Characterization of the paclitaxel loaded chitosan graft Pluronic F127 copolymer micelles conjugate with a DNA aptamer targeting HER-2 overexpressing breast cancer cells

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Abstract

In this work we report the isolation of DNA aptamer that is specifically bound to a HER-2 overexpressing SK-BR-3 human breast cancer cell line, using SELEX strategy. Paclitaxel (PTX) loaded chitosan graft Pluronic F127 copolymer micelles conjugate with a DNA aptamer was synthesized and its structure was confirmed by TEM image. This binary mixed system consisting of DNA aptamer modified Pluronic F127 and chitosan could enhance PTX loading capacity and increase micelle stability. Morphology images confirmed the existence of PTX micelles, with an average size of approximately 86.22 ± 1.45 nm diameters. Drug release profile showed that the PTX conjugate maintained a sustained PTX release. From in vitro cell experiment it was shown that 89%–93%, 50%–58%, 55%–62%, 24%–28% and 2%–7% of the SK-BR-3, NS-VN-67, LH-VN-48, HT-VN-26 and NV-VN-31, respectively, were dead after 6–48 h. These results demonstrated a novel DNA aptamer-micelle assembly for efficient detection and a system for the delivery of PTX targeting specific HER-2 overexpressing. We have also successfully cultivated cancer tissues of explants from Vietnamese patients on a type I collagen substrate. The NS-VN-67, LH-VN-48, HT-VN-26 and NV-VN-31 cell lines were used as cellular model sources for the study of chemotherapy drug in cancer.

Keywords: chitosan, DNA aptamer, HER-2, micelle, paclitaxel, Pluronic F127

Classification numbers: 2.04 2.05 5.08 5.09

1. Introduction

Ligand molecules which can be bound to a specific cancer cell type, by interacting with cancer cell surface proteins with high affinity and specificity is critical for developing cancer diagnostics and therapeutics. The ligand molecule in turn can be utilized to identify the cancer biomarker proteins [1]. Understanding the biology of these biomarkers can further facilitate the development of novel cancer therapeutics. HER-2/ErbB2/Neu (HER-2) is a transmembrane receptor tyrosine kinase, which is a member of the epidermal growth factor receptor family [2]. Overexpression of HER-2 is observed in 20% to 30% of breast cancers, and predicts for a poor clinical...
outcome [3]. A HER-2 ectodomain directed monoclonal antibody (trastuzumab or Herceptin) has been approved for the treatment of breast cancer. HER-2 is also an attractive target for in vivo imaging and targeted drug delivery for breast cancer.

Aptamers (Ap) are short single-stranded oligonucleotides of about 100 nt that bind their targets with high affinity and specificity [4]. They are randomly synthesized based on nucleic acid libraries by a procedure termed systematic evolution of ligands by exponential enrichment (SELEX) [5]. The attractive features of Ap including a relatively small physical size, lack of immunogenicity in vivo, easy and reproducible synthesis, high-binding affinity and molecular specificity to their ligand, easy modification, fast tissue penetration, low toxicity, and long-term stability make them very good candidates for clinical applications and ideal alternatives for antibodies in numerous applications [6–9]. Owing to these unique characteristics, Ap has been widely used to modify drugs and nanoparticles (NPs) for cancer therapy.

In recent years, there have been considerable interests in developing biodegradable NPs as effective drug delivery systems [10–12]. Amphiphilic block copolymer have been widely investigated as hydrophobic drug solubilizing agents in drug delivery systems. They can spontaneously self-assemble into polymeric micelles and NPs in aqueous environments [12, 13]. Most polymeric micelles are composed of a hydrophobic block as the inner core and a hydrophilic block as the outer shell. A hydrophobic drug can be encapsulated in the hydrophobic core of the micelles to increase the water solubility. The hydrophilic shell is able to prolong the circulation time due to a decrease in phagocytosis and renal clearance [14]. The polymeric micelles normally have average size of approximately 10–100 nm diameters, allowing the particles to accumulate in tumor tissue through a mechanism called enhanced permeation and retention effect allowing the particles to accumulate in tumor tissue through a mechanism called enhanced permeation and retention effect rather than in normal tissues [15, 16]. This is due to the fact that tumor vessels are structurally irregular and leaky compared to normal vessels.

