

A DNA sensor using gold-coated barcode silica nanotubes

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ABSTRACT

The bio barcode silica nanotubes (SNTs) was developed to detect pathogenic bacteria using quantum dots and beacon-type capture probe. Various quantum dots were embedded in SNT (QD-SNT) to generate a barcoding signal with different colors and orders. The QD-SNT was coated by colloidal gold nanoparticle to immobilize hairpin structured capture probe on outer walls. Pathogenic specific capture probes, which have fluorescent dye, were designed and immobilized on the outer wall of each QD-SNTs, respectively. As a result, these barcoded QD-SNTs could detect the *E.coli* specific nucleic acid products amplified by NASBA (Nucleic Acid Sequence Based Amplification).

Keywords: silica nanotube, quantum dots, gold nanoparticle, barcode

1 INTRODUCTION

A biosensor is an analytical device which converts a biological response into readable signal [1,2]. A biosensor consists of a bio-element and a sensor-element. The bio-element may be an enzyme, antibody, living cells, tissue, and so on [3-5]. A successful biosensor needs to possess at least some of the following beneficial features; the biocatalyst must be highly specific for the purpose of the analyses, be stable under normal storage conditions, and except in the case of colorimetric enzyme strips and dipsticks, show good stability over a large number of assays [6-8].

Hollow inorganic nanotubes are attracting a great deal of attention due to their fundamental significance and potential applications in bioanalysis and catalysis. Particularly, silica nanotubes are of great interest because of their hydrophilic nature, easy colloidal suspension formation, and surface functionalization accessibility for both inner and outer walls [9]. Silica nanotubes have been synthesized typically within the nanopores of porous alumina membrane templates using sol-gel coating technique [10-11]. These silica structures have a number of advantages over organic polymeric nano structures. First, the preparative processes involved require simple, ambient temperature conditions. These can be prepared with the desired size, shape, and porosity, and are extremely stable. These silica based nanostructures are also known as biocompatible materials and ease of surface modification for attaching targeting ligands, drugs, and imaging agents.

The silica nanoparticles were one of the representative materials in nanoscience. The nanoparticles have also been applied with nucleic acid sequence or protein in biologic system to research about the field of nanomedicine. The nanowires are usually applied as electronic elements whereas they can be replaced by nanotubes in the application of the other fields [12-15].

The silica nanotubes(SNTs) are typical example of inorganic nanotubes. The SNTs have been mostly studied at inorganic nanotubes related biology system, because they are more safety than other metals in human body. SNTs have many advantages that the length-to-diameter ratio was extremely high compared to any nanomaterials and the tensile stress is also strongest yet. These advantages were big issues of all nanomaterials whereas the surface property of carbon nanotube is superhydrophobic that contributes some problem that is bonding difficult with bio-molecules. In case of inorganic nanotubes, they are consisted of metal oxides with cylindrical nanostructure. The SNTs have a shape of a cylindrical nanostructure and this shape attach great importance as delivery materials to biotechnologic field. Therefore the research of gene delivery using SNTs has been studied actively. This way is that DNA was loaded in SNT using electrostatic forces that happens when electronic surface property of inner surface of SNT transfers positive charge and it enables SNTs to deliver target location. The biolabeling that can label target location using SNTs loaded by fluorescence such as quantum dots was one of application about SNTs. Furthermore SNTs embedded magnetite nanoparticles (magnetic nanotubes) were developed to be controlled their movement by magnetic field for delivering target and to investigate a track of drug delivery by magnetic resonance imaging (MRI). The application of SNTs as a biodetection for diagnosis of disease is still very rare. It is difficult for biomolecules such as DNA or antibody to immobilize with SNTs. Furthermore, the bioassay such as hybridization of DNA/RNA or antibody-antigen binding is performed in porous of nanosize like SNTs to be a biosensor not to be delivery material. These problems make waste of time and practical reasons that application of SNTs as a sensor was so rare [16-19].

In this study, we demonstrate the feasibility of silica nanobarcodes as DNA or RNA biosensors. The silica nanotube is coated by colloidal gold nanoparticle to introduce biological functions. The fabricated biobarcode silica nanotubes could detect the *E.coli* efficiently.

2 MATERIALS AND METHOD

2.1 Materials

Aluminum foils (99.99%) were purchased from Alfa Aesar. Perchloric acid (70%, DC Chemical), oxalic acid (Dihydrate, DC Chemical), phosphoric acid (85%, DC Chemical), silicon tetrachloride (SiCl_4 , 99.8%, Acros Organics) were used as supplied without further purification. Green, red and yellow CdSe/ZnS quantum dots(QDs) covered with trioctylphosphine oxide(TOPO) were provided by Prof. Son (Kyungwon University, Korea). Gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot \text{H}_2\text{O}$, Aldrich), trisodium citrate dehydrate (sigma-aldrich), sodium tetrahydridoborate (NaBH_4 , Fluka), (3-aminopropyl)-trimethoxysilane (APTMS, Aldrich), hydroxylamine hydrochloride (Aldrich) were purchased as supplied. E. coli and salmonella beacon were purchased from bioneer. E.coli0157:H7 (ATCC 43895) and Salmonella typhimurium (ATCC 6994) were purchased from American Type Culture Collection.

