Application of Gold Nanoparticles for Early Detection of Breast Cancer Cells*

Luu Manh Quynh, Tran Quoc Tuan, Nguyen Hoang Luong,[†] Nguyen Ngoc Long, and Nguyen Hoang Hai

*Center for Materials Science, Faculty of Physics,
Hanoi University of Science, 334 Nguyen Trai, Thanh Xuan, Hanoi, Vietnam*

Tran Thi Thanh Thoa, Nguyen Thi Van Anh, and Phan Tuan Nghia

*Key Laboratory of Enzyme and Protein Technology,
Hanoi University of Science, 334 Nguyen Trai, Thanh Xuan, Hanoi, Vietnam*
(Received 10 December 2009; Accepted 26 September 2010; Published 27 December 2011)

Gold nanoparticles (GNPs) have been synthesized by a chemical reduction method using sodium borohydride and functionalized with amino groups on their surface. The GNPs were then conjugated with the anti-HER2 human antibody (trastuzumab) for detecting breast cancer cells. The specific binding of trastuzumab-conjugated GNPs onto the breast cancer cells (KPL4 line) were observed by bright-field and dark-field microscopy and were for the first time observed by scanning electron microscopy and energy dispersive X-ray scanning. [DOI: 10.1380/ejsnt.2011.544]

Keywords: Gold nanoparticles; Trastuzumab; HER2; Breast cancer cells

I. INTRODUCTION

Nanoparticles have been studied and developed rapidly for application in diagnosis and treatment, because of their large surface area and unique optical properties. At the size ranging from 30 nm to 60 nm, the absorption peak of gold nanoparticles (GNPs) changes from 524 nm to 560 nm [1, 2]; and the light scattering shows more intense in organic dyes [3, 4]. Optical properties of GNPs change depending on the diffraction factor of the surrounding solutions, and on their surface's electronic properties [1]. In different synthesis methods, the surfactants such as cetyltrimethyl ammonium bromide (CTAB), citrate, AuCl_4 could link with the GNPs by non-covalent bonds, which make moderate changes on absorption peaks [5]; while the other groups such as ethiol ($-\text{SH}$) make more visible changes [6]. To apply GNPs in biology, most methods use the so-called bifunctional ethiol-R-amino group, in which $-\text{SH}$ group could contact rapidly with GNPs surface, while $-\text{NH}_2$ group remains free and exposes out side the surface for further conjugation with other chemical groups of biomolecules [7]. GNPs are potential candidates for cell imaging and cell-target drug delivery [2, 8–11], cancer diagnostics and therapeutic applications [12–14]. Nowadays, a number of bio-markers which are expressed at a high level on the surface of breast cancer has been reported, for example HER receptors belonging to a member of the epidermal growth factor (EGF) family of tyrosine kinase receptors. These include HER1, HER2, HER3, and HER4. While HER1, HER3, and HER4 are overexpressed in various types of cancer cells, such as head, neck, brain, stomach, breast, colon, gast, prostate, and so on, HER2 is a biomarker which is more specific for breast and ovarian [15, 16]. HER2 is super-expressed with several hundred folds higher in cancer cells of 20-30% breast cancer patients than in normal cells.

FIG. 1: Synthesis of GNPs using NaBH_4 reduction and functionalizing them with 4-ATP.

Therefore, HER2 is an interesting target for therapy of breast cancer. Anti-HER2 with generic name Trastuzumab or trade name Herceptin is a humanized monoclonal antibody (mAb), which has been approved by the FDA since 1998 for treatment of metastatic breast cancer [12, 13, 17]. In this study, we conjugated the GNPs with anti-HER2 antibody (Trastuzumab) through either non-covalent or covalent linkages. The Trastuzumab-conjugated GNPs were then used to specifically label breast cancer cells, KPL4 line, for imaging of the cells. This primary result is fundamental for further application in breast cancer tumor imaging.

II. EXPERIMENTAL

A. Synthesis of GNPs by NaBH_4 and functionalizing with 4-ATP ($\text{C}_6\text{H}_7\text{NS}$)

5 ml of 0.01 M NaBH_4 (MERCK) at 0°C was added to 25 ml of 1 mM HAuCl_4 (MERCK) in 50 ml ask with stirring for 15 min, until the color of the solution changed from lightly yellow to dark red (see Fig. 1). After 2

*This paper was presented at the International Workshop on Advanced Materials and Nanotechnology 2009 (IWAMN2009), Hanoi University of Science, VNU, Hanoi, Vietnam, 24-25 November, 2009.