Pluronic F127 (PF) is an A–B–A-type triblock copolymer consisting of polyoxyethylene (PEO) units (A) and polyoxypropylene (PPO) units (B) with a thermo reversible gelation property [17, 18]. With the increase in the temperature of PF aqueous solution, the PPO block tends to dehydrate and form a core with an outer shell of hydrated PEO chains that aggregate into spherical micelles. The micelle structure of this copolymer in an aqueous environment can be used for incorporation of hydrophilic and hydrophobic drugs and prolongs drug release. To overcome this limitation, grafting PF with chitosan (Chi) to form a copolymer was suggested. Chi is the cationic polysaccharide derived from chitin which stimulates cell growth and protein adsorption.

Paclitaxel (PTX), the first of a new class of microtubule stabilizing agents, has demonstrated significant antitumor activity in clinical trials against a broad range of solid tumors, including refractory ovarian cancer, metastatic breast cancer, non-small-cell lung cancer, AIDS-related Kaposi’s sarcoma [15, 19].

In this study, a total Ap was selected for a model SK-BR-3 breast cancer cell-line. We synthesized and characterized a novel NPs contain Ap for PTX delivery system using an ionic-gelation method comprised of Chi and PF. The properties of these polymeric micelles such as their appearance and stability, encapsulation efficiency, loading capacity, and in vitro drug release. Especially, comparison in vitro cytotoxicity level on SK-BR-3 (ATCC® HTB30™), and the four cell lines NS-VN-67 colon cancer cells, LH-VN-48 thyroid cancer cells, HT-VN-26 stomach cancer cells and NV-VN-31 tongue cancer cells were isolated from Vietnamese patients were also evaluated.

2. Materials and methods

2.1. Materials

Chitosan F-MMW 400 kDa was purchased from Sigma Aldrich (USA), Pluronic F127 (PF), paclitaxel (PTX), 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT), McCoy’s 5A medium, DMEM medium, fetal bovine serum (FBS), collagenase type I and trypsin-EDTA were purchased from Life Technologies (Singapore). Slide-A-Lyzer™ Dialysis cassettes (MWCO = 3.5 kD) was purchased from Thermo Fisher Scientific (USA). Oligonucleotide library 5′-TCA CCG GGA GGA GAC CCT GA-N40-3′, forward primer 5′-TCA CCG GGA GGA GAC CCT GA-3′ and reverse primer 3′-TTG TGG TGG TGG TTC AA-3′, forward primer 5′-TCA CCG GGA GGA GAC CCT GA-3′ and reverse primer 3′-TTG AAC CAC CAC CAA GCC AC-5′, forward primer 5′-AGC GAG CAG CCA AGT-3′, reverse 5′-TTG GTG GCC AGG TAG GTG AGT T-3′) and beta-actin primers (forward 5′-GAT GAG ATT GGC ATG GCT TT-3′, reverse 5′-CTC AAG TTG GGG GAC AAG AA-3′) were synthesized by IDT (Singapore). SK-BR-3 breast cancer cell-line (HTB-30™) was supplied from ATCC® (USA). Ceftriaxone was purchased from Bidasphar JSC (Vietnam). Sodium nitrite (NaNO₂), hydrochloric acid (HCl), sodium hydroxide (NaOH), acetic acid, sodium triphosphate (TPP), succinim anhydride (SA), 4-dimethylaminopyridine (DMAP), triethylamine (TEA), N,N-dimethylformamide (DMF), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), N-hydroxysuccinimide (NHS), 2-mercaptoethanol, diethyl ether, dimethylsulfoxide (DMSO) were all analytical reagent purchased from Merck Milipore Chemical Company (Germany). All other chemicals were of analytical grade and used without further purification.

