Functionalized Mesoporous Silica Nano-Particle for Optical Bio-Detection

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ABSTRACT

Mesoporous silica nano-particles were used as the matrix to immobilize antibodies and enzymes which were used as reporter elements for biomolecular interaction. Luciferases of Jamaican click beetles and antibodies were immobilized on mesoporous silica nano-particles for immune bioluminescence detection of *Staphylocuccus aureus* enterotoxin B (SEB) and carcinoembryonic antigen (CEA). Fluorescent labeled mouse IgG and green fluorescent protein (GFP) were used to test and confirm the protein immobilization on the nano-particles by fluorescent microscopic method. For the application of functionalized meso-porous bio-luminescence nanoparticle in bio-molecular detection, two sets of nano-particle report elements were prepared: anti-SEB antibody with luciferase-100 (red emission) and anti-CEA antibody with luciferase-99 (green emission). Antibody-antigen recognition was performed in 96 assay plates and on nitrocellulose membrane. In the presence of the target molecules, the enzymatic reaction on the nano-particles generated light that was detected by using a CCD camera. The enzyme activity and antibody biological function on the nanoparticles were maintained more than 30 hrs in dry. Bioluminescence reaction of this new report element was also demonstrated on a CMOS color imaging chip based device.

Key Words: mesoporous, nano-particle, luciferase, bioluminescence, detection

1 INTRODUCTION

Fluorescent technologies are well established and have been widely applied in molecular and cellular biology. Currently, most DNA and protein detection methods are based on fluorescent technologies. However, the disadvantage of fluorescent detection is the requirement of complicated and expensive optical equipment in addition to an external light source. Therefore, it is difficult to develop a simple, low cost and hand-held detector. Another disadvantage of fluorescent detection is that many biological materials have inherent fluorescence properties that can interfere with fluorescent assays. Moreover, photo-bleaching is a common problem in the fluorescent detection, which occurs when fluorescent molecules no longer respond or respond poorly when exposed to excitation light. In contrast, bio-luminescent assays are exquisitely sensitive and not limited by the overlap between the fluorescent properties of analytes and assay components. Therefore, bioluminescent assays are commonly used for detection of low concentration bio-agents *in vitro* and *in vivo* [1-4]. In addition, bioluminescence detection does not require an external light source. These characteristics make this technology attractive for development of a low cost portable luminescence detector. Various luciferases have been used as a reporter in the bio-luminescence detection. However, these enzymes are very sensitive to the changes of molecular conformation and lose their biological function in the conjugates. Because of this feature, most applications of luciferases in the bio-detection are in vivo through gene fusion approaches. Here we report a new luciferase report element for optical bio-detection. Mesoporous silicon nano-particles were chosen as a unique matrix for bio-molecular conjugation [5]. That did not change the molecular conformation of luciferases and maintained their biological function.

2 METHODS

2.1 Subcloning and Expression of Luciferases

The genes of luciferase-green and luciferase-red were PRC amplified from mammalian cell expression vectors (Promega) by using two sets of primers with additional restriction sites BamHI in forward primers and NdeI in reverse primers. Both amplified DNA fragments were separately inserted into the multiple cloning site of an *E. coli* expression vector pET-15. Positive clones were identified by restriction enzyme digestion. Expression of luciferases was induced in the presence of 3 mM IPTC by using standard protocol from manufacturing instruction (Novagen).

2.2 Purification and Characterization of luciferase

Isolation and purification of luciferases was carried out by using nickel-agarose column. Purified luciferases were dialyzed in PBS, and then quantified by using BCA assay. Characterization of luciferases was performed by denaturing gel electrophoresis. Bioluminescence detections were used to confirm the biological activity of the luciferases.

