Attaching quantum dots to HER2 specific phage antibodies

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Abstract
This work presents the results of the attachment of Qdot 655 ITK™ amino (PEG) quantum dots (QDs) (Invitrogen) and CdTe QDs (provided by Institute of Materials Science, VAST) to HER2 (Human Epidermal growth factor Receptor 2) specific phage antibodies (Abs) (provided by Institute of Biotechnology, VAST) in solution. The QDs were attached to the phage display specific HER2 Abs to form a complex QD–Ab. The QDs and complex QD–Ab were characterized by UV-VIS spectroscopy, transmission electron microscopy (TEM) and fluorescence microscopy. The fluorescence images show the QDs conjugated to the phage. Due to the QDs attaching to the surface, the phage dimensions were amplified, so its shape could be observed by optical microscopy. The complex QD–Ab was stable and lasted for a month. The results illustrate the value of the HER2 phage–QD complex as a cancer detection platform.

Keywords: quantum dot, nanostructure complex, fluorescence of molecules

Classification numbers: 2.04, 4.01, 5.04, 5.08

1. Introduction
Quantum dots (QDs) or semiconductor nanocrystals are well known as a new class of fluorescent label for biology and medicine [1, 2]. Because of their nano structure, QDs have interesting optical and electronic properties, such as size-dependent light emission, superior signal brightness, resistance to photobleaching and simultaneous excitation of multiple fluorescence colors [3, 4]. These properties are most promising for improving the sensitivity and multiplexing capabilities of molecular histopathology and disease diagnosis [5].

HER2 stands for Human Epidermal growth factor Receptor 2 and is a protein that offers greater aggressiveness in the treatment of breast cancers. Overexpression of this receptor in breast cancer is associated with increased disease recurrence and a worse prognosis, so breast tumors are routinely checked for overexpression of HER2 [6].

In order to detect tumors before they turn into late stage cancer, virus-based nanoparticles are becoming more and more popular in diagnostic imaging. The use of ‘smart’ nanoparticles, which combine multiple functions of targeting, imaging and drug delivery, has great potential to increase the sensitivity and specificity of therapies [7]. The bacteriophage M13 offers many attractive features, such as having a high surface density (300–400 m² g⁻¹) so it can stick to whatever it has been designed to target. Furthermore, a phage has 2700 copies of the major coat protein P8 and 5 copies of two different minor coat proteins (P9, P6 and P3). These proteins can be genetically engineered to express peptides that have a high affinity for cancer markers, other proteins and inorganic materials [8, 9]. The QDs attached to phages are used as a
This work presents the results of attaching QDs to HER2 specific M13 bacteriophage Abs with the purpose of detecting cancer lesions and cell imaging. The HER2 specific Abs are conjugated with end coat proteins of the phage to create HER2 specific monoclonal Abs. The attachment of QDs to phages was investigated using fluorescence and transmission electron microscopy.

2. Experimental

2.1. Materials

2.1.1. Quantum dots (QDs). Qdot 655 ITK™ amino (PEG) QDs (Invitrogen) and CdTe QDs (provided by Institute of Material Science, VAST) are used in conjugation with HER2 specific phage Abs. The commercial Qdot 655 ITK™ amino (PEG) QDs are CdSe/CdS QDs having an amine-derivatized polyethylene glycol (PEG) outer coating that can react directly with amine-reactive groups, such as isothiocyanats and succinimidyl esters, or with carboxylic acids of proteins and other water-soluble biopolymers in aqueous solution using water-soluble carbodiimides, such as EDC (N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride). TEM image JEM 1011 (figure 1) indicates that QDs have a prolate spheroid shape with about 11–12 nm major and 5–6 nm minor axes, respectively. Each QD has a circle of 2–3 nm thickness, which is the PEG capping layer. Therefore, the effective dimensions of QDs are 18–20 nm and 10–12 nm of axes.

The absorption and photoluminescence spectra of Qdot ITK™ 655 are presented in figure 2. It is clear that the Qdot 655 ITK™ has a narrow emission of about 30 nm full-width at half-maximum (FWHM) and a large Stockes shift.

The colloidal CdTe QDs (provided by Institute of Materials Science, VAST) prepared in TOPIDDA (trioclylphosphineldodecylamine) are transferred into water by the use of mercaptopropionic acid (MPA), so these QDs have the negative charged carboxyl groups on their surface.
Figure 5. Transmission and fluorescence images of HER2 phage Abs before (A, B) and after (D, E) Qdot 655 ITK\textsuperscript{TM} conjugation. Fluorescence image of Qdots ITK\textsuperscript{TM} alone (C). Epifluorescence microscope (Olympus IX71, objective ×100 NA 1.3) excitation at 480 nm of mercury light (the horizontal axis of each image is 15 μm).