[†]Corresponding author: luongnh@vnu.edu.vn

days, these GNPs were functionalized with 4-ATP. Different volumes of 2 mM 4-ATP were added into 20 ml of the GNPs containing solution. The color of the solutions changed from dark red to dark blue, and the solution was then incubated for at least 1 day.

B. Conjugation of Trastuzumab to GNPs

Trastuzumab (Dakko Co.) was non-covalently conjugated to colloidal GNPs [2], which were prepared by NaBH_4 reduction (named as NaBH_4 *GNPs). The colloidal gold suspension was adjusted by 0.1 M NaOH to pH 6.5 to react with a mixture of non-labeled Trastuzumab and FITC-Trastuzumab (480 nm/520 nm) at mole ratio 10:1 of final concentration 6 $\mu\text{g}/\text{ml}$ at room temperature (RT) for 5 min. The Trastuzumab- NaBH_4 *GNPs were collected by centrifugation at 4°C, at the rate of 30,000 rpm, for 30 min. The pellets were washed twice and then resuspended in phosphate buffer solution (PBS) with pH 7.4 containing 0.2% bovine serum albumin (BSA).

Trastuzumab was covalently linked to 4-ATP functionalized GNPs (amino-GNPs) through 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) connection, similarly described for magnetic nanoparticles [18]. Briefly, the above prepared amino-GNPs was reacted with 0.2 mM EDC in MOPS buffer with pH 6.0, for 20 min at RT. Then, the EDC*GNPs were reacted with 6 $\mu\text{g}/\text{ml}$ non-labeled Trastuzumab and FITC-Trastuzumab (480 nm/520 nm) at mole ratio 10:1, for 30 min at RT. The Trastuzumab-EDC*GNPs were washed three times by centrifugation at the rate of 13,000 rpm, at 4°C, for 13 min with PBS and then was stocked in PBS with pH 7.4 containing 0.2% BSA. The absorbance profile of the covalent Trastuzumab-EDC*GNPs was measured and compared with that of GNPs using Spectrophotometer (Nanodrop).

C. Culture of KPL4 and Hela cells

KPL4 and Hela cells were cultured in 24-well ELISA plates containing DMEM (Difco Modified Eagle Medium, Gibco Co.) plus 10% FBS (Fetal Bovine Serum, Gibco Co.). Glass coverslips were added in each well and the cells were incubated at 37°C, 5% CO_2 to reach a population of about 4×10^5 to 10^6 cells/ml. The coverslips were then picked out and put into another 24-well ELISA plate for further immuno-nanogold incubation.

D. Incubation of Trastuzumab-conjugated GNPs with KPL4, Hela cells

Coverslips containing KPL4 cells were washed 3 times with 500 μl PBS before being fixed by 3% paraformaldehyde at RT, for 15 min and then treated with 5% Triton X-100 at RT for 5 min. After being blocked with 500 μl of 2% BSA, the coverslips were incubated with 200 μl either of Trastuzumab- NaBH_4 *GNPs and Trastuzumab-EDC*GNPs containing solution at a concentration of 3 $\mu\text{g}/\text{ml}$ at RT, for 60 min. Schematic diagram of incubation of GNPs with KPL4 and Hela cells is shown in Fig. 2.

FIG. 2: Schematic diagram of incubation of GNPs with KPL4 and Hela cells.

FIG. 3: TEM images of NaBH_4 reduced (A) and of 4-ATP functionalized GNPs (B); UV-vis spectra of GNPs before and after 4-ATP functionalizing (C) and of GNPs before and after conjugation with Trastuzumab (D).

The FITC-GNPs containing coverslips were observed under AxioPlan epifluorescent microscope (Carl Zeiss), while the non-fluorescent GNPs containing coverslips were observed under light microscope with either bright- or dark-field phases. Next, the scanning electron microscope (SEM) and energy dispersive X-ray spectroscopic (EDS) scanning of KPL4 cells were used for detecting the GNPs distributed positions.

III. RESULTS AND DISCUSSION

A. Red-shift of GNPs UV-vis spectra after functionalizing and conjugation

Due to the change of the surfactant, the so-called surface plasmon resonance (SPR) band of the nanoparticles changes, and it could be observed through the change of their UV-vis spectra [18, 19]. Higher dielectric factor (ϵ) of the surrounding surfactant makes the peak of the UV-vis spectra red-shift. Figure 3 shows the transmission electron microscope (TEM) image of GNPs before (pallet

FIG. 4: Fluorescence image of FITC-Trastuzumab conjugate with NaBH_4 reduced (E serial) and 4-ATP functionalized (F serial) GNPs after 5 min incubation with Hela (2 serial) and KPL4 (1 serial) cell lines.