2.3 Nano-article Modification and Bio-molecule Immobilization

Mesoporous nano particles were mixed with 11-mercaptoundecanoic and 3-mercaptopropionic acid for 24 hrs. These particles were washed with ethanol and sonicated in 100% ethanol for 30 minutes. The modified silica particles were incubated with an aqueous solution of 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide (EDAC) and *N*-hydroxysuccinimide (NHS) (1:1 v/v) at room temperature for 1 hr and thoroughly washed with water and sonicated in water for 1 hrs. These modified silica nano particles were stored in a trehalose solution. These stored mesoporous nano-particles were used for bio-molecular immobilization. Fluorescent labeled mouse IgG, goat anti-mouse IgG and green fluorescent protein (GFP) were used to test and confirm the immobilization by using a fluorescent microscopic method. 100 ul of 10 ug/ml goat anti-mouse IgG- AF-488 or GFP were incubated with 50 ul nano-particles at room temperature for 3 hrs. After washing with 5 x 200ul PBS, the particles were visualized under fluorescent microscope. Mouse IgG-AF555 was used to test the biological function of goat anti-mouse IgG antibody on the nano-particles through an antibody- antigen reaction.

2.4 Characterization of the report elements

Streptavidin-Alkline phosphatase (SA-AP) was used to test enzyme immobilization on nano-particles. 100 ul 0.1 mg/ml SA-AP incubated with 50 ul nano-particles at room temperature for over night. These functionalized particles were washed with PBS and tested through a chemiluminescence detection of biotin labeled mouse IgG. To construct luciferase functionalized nano-particles, luciferase and fluorescent labeled antibody, at 1:1 ratio, were co-immobilized on nano-particles as described above. After immobilization, fluorescent microscopic method was used to confirm the presence of antibody on the nano-particle. Bio-luminescence detection was used to verify luciferase on the nano-particles and maintained their biological function. Anti-SEB polyclonal antibody and luciferase-red were used to construct the report element-1. Anti-CEA monoclonal antibody 1146 and luciferase-green were used to construct the report element-2.

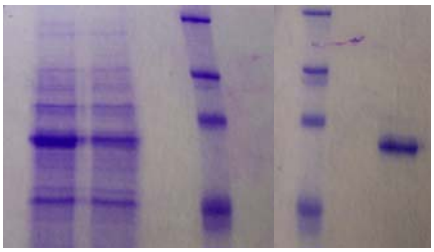
2.5 Bio-detection

0.5 ul of 10 ug/ml anti-SEB monoclonal antibody and anti-CEA monoclonal antibody 1147 were spotted on a nitrocellulose membrane. After locking with AP-blocking buffer, the membrane was incubated with SEB and CEA samples at room temperature for one hr. After washing with PBS, the membrane was incubated with report element 1 and 2 at room temperature for one hr. Unbound particles were washed away. Bio-luminescence detection was carried out by adding 80 ul luciferase substrate to the membrane and CCD images were taken with 60 seconds exposure time.

3 RESULTS AND DISCUSSION

3.1 Expression of luiciferase

Expression of luciferase-red was analyzed by denaturing polyacraymide gel electrophoresis (PAGE). A thicker band in 60 kD region as observed in the induced sample (lane 1) compared with that in non-induced sample (lane 2). This indicates that the cloned protein gene was induced and expressed in *E. coli*. A single band of a 60 kD protein was showed in nickel column purified sample (lane 5). The same result was obtained in the analysis of expression of luciferase-green in *E. coli* (data not shown)



1 2 3 4 5

Figure 1. Gel Images of luciferase protein expressed in *E. coli* Expression of luciferases in *E. coli* was analyzed on a 10% SDS PAGE. Lane 1 is crude bacterial protein sample from an induced culture. Lane 2 is crude bacterial protein sample from a non-induced culture. Lane 3 and 4 are molecular weight markers. Lane 5 is nickel column purified protein sample.

To confirm these proteins are luciferases, bio-luminescence detection of luciferase activity was performed in the presence of luciferase substrate. The results in Fig.2 showed luciferase activities were detected in both proteins by CCD camera (left) and CMOS color imaging device (right).