2.2. Primary explants culture procedures

The NS-VN-67 colon tissues cancer, LH-VN-48 thyroid tissues cancer, HT-VN-26 stomach tissues cancer and NV-VN-31 tongue tissues cancer of Vietnamese patient were kindly provided by the Oncology Hospital, Ho Chi Minh City, Vietnam. Biopsies were obtained at the time of exploratory surgery or surgical placement. A representative portion of the surgical specimen was taken aseptically and transported from the surgical suite in cold (4°C) tissue culture medium for
immediate processing. Fresh tissues were minced with apposed scalpels into 0.5 mm pieces. Individual pieces of tissue were placed 6 to 8 per dish on a previously formed collagen I substrate. During the attachment process only medium sufficient to wet the substrate surface was used. After 10 to 30 min an additional 2 ml of DMEM medium containing 10% (v/v) fetal bovine serum and 0.25 µg ml⁻¹ ceftriaxon was added to each dish. Explants were incubated in a humidified atmosphere of 5% CO₂ in air at 37 °C. Cultures were observed daily, and medium was changed three times a week. Epithelial outgrowth at the periphery of the explants was evident within 24 to 48 h of attachment. This outgrowth increased over time but never became confluent.

2.3. SELEX procedures

SK-BR-3 cell-line were harvested by trypsinization and 1 x 10⁶ cells were recovered in complete media at 37 °C for 30 min 200 pmol ssDNA pool dissolved in 500 µl of binding buffer (4.5 g l⁻¹ glucose, 5 mM MgCl₂, 0.1 mg ml⁻¹ yeast tRNA, 1 mg ml⁻¹ bovine serum albumin in phosphate buffered saline (PBS)) was denatured by heating at 95 °C for 5 min and cooled on ice for 10 min before binding. Then the ssDNA pool was incubated with a cell monolayer in a T25 flask (target cells) at 4 °C for 45 min Cells were washed twice with washing buffer, and then the bound Ap were eluted by heating at 95 °C for 5 min and separated by phenol:chloroform:isoamyl alcohol (Tripure, Roche) and chloroform extraction. The obtained DNA was PCR amplified with primers (25 cycles of 30 s at 94 °C, 30 s at 58 °C, and 30 s at 72 °C, followed by 5 min at 72 °C; the Taq polymerase and deoxynucleotide triphosphate (dNTP) were obtained from Promega. In the first round selection, the amount of initial ssDNA pool was 12 nmol, dissolved in 1 ml of binding buffer, and the counter selection step was eliminated. In order to acquire Ap with high affinity and specificity, the wash strength was enhanced gradually by extending wash time (from 1 to 10 min), increasing the volume of wash buffer (from 1 to 5 ml) and the number of washes (from 3 to 5). Additionally, 20% FBS and 50–300 fold molar excess 45-mer random DNA library (a completely different library) were added to the incubation solution to reduce the nonspecific binding of the selected pool. After 20 rounds of selection, the selected ssDNA pool was PCR amplified using unmodified primers.

2.4. Preparation of Chi-TPP-PF copolymer

To obtain a low molecular weight Chi, medium molecular weight Chi was depolymerized according to the method described in [18, 20]. The preparation of Chi NPs was achieved via the ionic-gelation method [21]. A Chi solution (0.1% w/v) was obtained by dissolving low molecular weight Chi in 1% v/v acetic acid. Chi NPs were prepared spontaneously upon addition of various concentrations of TPP (0.01%, 0.015%, 0.02%, 0.025%, and 0.03% w/v) to Chi solution under gentle magnetic stirring at room temperature for 1 h. In all cases, the volume ratio of Chi-TPP solution was 2:1. PF was incorporated into NPs by adding 0.025% w/v TPP solution to Chi aqueous solution containing different concentrations of PF (10%, 15%, 20% w/w).