A) and after (pallet B) functionalizing with 4-ATP. After functionalizing, the peak of UV-vis spectra red-shifted from 530 nm to 544 nm (pallet C). After conjugating with HER2, that peak red-shifted once more, from 544 nm to 555 nm (pallet D). These results agree with recent publications [1, 2, 4, 19].

B. Specific binding of HER2 and Trastuzumab

The specific interaction between Trastuzumab and HER2 directs the Trastuzumab molecules to concentrate onto the surfaces of the KPL4 breast cancer cells, where HER2 molecules are overexpressed and uniformly distributed. Figure 4 shows significant bright signals of FITC labeled Trastuzumab on the membranes of KPL4 cells, indicating the specific binding of Trastuzumab on HER2 overexpressed on the membranes of KPL4 cells. The fluorescent intensity of KPL4 cells incubated with FITC-Trastuzumab was about 10 times higher than that of Hela cells incubated with FITC-Trastuzumab, confirming specific interaction of Trastuzumab toward HER2 of KPL4 where the level of HER2 expression is much higher. This result also shows good purity of Trastuzumab, which is important for next experiments.

C. Light scattering of GNPs incubated breast cancer cells

In Fig. 5, the dark-field images (Pallet H2 and K2) showed distribution of GNPs on cell surfaces through their scattering light. When the GNPs were linked with the Trastuzumab, they bound onto the surfaces of KPL4 cells,

FIG. 5: Bright-field (1 serial) and dark-field (2 serial) spectroscopy images of non-labeled GNPs incubated with KPL4 (G serial), of Trastuzumab conjugated GNPs incubated with KPL4 (H serial) and of Trastuzumab conjugated 4-ATP-GNPs incubated with KPL4 (K serial).

where HER2 proteins are overexpressed. The concentration of GNPs-Trastuzumab nanoparticles on cell membranes created light scattering, reflecting the shape of the cells. When the GNPs were not linked with Trastuzumab, dark-field image showed no signal of GNPs (Pallet G2), indicating GNPs did not bind non-specifically onto KPL4 and that binding of GNPs-Trastuzumab onto KPL4 was due to specific interaction between Trastuzumab and membrane protein of KPL4.

The bright-field images showed the morphology of the cells, confirming co-localization of GNPs bound exactly onto the cells. As seen in Fig. 5, pallet H2 and G2 showed the light scattering in both cases, of NaBH_4 reduced GNPs and of 4-ATP functionalized GNPs, respectively. Thus, using both conjugations of Trastuzumab with fluorescent dye FITC and with GNPs, dual data of fluorescent images and dark-field images of KPL4 labeled with FITC and GNPs certain that Trastuzumab were successfully conjugated with GNPs and that Trastuzumab-GNPs could be used for specific labeling KPL4.

FIG. 6: SEM image and EDS scanning image of Trastuzumab-conjugated GNPs incubated with KPL4. The scale bars in all the images are 20 μm .

D. SEM image and EDS scanning of GNPs incubated breast cancer cells

In higher magnification, SEM and EDS scanning pattern of the GNPs incubated KPL4 cells were obtained. While the detector scanned on the surface of the sample, the energy dispersive X-rays of only gold element were collected. Figure 6 shows the SEM image and EDS scanning image of the KPL4 cells after 1 day incubation with

NaBH_4 reduced GNPs. The concentrated places of the GNPs imaged mirrored the location of the HER2 proteins which shows that these proteins de-located and re-distributed after 1 day incubation with GNPs.

IV. CONCLUSION

In this work, we are successful in synthesizing GNPs in solution and functionalizing them with 4-ATP to have free amino ($-\text{NH}_2$) groups. The GNPs then were applied for imaging KPL4 breast cancer cells after conjugating them with Trastuzumab. High resolution images of GNPs-Trastuzumab incubated KPL4 cells were observed by EDS scanning, and showed where the GNPs were concentrated, which could help study the time dependent mobility of HER2 on the surface of cells.

Acknowledgments

The authors would like to thank Ministry of Science and Technology of Vietnam (Contract No 38/355/2008/HD-NDT for Task of Protocol with Israel) and Vietnam National University, Hanoi (Key Project QGTD.08.05) for financial support.

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