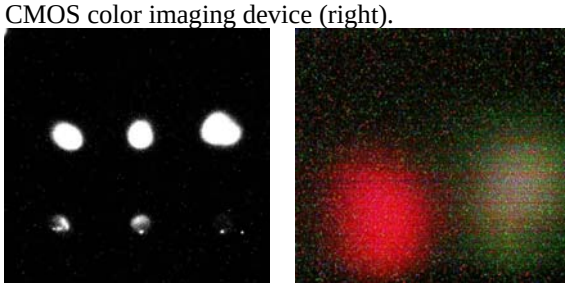


Figure 2. Bioluminescence detection of luciferase activities Left: top are luciferase-red, and bottom are luciferase-green. Image on the right is bioluminescence detection of luciferase-red and Luciferase-green on CMOS color imaging chip.

3.2 Immobilization Verification

Bio-molecular immobilization on mesoporous nano-particles was characterized by using fluorescent microscope. Green fluorescent protein (GFP) and goat anti-mouse IgG-Texas red conjugates were used to test the immobilization method (Fig. 3). GFP (top left) and goat anti-mouse IgG-TR (top right) immobilization on nano-particles was confirmed. To investigate biological function of immobilized antibody, goat anti-mouse antibody-AF488 (green) on nano-particles was used to capture mouse IgG-AF-555 (red). Images in Fig. 3 showed that antibodies were on the particles (bottom left) and were able to capture the antigen (bottom right). These results demonstrated that bio-molecules immobilized on the mesoporous nano-particles maintained their biological functions.

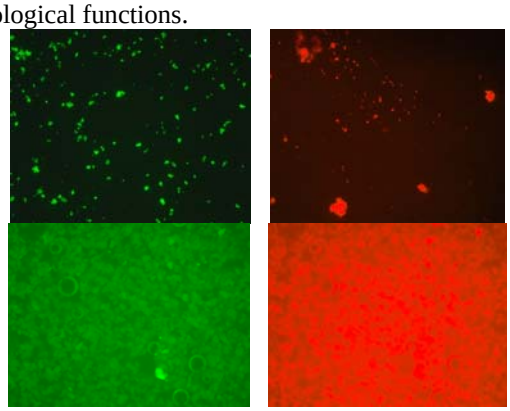


Figure 3. Characterization of bio-molecules on nano-particles Top GEP (left) and goat anti-mouse IgG (right) immobilized nano-particles. Bottom left and right are the same image of goat anti-mouse IgG-AF488 captured mouse IgG-AF555 with red and green filter under UV.

3.3 Luciferase and Antibody Immobilization

Co-immobilization of luciferase and antibody on mesoporous nano-particles was investigated by using fluorescent microscopic and bioluminescence detection methods. Fig. 4 top are fluorescent microscopic images which indicate that fluorescent labeled antibody were on the nano-particles. Fig. 4 bottom are bioluminescence CCD images which indicate luciferase activity was detected on the same nano-particles. Combining two test results, co-immobilization of luciferase and antibody on the mesoporous nano-particles was confirmed.

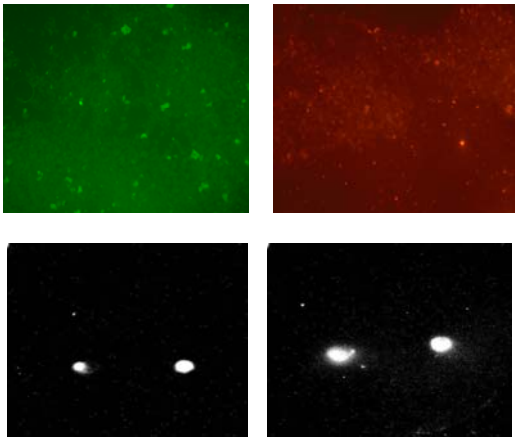


Figure 4. Characterization of co-immobilization of luciferase and antibody on nano-particles Fluorescent microscopic images of immobilized nano-particles: top left is luciferase-red and antibody-AF488 nano-particles and top right is luciferase-green and antibody-AF555 nano-articles. Bioluminescence detection of luciferase activity of above nano-particles: luciferase-green (left spot) and luciferase-red (right spot) on both images which were taken at 6minutes and 60 minutes after the reaction start.

