Colorimetric Detection of Human Lung Carcinoma Cell by Enlarging Au-Nanoparticles in situ

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A new approach to the colorimetric detection of human lung carcinoma cell by enlarging Au-nanoparticles in situ has been reported. The results show that the sensitivity of detecting lung carcinoma cell is ca. 100 cell mL^{-1}. By enlarging Au-nanoparticles immobilized on the nitrocellulose strips in situ with 0.01% $HAuCl_4$ and 0.4 mM NH_2OH, detection of lung carcinoma cell by naked eye is available. It will be of great significance in rapid and early clinical diagnosis.

The development of simple, rapid and sensitive approach to detect biomolecules has been a topic of significant interest because of its potential applications in the diagnosis of disease.[1–4] Many detection techniques have been developed which rely upon target hybridization with radioactive, fluorescent, chemiluminescent, and other types of labeled probes.[5–7] Colorimetric systems are especially attractive detection formats since they do not pose safety problems, are simple to monitor, and are relatively inexpensive.

Recently, the use of gold nanoparticles as amplifier has attracted considerable attention in biosensor development[8–10] due to its special properties including ease of preparation, high density, large dielectric constant, and biocompatibility. Smaller Au-nanoparticles are usually used to fabricate the conjugate of antibody- or DNA-Au-nanoparticles because of its highly labeling efficiency. However, smaller Au-nanoparticles are not beneficial to observation. Therefore, it is necessary to enhance the smaller gold nanoparticles usually by silver staining. Although silver staining has been widely used to visualize protein-, antibody-, and DNA-conjugated Au-nanoparticles in histochemical electron microscopy studies,[11] it needs to avoid daylight in most cases. It has not been reported that enlarging Au-nanoparticles by 0.01% $HAuCl_4$ and 0.4 mM NH_2OH (HN) in situ rather than silver staining was used to detect cell by naked eye. The present method is less light sensitive than silver staining. It can be performed on a laboratory desk and need not be shielded from daylight.

The principle of the method used in the study was shown in Figure 1. While NH_2OH is thermodynamically capable of reducing Au^{3+} to bulk metal,[12] the reaction is dramatically accelerated by Au surfaces.[13] As a result, no new particle nucleation occurs in solution and all added Au^{3+} goes into production of larger particles. In this case, Au-nanoparticles will be specifically enlarged by HN. We choose 2 min for the following enlargement step one time since the enlarging reaction will be completed within 2 min.[14] A 2 μL of monoclonal antibody (ALT-04) against surface antigen on human lung carcinoma cell (LCC) solution (1 mg mL^{-1}, 20 mM Tris-HCl buffer, 0.15 M NaCl, pH 7.4) were spotted on the nitrocellulose strip (NCS). After the non-specific adsorption was blocked with 3% bovine serum albumin (BSA), these NCSs were treated sequently with LCC suspension and conjugate of ALT-04/$Au_{10\,nm}$. In this case, LCCs were specifically adsorbed on the NCS through the specific interaction between ALT-04 pre-immobilized on NCS and the surface antigens on LCC. Similarly, the Au-nanoparticles were immobilized on the surface of LCC pre-adsorbed on NCS through the specific interaction between ALT-04 conjugated with Au-nanoparticles and surface antigens on LCC. Finally, these immobilized Au-nanoparticles were dramatically and specifically enlarged by HN. As a result, the staining was intensely strengthened. When the concentration of LCC was less than ca. 100 cell mL^{-1}, there was no detectable staining after enlarging Au-nanopartices twice with HN as shown in Figure 2e, showing that the detecting limit is ca. 100 cell mL^{-1}.

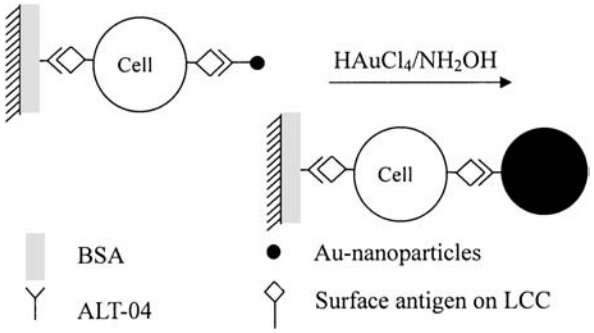

Figure 1. Schematic illustration of the detection of LCC by enlarging Au-nanoparticles with HN in situ.