The absorption and photoluminescence spectra of CdTe QDs are shown in figure 3.

2.1.2. HER2 specific phage display Abs (HER2 phage). HER2 specific phage display Abs (provided by Institute of Biotechnology, VAST) are M13 phages displayed HER2 specific Abs. Anti HER2 phage single-chain Fv variable (scFv) fragment Abs have been panned and selected from the Griffin.1 highly diverse human scFv library, based on its affinity with HER2 target antigens. The Griffin.1 library includes 10\textsuperscript{9} different Ab cloned fragments with heavy chain (V\textsubscript{H}) and light chain (V\textsubscript{L}) variable regions. These scFv fragments were fused with shell protein P3 of phage M13 to create the anti HER2 fragments displayed on the phage. The bioactivity of HER2 specific phage Abs (HER2 phage) was verified by the ELISA (Enzyme-linked immunosorbent assay) test (results are not shown). HER2 phages are dispersed in PBS buffer (phosphate buffered saline) of pH 7.4. The TEM image of HER2 phages is shown in figure 4. It is clear from this figure that the size of the phages is about 6–8 nm wide and 1000 nm long. The concentration of phages is high, such that they wind and join in parallel to form larger filaments.

2.2. QD bioconjugation to HER2 phage

The direct conjugation of Qdot 655 ITK\textsuperscript{TM} to HER2 phage Abs through amine-carboxylic acid coupling was used with EDC (N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride) as a catalyzer.

- Mix QDs (10 μl, 32 nM) with EDC activated Abs (100 μl, 10\textsuperscript{12} CFU in PBS) and react for 1 h at 4 °C temperature; then shake the mixture at 200 rpm for 1 h maintaining the 4 °C temperature.

- Separate QD–Ab conjugates from excess free Abs and QDs via centrifugation of 12000 rpm at 4 °C temperature in NaCl (2.5 M) solution containing 20% PEG (twice). The concentrated QD–Ab complex was rediluted in 100 μl PBS buffer.

The conjugation of CdTe QDs to HER2 phage was also carried out by the above route. The HER2 phage before and after QD conjugation were imaged on an epifluorescence microscope (Olympus IX71, objective X100 NA 1.3).

3. Results and discussion

Figure 5 shows the transmission and fluorescence images of HER2 phages before and after Qdot 655 ITK\textsuperscript{TM} conjugation. It is clear from figure 5 that, because of their small diameter (8 nm), the bare filamentous phages cannot be observed by an optical microscope having a resolution of about 200 nm (A), and the bare phages do not fluoresce at 480 nm excitation (B). A fluorescence image of QDs alone is an intense, homogeneous light halo without any objects (C). The HER2 phages after QD conjugation were observed by optical microscope in both transmission and fluorescence images (figures 5(D) and (E)). This phenomenon can be explained by the amine-terminated QDs showing affinity for the negatively charged HER2 phages and attaching to them. Each phage is composed of approximately 2700 copies of the major coat protein P8 containing 50 amino acids, so the number of carboxyl groups on the phage surface is very large (about 1 350 000 groups), which allows many QDs to stick. Due to the QDs attaching to the surface of the phages, the phage dimensions are amplified and their shape can be observed by an optical microscope.

Similar images were obtained for the CdTe QDs (figure 6). In this case, the negatively charged carboxyl groups...
on CdTe QDs reacted with the amino groups on the phage surface.

In both figures 5 and 6, we can see that the fluorescence QDs attach along the body of the phages, forming many fluorescence filaments. The optical transmission images also show these filamentous shapes of the phages as they appeared in the TEM image (figure 4), but with the amplified dimensions.

The QD-HER2 phage samples were kept in the dark at 4 °C temperature for 1 month and were visualized in the same conditions as before. The results are shown in figure 7. We can see that the fluorescence phage filaments (figure 7(B)) are still observed clearly as if having just been prepared.

4. Conclusion

The QDs (commercial Qdot ITK™ 655 and laboratory manufactured CdTe QDs) were successfully attached to HER2 specific phage display Abs in solution. Due to QDs attaching to the surface, the phage dimensions were amplified and their shape could be observed by optical microscope in both transmission and fluorescence images. These results highlight the quality of QDs (commercial and laboratory) as fluorescence probes, and the value of complex phage-QDs as a cancer detection platform. Much work must be done before they can be properly adopted in medicine, but these initial experiments have created a foundation for combining QDs and other nanoparticles [12] to make from a phage a single multifunctional cancer detection system.

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