensor: Single crystalline Au NWs were synthesized using the vapor transport method reported previously.[8,12] As-synthesized Au NWs had diameters of ≈100 to 200 nm and lengths of tens of micrometers. The absorption band of the Au NWs was about 570 nm (Supporting Information,Figure S3). For the immobilization of capture DNA, 5 μM capture DNA in 1 M KH_2PO_4 (pH 6.7) was incubated on as-grown Au NWs at room temperature for 18 h and excessive DNA was washed with 0.2% (w/v) sodium dodecyl sulfate (SDS) for 5 min. The capture-DNA-modified Au NWs were then transferred onto a Au film that was fabricated by electron beam assisted deposition of 10 nm Cr followed by 300 nm Au. For the patterning of NWs, a custom-built nanomanipulator with a tungsten tip (≈100 nm diameter at the end) mounted on a 3D piezoelectric stage (Sigma-Koki) was used.[5h] By using a nanomanipulator, a single NW can be transferred routinely and the positional address of the NW provides the multiplex detection capability.[6]

Preparation of Pathogenic Fungal DNA: Reference fungi used in this study (Supporting Information, Table S1) were obtained from the Korea Culture Center of Microorganisms (KCCM, Seoul, Korea) and the Korean Collection for Type Cultures (KCTC, Daejeon, Korea). All clinical isolates were provided by the Yonsei University College of Medicine, Seoul, Korea. Genomic DNAs from reference strains and clinical isolates were extracted using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Blood culture samples were treated as follows: 200 μL of blood culture sample was mixed with 1 mL of tris(hydroxymethyl)aminomethane-ethylenediaminetetraacetic acid (Tris-EDTA) buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and vortexed to lyse the red blood cells (RBCs). Then, it was centrifuged at 13 000 g for 1 min for the removal of red blood cells (RBCs).

The above steps were repeated twice and the pellet was collected. Genomic DNAs from the resultant samples were extracted and eluted with 100 μL of elution buffer.

The target DNAs were prepared by amplifying 18S ribosomal DNA (rDNA) from reference strains (Supporting Information, Table S1). Polymerase chain reaction (PCR) for target DNA preparation was performed with the universal and species-specific primers by using the genomic DNAs from reference fungi and clinical isolates. PCR was performed in 50 μL reactions containing 1× reaction buffer, 0.2 mM deoxyribonucleotide triphosphate (dNTP), 2 units of *Thermus aquaticus* (*Taq*) DNA polymerase (Takara Shuzo Co., Shiga, Japan), 5 pM forward primer, and 25 pM reverse primer. Species-specific primers have the same sequences as those of species-specific probes. PCR using species-specific primers (Asper03 for *A. fumigatus*, Cgla01 for *C. glabrata*, Ckru001 for *C. krusei*, and Cne12 for *C. neoformans*) and universal primer (FunR, 5′-CTACGACGGTATCTGATCAT-3′) was performed under the following amplification conditions: 94 °C for 4 min, followed by 40 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min.

Detection of Pathogenic Fungal DNA: The probe DNA labeled with Cy5 and pathogenic fungal DNA was incubated with exonuclease III (New England BioLabs Ltd.) in enzyme specific buffer at 37 °C. The resultant solution was incubated at 75 °C for 10 min in order to inactivate enzyme and sequentially incubated on the Au NW-on-film sensor at 37 °C for 1 h. After washing with 2× saline-sodium citrate (SSC) containing 0.1% (w/v) SDS for 5 min and drying under slight N_2 stream, SERRS spectra of the NW-on-film sensor were measured under ambient conditions.