2.5. Preparation of core-shell NPs

A carboxylic acid group was introduced to the chain end of PEO-PPO-PEO (PF, MW = 12 500) by reaction of the terminal hydroxyl group of PF with succinic anhydride as described in the previous study [22]. Briefly, Chi-TPP-PF copolymer (5 g, 0.75 mmol), succinic anhydride (0.56 g, 3 mmol), DMAP (0.5 g), and TEA (0.5 ml) were dissolved in 30 ml anhydrous 1,4-dioxane and stirred overnight at 30 °C. Then the 1,4-dioxane was removed under a centrifuge vacuum. The residue was dissolved in chloroform, precipitated into an excess of diethyl ether, and then filtered to remove un-reacted succinic anhydride, DMAP and TEA. With repeating the process twice, the precipitate, carboxyl terminated PF (about 85% yields), was obtained after filtering and drying in a vacuum for 24 h. The procedure of the reaction between carboxyl-terminated Chi-TPP-PF copolymer and PTX was as follows: 4 g (0.7 mmol of −COOH groups) of carboxyl-terminated Chi-TPP-PF copolymer was dissolved in 2 ml of N,N-dimethylformamide (DMF) at room temperature. 0.1 g of EDC and 0.2 g of NHS was added in carboxyl terminated Chi-TPP-PF copolymer solution. After 15 min reaction, 1.5 ml of 2-mercaptoethanol was added to quench the EDC followed by an addition various concentrations of PTX (0.2, 0.4, 0.6, 0.8 and 1.0 mg ml⁻¹) to the activated carboxyl terminated Chi-TPP-PF copolymer. The activated carboxyl-terminated Chi-TPP-PF copolymer in DMF was reacted with PTX in the presence of triethylamine (1 mg) and stirred at room temperature for 12 h under nitrogen. The resultant was dialyzed against DMF by using a dialysis cassettes (MWCO = 3.5 kDa) for 24 h and finally lyophilized to gain the product.

2.6. Characterization of NPs

Size and zeta potential (surface charge) of NPs were measured at least in triplicate using Nano Partica SZ-100 (Horiba, Japan). For both of the above measurements, 0.2 mg NPs was suspended in 10 ml milli Q water.

Transmission electron microscopy JEM 1230 (Joel Ltd, Tokyo, Japan) was used to examine and compare the topography of the NPs. Freeze dried NPs were suspended in milli Q water before observation.

2.7. Evaluation of drug encapsulation

0.5 mg NPs were dissolved in 5 ml milli Q water and centrifuging at 14 000 rpm for 30 min at 15 °C. The amount of PTX released in the supernatant was measured spectrophotometrically at 247–249 nm. Each sample was measured in triplicate. The following equations were used to evaluate
the encapsulation efficiency (EE) of the NPs [15]

\[
EE(\%) = \frac{\text{total PTX} - \text{free PTX}}{\text{total PTX}} \times 100.
\]

2.8. In vitro release of aptamer

Aptamer (Ap) release was determined by incubating the NPs with 5 g ml⁻¹ heparin (37°C, horizontal shaking 100–110 cycles min⁻¹). At time intervals of 1 h and 4 h, individual samples were isolated by centrifugation at 10 000 rpm for 40 min. Supernatant samples were analyzed by 1.5% agarose gel electrophoresis using unencapsulated Ap free as positive control sample [23].

2.9. Comparison in vitro cytotoxicity level on SK-BR-3, NS-VN-67, LH-VN-48, HT-VN-26 and NV-VN-31

The cytotoxicity of PTX NPs and free PTX were performed on SK-BR-3, NS-VN-67, LH-VN-48, HT-VN-26 and NV-VN-31. The cell-lines were cultured in Mc Coy 5A and DMEM supplemented with 10% FBS, L-glutamine, and 0.25 μg ml⁻¹ ceftriaxon solutions, and incubated at 37°C at 5% CO₂. Cells were seeded at a density of 12 000 viable cells per well in 96-well tissue culture plates (Corning Incorporated Costar®) incubated for 24 h to allow cell attachment. The cells were then incubated for another 12, 24 and 48 h with PTX copolymer NPs and free PTX. Cells were then washed in PBS, and 20 μl of MTT solution (5 mg ml⁻¹) was added to each well. The plates were incubated for an additional 4 h, and then the medium was discarded. 200 μl DMSO was added to each well, and the solution was vigorously mixed to dissolve tetrazolium dye. The absorbance of each well was measured by PlateReader AF2200 (Eppendorf, Germany) at 570 nm.