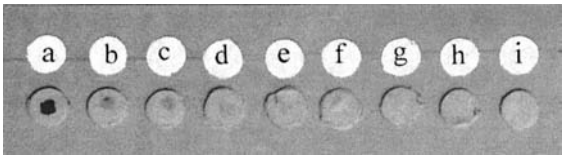

Figure 2. Immunoblot of LCC detection. A 2 μL ALT-04 solution (1 mg mL^{-1}, 20 mM Tris-HCl buffer, 0.15 M NaCl, pH 7.4) were applied onto NCSs. They were treated sequentially with 5% BSA, LCC suspension, conjugate of ALT-04/$Au_{10\,nm}$, and HN twice. The concentrations of LCC suspension from a to g: 1×10^6, 1×10^5, 1×10^4, 1×10^3, 1×10^2 cell mL^{-1}, buffer blank, and supernatant of cell suspension instead of cell suspension. Similar procedures involved treating NCS as above mentioned except treatment of NCS without conjugate ALT-04/$Au_{10\,nm}$ (h). Hela cell suspension with high concentration (1×10^5 cell mL^{-1}) instead of LCC suspension was detected (i).

To address the non-specific adsorption of conjugate of ALT-04/$Au_{10\,nm}$ and the non-specific enlargement of Au-nanoparticles, the following control experiments were performed. A 2 μL of Tris-HCl buffer solution (solvent of the ALT-04 solution) instead of ALT-04 solution was spotted on NCS. No detectable staining can be observed as shown in Figure 2f. Similarly, no detectable staining appeared for similar procedures involved treating NCS with the exception of treating NCS using supernatant of LCC suspension instead of LCC suspension (Figure 2g). Similar procedures involved treating NCS except treatment of NCS without conjugate ALT-04/$Au_{10\,nm}$, there was no detectable staining (Figure 2h). As a control, the Hela cell (human cervical carcinoma) suspension with high concentration (1×10^5 cell mL^{-1}) instead of LCC suspension was detected. There was no detectable staining (Figure 2i). These results demonstrated the specificity of the present method.

Table 1. ELISA results of LCC detection[a]

Cell mL^{-1}	Absorbance at 490 nm (OD)	Effect[b]
5000	1.013	+
500	0.856	+
250	0.672	+
125	0.473	−
68	0.450	−
34	0.463	−

[a]All data are presented as a mean of three independent experiments ±SE. Critical value $= \bar{N} + 2$ S.D. $= 0.490$. $\bar{N}$ and S.D. represent a mean of negative control and standard deviation, respectively. [b]OD > 0.490 represent positive response.

Compared with the results of the present method, for the standard enzyme-linked immunosorbent assay (ELISA) to detect LCC, the detecting limit was ca. 250 cell mL^{-1} as shown in Table 1, which is lower than that of the present method (100 cell mL^{-1}). In addition, it will only take about 3 h for the method described in the study, which is more simple and rapid than ELISA.

In order to understand the reason to enhance sensitivity, the increase of the surface area of the Au-nanoparticles induced by the enlargement with HN was studied using 10 nm Au-nanoparticles as seeds in aqueous solution. The diameters of the Au-nanoparticles were increased to ca. 200 nm after twice enlargements as shown in Figure 3. Compared with the surface area of 10 nm Au-nanoparticles without enlargement, these results mean that the surface area of the Au-nanoparticles was remarkably increased by ca. 400-fold.

In summary, by enlarging Au-nanoparticles immobilized on the nitrocellulose strips in situ with 0.01% $HAuCl_4$ and 0.4 mM NH_2OH detection of human lung carcinoma cell by naked eye is available. This method is simple, rapid and more importantly suitable for the application in the detection with the naked eye. It will be of great significance in rapid and early clinical diagnosis.

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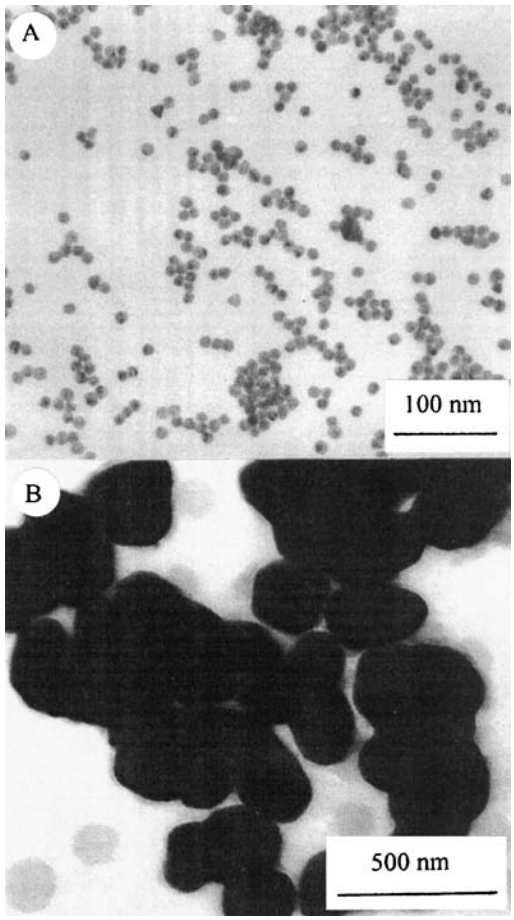

Figure 3. TEM images of Au-nanoparticles enlarged by HN. (a) seeds, (b) after two treatments.

References and Notes

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