3.4 Detection of SEB and CEA

To demonstrate functionalized mesoporous silica nano-particle for optical bio-detection, *Staphylococcus aureus* enterotoxin B (SEB) and a cancer biomarker carcinoembryonic antigen (CEA) were used as biological target agents in the experiments. Two sets of antibodies were used to capture and identify target molecule. Sandwich immune-detection was performed on nitrocellulose membranes. Streptavidin-AP functionalized nano-particles was used to detect biotin labeled anti-SEB and biotin labeled anti-CEA in the sandwich

immune-detection (Fig.5 left). Luciferase-Red / anti-SEB antibody and luciferase-green / anti-CEA antibody nano-particles were used to detect the presence of the targets (Fig. 5 right). These results demonstrated that this self-illuminated optical element can be used to report bio-molecular recognition events.

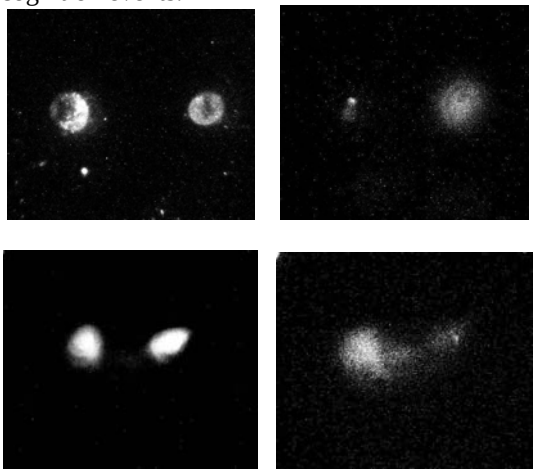


Figure 5. Immuno-detection of SEB and CEA Top: AS-AP nano-particle (left) was used to report the presence of SEB and CEA (right). The dot on the left is SEB and on the right is CEA. Bottom: Luciferase / antibody nano-particles were used to detect SEB and CEA.

3.5 The Stability of Luciferase

The stability of luciferase on nano-particles was also investigated. The literature from the vector manufacturing company showed that the activity of luciferase-green is about 30 minutes, and the activity of luciferase-red is shorter. Our results showed that the activity of luciferase on nano-particles lasts much longer (Figure 6). Most enzymes stored at room temperature in dry lost their biological function, unless went through a freeze dry process. In the results present herein, luciferase on mesoporous nano-particles maintained its activity after 40 hrs at room temperature in dry. It is possible that a semi-liquid nano-environment is created on the mesoporous surface which is similar to the nature sub-cellular environment for bio-molecules.

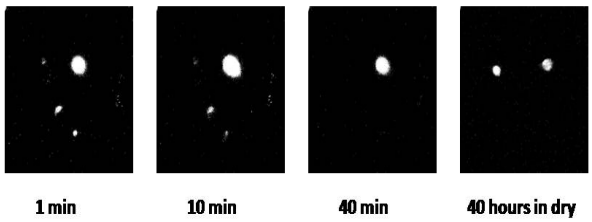


Figure 6. Stability test of luciferase nano-particles Images 1, 2, and 3 are luciferase activity detection of the functionalized nano-particles in the different time period. Image 4 is luciferase nano-particle enzymatic activity test after stored at room temperature and in dry for 40 hrs.

4 CONCLUSION

Mesoporous nano-particles have showed potential as a new matrix for bio-molecular immobilization. It was also used as a linkage for bio-molecular conjugation with a much simpler process compared with conventional chemical conjugation methods. Its unique surface feature maintained biological functions of the immobilized molecules on a solid surface.

5 ACKNOWLEDGEMENTS

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