2.10. Reverse transcription polymerase chain reaction and image analysis

RNA is prepared as follows: total RNA was extracted from the excised SK-BR-3, NS-VN-67, LH-VN-48, HT-VN-26 and NV-VN-31 cells and subjected to reverse transcription polymerase chain reaction (RT-PCR). For extraction of total RNA from small amounts (up to 10⁵ cells) of cultured cells, the RNeasy Mini Kit (Qiagen, Chatsworth, CA) is used according to the manufacturer’s instructions. The measure concentration of total RNA by a spectrophotometric method (GeneQuant, Amersham Biosciences; Biochrom Ltd, England), total RNA was transcribed into cDNA using the procedure Promega (Promega Corporation, Madison, USA). 2 μg of total RNA were combined with a reaction mixture containing 30 μl of 10 μl tampon RT 5X, 10 μl of dNTP’s 10 mM each, 2 μl of random primer, 1.2 μl of RNase inhibitor 40 U μl⁻¹, 2 μl of M-MLV reverse transcriptase, and 4.8 μl of sterile water. Reverse transcription was performed under the following conditions: 10 min at 27°C, 60 min at 42°C, 5 min at 95°C and 5 min at 5°C in an Master Cycler (Eppendorf, Hamburg, Germany). The cDNA was stored at 20°C.

PCR procedures, HER-2 and beta-actin gene detection with primers HER-2 and beta-actin were performed. In brief, PCRs were performed in a final volume of 50 μl. Each PCR mixture contained 50 mM KCl, 10 mM tris-HCl (pH 8.5), 6 mM MgCl₂, a 200 μM concentration of each deoxynucleoside triphosphate (dNTP), 5 U (instead of 7 to 10 U) of taq gold DNA polymerase (Promega Corporation, Madison, USA), 50 pmol of primers HER-2 for the simultaneous amplification of a 247 bp product and primers beta-actin for the simultaneous amplification of a 431 bp product of the human beta-actin housekeeping gene, used as an internal control for PCR reactions. Amplifications were performed with the following cycling profile: taq gold activation was performed by incubation at 94°C for 10 min followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, and 1 min elongation at 72°C. The last cycle was followed by a final extension step of 7 min at 72°C (instead of 5 min). The PCR products were fractionated by 1.5% agarose gel electrophoresis (Promega Corporation, Madison, USA) and stained with 0.5 μg ml⁻¹ ethidium bromide (Sigma Aldrich, USA), and the identity of the PCR products was confirmed using a 100 bp ladder (Promega Corporation, Madison, USA) as the DNA standard.

2.11. Statistical analysis

Results are shown as mean ± standard deviation and each experiment was measured in triplicate. Statistical data analyses were performed by applying one-way ANOVA tests and P-value <0.05 was considered significant.

3. Results

3.1. Characterization of the conjugation of aptamer to Pluronic F127

To isolate aptamer (Ap) with high affinity and specificity to the native, membrane presented form of HER-2, we performed SELEX using a well-known HER-2 overexpressing breast cancer cell line, SK-BR-3. We started the SELEX procedure by using a DNA library of 45 nt randomized region, with 10¹⁴ complexity. We performed positive selection by retrieving Ap that bind to SK-BR-3 cells. After 20 rounds of positive selection, the binding affinity of DNA library reached saturation (data not shown). After the successful enrichment of Ap with high affinity to SK-BR-3 cells, individual Ap was sequenced (table 1).

Aptamer modified Pluronic F127 (PF-Ap) was synthesized by the reaction of carboxylated Pluronic F127 (PF) with the amino groups at the ends of Ap (figure 1(a)). PF-Ap containing a scrambled Ap was obtained by the same method. To confirm the conjugation and determine the maximum conjugation content of Ap on PF, novedx® 12% tris-glycine mini gel electrophoresis was carried out. The samples including free Ap, Chi-TPP-PF, and Chi-TPP-PF-Ap with or without EDC-NHS used in the conjugation reaction were separately subjected to 2% tris borate edta sodium dodecyl
sulfide polyacrylamine gel electrophoresis (TBE SDS PAGE). Then the electrophoresis was performed at 125 V for 30 min, and the base pair (bp) band on the gel was displayed by SYBR® green nucleic acid stain (figure 1(b)). The detailed synthesis procedure, chemical compositions, and characterization of the materials were given in supplementary information.