Instrumentation: SERRS spectra were measured with a home-built micro-Raman system based on an Olympus BX41 microscope. The 633 nm radiation of a He–Ne laser (Melles Griot) was used as an excitation source and the laser light was focused on a sample through a ×100 objective (numerical aperture NA = 0.7, Mitutoyo). The SERRS signals were recorded with a thermodynamically cooled electron multiplying charge coupled device (EMCCD, Andor) mounted on the spectrometer with a 1200 groove mm^{-1} grating. A holographic notch filter was used to reject the laser light.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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[1] L. G. Reimer, M. L. Wilson, M. P. Weinstein, *Clin. Microbiol. Rev.* **1997**, *10*, 444.
[2] a) J. A. M. Vet, A. R. Majithia, S. A. E. Marras, S. Tyagi, S. Dube, B. J. Poiesz, F. R. Kramer, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6394; b) R. Eriksson, M. Jobs, C. Ekstrand, M. Ullberg, B. Herrmann, U. Landegren, M. Nilsson, J. Blomberg, *J. Microbiol. Methods* **2009**, *78*, 195; c) H. A. Erlich, D. Gelfand, J. J. Sninsky, *Science* **1991**, *252*, 1643.
[3] a) X. L. Zuo, F. Xia, Y. Xiao, K. W. Plaxco, *J. Am. Chem. Soc.* **2010**, *132*, 1816; b) J. W. J. Li, Y. Z. Chu, B. Y. H. Lee, X. L. S. Xie, *Nucleic Acids Res.* **2008**, *36*, e36; c) W. Xu, X. J. Xue, T. H. Li, H. Q. Zeng, X. G. Liu, *Angew. Chem. Int. Ed.* **2009**, *48*, 6849; d) K. Hsieh, Y. Xiao, H. T. Soh, *Langmuir* **2010**, *26*, 10392; e) T. T. Goodrich, H. J. Lee, R. M. Corn, *Anal. Chem.* **2004**, *76*, 6173; f) T. T. Goodrich, H. J. Lee, R. M. Corn, *J. Am. Chem. Soc.* **2004**, *126*, 4086; g) H. J. Lee, Y. Li, A. W. Wark, R. M. Corn, *Anal. Chem.* **2005**, *77*, 5096; h) S. Bi, J. L. Zhang, S. S. Zhang, *Chem. Commun.* **2010**, *46*, 5509; i) J. H. Chen, J. Zhang, J. A. Li, F. F. Fu, H. H. Yang, G. N. Chen, *Chem. Commun.* **2010**, *46*, 5939; j) S. Sando, T. Sasaki, K. Kanatani, Y. Aoyama, *J. Am. Chem. Soc.* **2003**, *125*, 15720.
[4] a) M. Moskovits, *Rev. Mod. Phys.* **1985**, *57*, 783; b) R. Gunawidjaja, E. Kharlampieva, I. Choi, V. V. Tsukruk, *Small* **2009**, *5*, 2460; c) H. Ko, V. V. Tsukruk, *Small* **2008**, *4*, 1980.
[5] a) R. A. Alvarez-Puebla, L. M. Liz-Marzan, *Small* **2010**, *6*, 604; b) K. J. Jang, H. Lee, H. L. Jin, Y. Park, J. M. Nam, *Small* **2009**, *5*, 2665; c) J. Hu, P. C. Zheng, J. H. Jiang, G. L. Shen, R. Q. Yu, G. K. Liu, *Analyst* **2010**, *135*, 1084; d) H. Xu, E. J. Bjerneld, M. Käll, L. Börjesson, *Phy. Rev. Lett.* **1999**, *83*, 4357; e) H. Xu, J. Aizpurua, M. Käll, P. Apell, *Phy. Rev. E.* **2000**, *62*, 4318; f) H. Wei, F. Hao, Y. Huang, W. Wang, P. Nordlander, H. Xu, *Nano Lett.* **2008**, *8*, 2497; g) Y. Fang, H. Wei, F. Hao, P. Nordlander, H. Xu, *Nano Lett.* **2009**, *9*. 2049; h) T. Kang, I. Yoon, K.-S. Jeon, W. Choi, Y. Lee, K. Seo, Y. Yoo, Q.-H. Park, H. Ihee, Y. D. Suh, B. Kim, *J. Phys. Chem. C* **2009**, *113*, 7492; i) Y. C. Cao, R. Jin, C. A. Mirkin, *Science* **2002**, *297*, 1536.
[6] T. Kang, S. M. Yoo, I. Yoon, S. Y. Lee, B. Kim, *Nano Lett.* **2010**, *10*, 1189.
[7] M. D. Richardson, *J. Antimicrob. Chemother.* **2005**, *56*, 5.
[8] a) T. Kang, S. M. Yoo, I. Yoon, S. Lee, J. Choo, S. Y. Lee, B. Kim, *Chem. Eur. J* **2011**, *17*, 2211; b) I. Yoon, T. Kang, W. Choi, J. Kim, Y. Yoo, S.-W. Joo, Q.-H. Park, H. Ihee, B. Kim, *J. Am. Chem. Soc.* **2009**, *131*, 758.
[9] Y. G. Li, Y. T. H. Cu, D. Luo, *Nat. Biotechnol.* **2005**, *23*, 885.
[10] a) T. J. Morrow, M. W. Li, J. Kim, T. S. Mayer, C. D. Keating, *Science* **2009**, *323*, 352; b) B. He, T. J. Morrow, C. D. Keating, *Curr. Opin. Chem. Biol.* **2008**, *12*, 522; c) J. Lee, A. A. Wang, Y. Rheem, B. Yoo, A. Mulchandani, W. Chen, N. V. Myung, *Electroanalysis* **2007**, *19*, 2287.
[11] L. Sun, C. X. Yu, J. Irudayaraj, *Anal. Chem.* **2007**, *79*, 3981.
[12] Y. Yoo, K. Seo, S. Han, K. S. K. Varadwaj, H. Y. Kim, J. H. Ryu, H. M. Lee, J. P. Ahn, H. Ihee, B. Kim, *Nano Lett.* **2010**, *10*, 432.

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