2.2 Preparation of silica nanotubes

Anodic aluminum oxide (AAO) templates prepared according to previously published protocols were coated with silica using the surface sol-gel method as reported previously. The AAO templates were immersed in SiCl_4 , rinsed with hexane, dipped in a mixture of hexane and methanol, then rinsed with ethanol. The templates were immersed in water after being dried with nitrogen gas. Nine deposition cycles were performed as above. Schematic diagram was shown in figure 1.

2.3 Quantum dots in silica nanotube

After plasma treatment of silica nanotube, the resultant templates were taken functional group to load quantum dots using reactant solution by reflux apparatus. The silica-coated AAO templates that treated reflux were then immersed in quantum dot solution (quantum dots in toluene) in oven (40 °C). The templates were immersed in a 10% TEOS solution with 95% ethanol for 1h at 40 °C to form silica layer. The templates were immersed the other quantum dot solution again and then repeat as above as a template had diverse colors. Completed templates embedded quantum dots were mechanically polished using aluminum oxide for 24h.

2.4 Preparation of colloidal gold nanoparticles

To make a colloidal gold nanoparticles, the solution of 1% $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$ was added to D.I. water with vigorous stirring and 1% sodium citrate was then added to the resulting

solution 1min later. The solution of 0.075% NaBH_4 was also added to the mixture

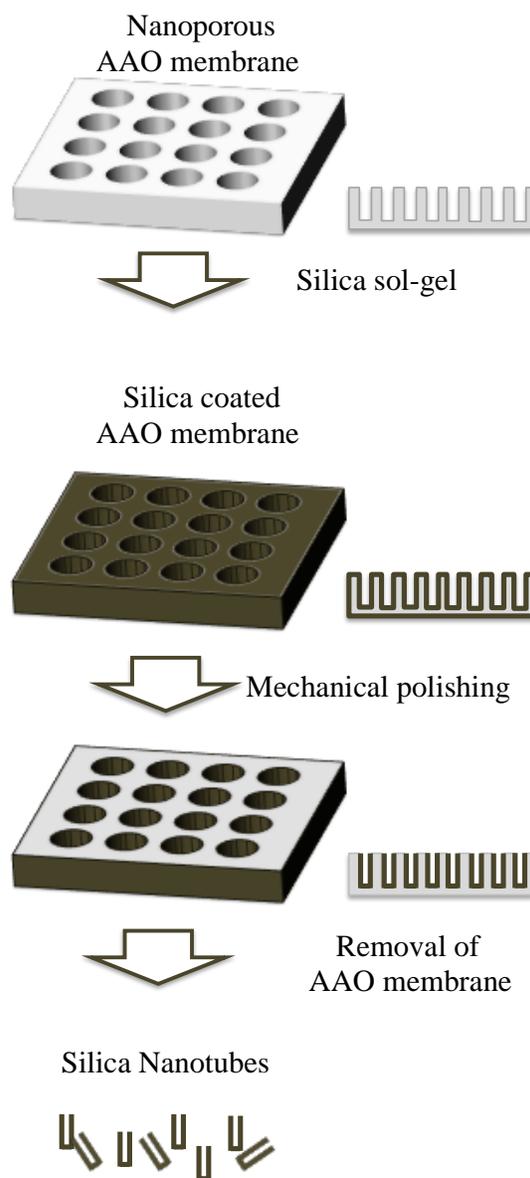


Figure 1. The schematic diagram of the fabrication process of silica nanotubes

2.5 Cell lysate and Nucleic Acid Sequence Based Amplification (NASBA) of concentrated RNA

The E.coli 0157:H7 sample was thermally treated at 50 for 5min prior to being subjected to different lysis methods; thermal lysis at 95 °C for 5min. To measure the viable E.coli, mRNA for the heat shock protein, *clpB*, was chosen as the target sequence. The sensitivity and specificity of primer set for the amplification of the target sequence

specific to *E. coli* was already confirmed in my previous research [20].

2.6 RNA hybridization

The each SNT was hybridized with RNA of *E. coli* and salmonella in SSC buffer for 5h at 43 °C with shaking. The operation of bio-SNTs was confirmed using confocal microscope (EZ-C1, Nkkon, Japan) and fluorescence signal of reacting bio-SNTs was compared with bio-SNTs in normal condition.

2.7 TEM measurement

TEM was utilized to investigate the length of SNTs and the size of gold nanoparticles and to confirm quantum dots in SNT. Topological images were obtained by a HR-TEM (JEOL 3010 300kV). The sample in grid was treated in 25% ethanol for dispersion of SNTs.