### 3.2. Characterization encapsulation efficiency of PTX core–shell NPs

The general addition of PTX increased the sizes of Chi NPs, but did not affect their zeta potential significantly (P < 0.02). The effect of PTX concentration on particle size is more significant than when the concentration raises 0.2–0.4 mg ml⁻¹ (P < 0.015). The PTX-loaded Chi-PF NPs size did grow significantly at concentrations up to 0.6–1 mg ml⁻¹, but there was a change in size when the concentration of PTX 0.4 mg ml⁻¹ were increased maximum (EE = 83.28% ± 0.13) and mean diameter 86.22 nm ± 1.45. PTX was a low molecular weight anticancer drug. Therefore, it might not be possible to increase the PTX particle diameter severely until it reached its maximum capacity inside the NPs. The PTX concentration did not influence the zeta potential of the prepared NPs (P < 0.04); Chi 1%-TPP 0.025%-PF 15% NPs were loaded in different concentrations of PTX (table 2). The differences in shape between NPs may be understood on the basis that the formation of Chi-TPP NPs is governed not only by electrostatic interactions between the PF and Chi, but also between TPP

| PTX (mg ml⁻¹) | Mean diameter (nm) | Zeta potential (mV) | EE (%) |
|---------------|--------------------|---------------------|-------|
| 0.2           | 65.11 ± 3.81       | 23.22 ± 1.56        | 67.31 ± 0.51 |
| 0.4           | 86.22 ± 1.45       | 60.12 ± 0.55        | 83.28 ± 0.13 |
| 0.6           | 89.55 ± 2.05       | 48.35 ± 0.24        | 68.01 ± 3.77 |
| 0.8           | 99.32 ± 7.14       | 40.21 ± 1.43        | 80.62 ± 1.28 |
| 1.0           | 110.46 ± 4.32      | 38.33 ± 1.12        | 59.36 ± 1.53 |

### Figure 1. Synthesis and confirmation of polymers. (a) Synthesis route of Pluronic F127-aptamer, (b) determination of the conjugation of aptamer to Pluronic F127 via noverx® 12% tris-glycine mini gel electrophoresis [18].

### Table 1. All of the oligonucleotides used in this work [18].

| Name      | Sequence                      |
|-----------|-------------------------------|
| Library   | 5′-TCA CCG GGA GGA GAC CCT GA-N40-GTG GCT TGG TGG TGG TTC AA -3′ |
| Aptamer   | 5′-AAC TTG GTG GTG GTT CGG TGG CTG TTC AGG GTC TCC TCC CGG TGA-3′ |

N is base number which is equal to the corresponding aptamer sequences.
and Chi, the latter interaction being responsible for the controlled gelation of Chi in a nanoparticulate form. This controlled gelation and reticulation process could explain why the resulting NPs are more spherical and compact than simple complexes.

PTX, hydrophobic anti-cancer drug, was encapsulated at the core of the polymeric micelles in the hydrophobic block owing to its hydrophobicity. The shape of PTX encapsulated Chi-PF NPs was investigated using TEM. Independent of the molecular weight, PTX NPs showed a very well defined spherical shape (figure 2), showed that particles were spherical and uniform with mean diameter 86.22 nm. In this study, PTX was successfully loaded into the hydrophobic core of the micelles via physical entrapment.

3.3. In vitro release of aptamer from NPs

An additional assay was performed in order to evaluate if the associated Ap could be displaced from Chi-TPP-PF NPs. For this purpose, Ap loaded NPs were incubated in an aqueous medium containing heparin as a competitive anion. Overall, these results show that Ap is efficiently and tightly associated to the NPs, however, it is not irreversibly bound since it could be released upon the degradation of the polymeric matrix, which is envisaged to occur in a biological environment following in vivo administration.

The results of the electrophoresis analysis (figure 3) showed that, as expected, Ap is easily displaced from NPs due to the presence of heparin. Ap free was also used as controls. This competitive displacement indicates that the Ap association to PF is labile in these polyplexes. Interestingly, this was not the case for PF trapped in NPs, a result that was independent of the size of NPs. This result corroborates that Ap association to the NPs is not simply mediated by an ionic interaction process, but rather that Ap is well entrapped within the polymeric mesh formed by the ionically cross-linked Chi. Thus, the results from the TEM (figure 2) and heparin displacement studies suggest that TPP-PF NPs differ significantly in the physical entanglement of Ap into their structures.

3.4. Comparison in vitro cytotoxicity level on SK-BR-3, NS-VN-67, LH-VN-48, HT-VN-26 and NV-VN-31

All cell lines were established from epithelial outgrowth from tissue explants cultivated on a type I collagen substrate. Using similar methods, finite cell lines were routinely established from carcinomas have been expanded in T25 flasks. High, 10%, concentrations of FBS in the growth medium were associated with minimal fibroblast contamination. We had developed successful techniques for the cultivation of cell lines from human cancer.

The NPs Chi 1%-TPP 0.025%-PF 15%-PTX 0.4 mg ml⁻¹, the SK-BR-3, NS-VN-67, LH-VN-48, HT-VN-26 and NV-VN-31 were dead 89%–93%, 50%–58%, 55%–62%, 24%–28% and 2%–7%, respectively, after 6–48 h. The partition was destroyed and led to broken cells after 6 h (figure 4). PTX was a hydrophilic anti-cancer drug that needed membrane transporters to enter the cells. Cells could uptake NPs by endocytosis mediated by a clathrin-mediated

![Figure 2](link). Micrographs of PTX 0.4 mg ml⁻¹ TEM image [18].

![Figure 3](link). Agarose gel electrophoresis retardation assay following incubation of Ap micelles anionic heparin. Lane M: DNA ladder 100 bp; lane 1: for 1 h; lane 2: for 4 h; lane 3: Ap free; lane 4: distilled water.

![Figure 4](link). Cell viability of SK-BR-3, NS-VN-67, LH-VN-48, HT-VN-26 and NV-VN-3 with Ap-micelle-PTX at different times.
process. Therefore, the NPs could act as drug delivery systems that facilitated drug entrance into the cells. The PTX 1 mg ml\(^{-1}\) free, the cells were dead 18\%–20\%. The particles PTX were not uptake into the cells efficiently.

Figure 5 showed the HER-2 and beta-actin mRNA were detected in SK-BR-3, NS-VN-67, LH-VN-48, HT-VN-26 and NV-VN-3 cells. HER-2 strongly expressed in SK-BR-3 and high expressed in LH-VN-48. Relative expression levels of HER-2 mRNA were significantly higher in LH-VN-48 than in NS-VN-67 and HT-VN-26, but significantly lower in SK-BR-3 (P < 0.01). HER-2 mRNA was no significantly expression in NV-VN-3 (P < 0.01). Relative expression levels of Beta-actin mRNA were significantly equal in all cells (P < 0.05). These data confirmed that the enhancement effect specifically of NPs for different expression levels of HER-2 receptor, not other proteins expressed on the surface of cells. The results of the in vitro cytotoxicity test showed above that the NPs were biocompatible and used as anti-cancer drug carriers.

Ap-micelle was used to synthesize anew Ap-PF conjugate and loaded PTX successfully. The PTX loaded Ap-micelles showed higher cytotoxicity which compared to blank Ap-micelles and free drug. The Ap-micelles loaded PTX were spherical and uniform with mean diameter 86.22 nm and high encapsulation efficiency.

All of these advantages endow this unique assembly with the capacity to function as an efficient detection and delivery vehicle in the biological living system. However other studies should be performed to improve the efficacy of these Ap-micelles.

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4. Conclusions

In this study, we had successful tissues cancer of Vietnamese patients explants cultivated on a type I collagen substrate. The NS-VN-67, LH-VN-48, HT-VN-26 and NV-VN-3 cell lines were using cellular model sources for the study of chemotherapy drug in cancer.

The SELEX procedure, we isolated Ap which can bind with high affinity and specificity to HER-2. This Ap-micelle enhances the binding ability of the Ap moiety at physiological temperature, even though the corresponding free Ap loses its binding ability under the same condition. In addition, minimal domain required for HER-2 binding can be identified, and could be multimerized to maximize the avidity of Ap against the target. We successfully developed a novel Ap-micelle assembly for efficient detection and delivery system for PTX targeting specific HER-2 overexpressing breast cancer cells.

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