3 RESULTS AND DISCUSSION

3.1 The nanoporous AAO templates and silica nanotubes derived from AAO

Figure 2 showed the SEM images of nanoporous AAO templates. The distance between center and center was exactly 110 nm. The nanopore was designed for nanotube to have 50 nm of diameter. The alumina nanostructure surface was fabricated by two-step electrochemical anodization on aluminum plate. As pre-treatment, aluminum plates were degreased for 1h in acetone and then electropolished in fresh mixture of perchloric acid and ethanol. The first anodization was accomplished in 0.3M solution of oxalic acid. The second anodization was performed .

We fabricated silica nanotubes with nanoporous AAO templates based on the methods described in section 2.2. The typical silica nanotubes were shown in figure 3. High concentration of silica nanotubes was achieved after membrane removal process. As expected, Not only single nanotubes were achieved and few bundles of silica nanotubes were coincidentally formed. It is likely that mechanical polishing process were not fine tuned. Therefore, simple filtration was performed to separate individual nanotube from bundles.

3.2 Quantum dot embedded silica nanotube

Two different quantum dots were embedded in nanotube, in which Ag nanoparticles were also inserted between two quantum dot. This is one of the merits of hollow type silica nanotube. We can design quantum dot color and order in nanotube when we need to discriminate nanotubes. By the way, embedding efficiency in silica

nanotube did highly depend on the size of quantum dot as expected.

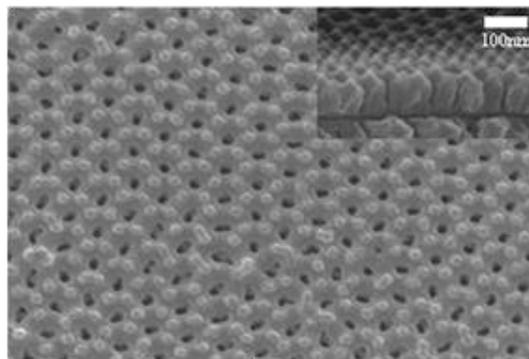


Figure 2. The SEM image of typical nanoporous AAO template

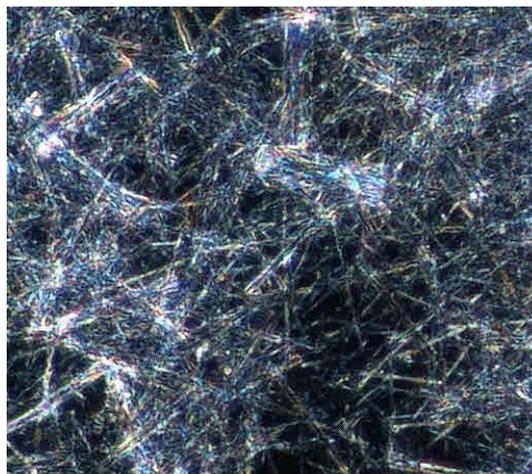


Figure 3. The fluorescent microscopic image of silica nanotubes

3.3 *E. coli* detection with silica nanotube

The silica nanotube containing quantum dots were functionalized by the introduction of capture probe on outer membrane. The capture probe was designed to absorb on the gold surface by employment of thiol group at the 3' end of probe. Therefore, the gold nanoparticle was adsorbed on the outer silica nanotube and serially capture probe was immobilized. This bio functionalized silica nanotube was prepared. Figure 5 showed the detection signal of *E. coli* with quantum dot embedded silica nanotube. The silica nanotube has green colored quantum dot as barcodes and red fluorescent dye immobilized hairpin structured capture probe. As shown in figure, the red signal was detected when nucleic acid sample introduced whereas green color indicated *E. coli* specific nanotube.

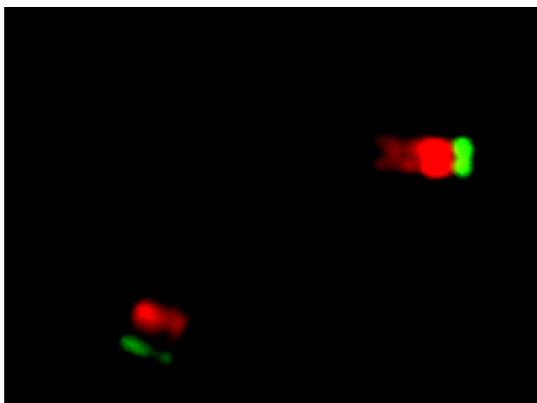


Figure 5. Confocal microscopic images of bio functionalized silica nanotube containing green quantum dot.

4 CONCLUSION

In this study, we demonstrated the application of silica nanotube to biosensing fields. As described before, silica nanotube has specific merits. First is that we could utilize both of an inner space and an outer space at the same time.

In here, we showed the inner space was utilized as a barcoding region with quantum dot and the outer space was used as bio functionalization. This result implies we could develop 8 different nanosensors by just control of color and order of quantum dot embedded in nanotube.

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