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Circumventing paclitaxel resistance in breast cancer cells using a nanoemulsion system and determining its efficacy *via* an impedance biosensor

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One of the best strategies to circumvent drug resistance is the employment of nanocarriers. For the current study, we have employed a nanoemulsion formulation of paclitaxel (PTX) to bypass drug resistance in the MDA-MB-231 cell line and impedance sensing biosensors to determine the exact time that PTX-NE induced apoptosis. Our MTT results demonstrated that PTX treatment could not reduce MDA-MB-231 cell viability to IC_{50} even after three days. However, the employment of the reagent TPGS (inhibitor of drug resistance) combined with paclitaxel could partially obviate PTX resistance. Next, the nanoemulsion form of PTX (PTX-NE) was fabricated employing the essential oil of the *Satureja khuzestanica* plant and was characterized using DLS and TEM methods. Our data showed that after 72 hours, PTX-NE at 250 nM concentration could induce a 50% reduction in cell viability. Moreover, annexin/PI and cell cycle analysis confirmed the apoptotic effect of PTX-NE on cancer cells. Lastly, we measured the impedance of MDA-MB-231 cells treated with the free and nanoemulsion forms of PTX. A significant decrease in the mean impedance of PTX-NE treated cells could be observed after 40 hours. To conclude, we have demonstrated here that PTX-NE could circumvent resistance and induce apoptosis in PTX-resistant breast cancer cells, which could be inferred from their impedance measurement.

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Introduction

Breast cancer is one of the most common cancers among women, which is responsible for approximately 620 000 deaths annually in the world.[1] Triple-negative breast cancer (TNBC), which lacks ER, PR, and HER2, is the most invasive form of breast cancer with a prevalence of 20% and with the least survival rate.[2] Since it lacks three receptors, it is the most resistant one to respond to drugs.[3] Paclitaxel (PTX) inhibits microtubule polymerization, thus inducing cell cycle arrest followed by apoptosis occurrence.[4] PTX resistance in the primary or acquired form was observed in breast cancer patients and is considered a major clinical problem.[5,6]

Drug resistance is the chief reason for tumor relapse and metastasis death in breast cancer patients.[7] One of the reasons for drug resistance is overexpression of P-glycoprotein (P-gp), also known as ABCB1 or MDR-1.[8,9] That is why inhibitors of this pump such as TPGS (D-α-tocopherol polyethylene glycol) have been successfully utilized to overcome drug resistance to doxorubicin and other therapeutics.[10] Moreover, PTX has very low water solubility (less than 1 μg mL^{-1}), which should be solubilized in alcohol and Cremophor®EL (polyoxyethylated castor oil) for better delivery. However, it was proved that due to the presence of Cremophor in the Taxol formulation, severe adverse effects accompany the usage of this drug, including hypersensitivity reactions and neuropathy.[11,12] Therefore, with the purpose of reducing side effects, other PTX formulations were presented, including Abraxane, Genexol, and Lipusu.[13] Nonetheless, the mentioned formulations were partially successful to overcome PTX resistance.[14] One of the best strategies to circumvent drug resistance is the application of nanocarriers. Free drugs enter the target cells *via* diffusion and the MDR pumps can efflux them easily out of the cells; however, nanoparticles employ a different tactic for internalization called endocytosis, which efficiently delivers their cargo deep into the cytoplasm and does not allow them to be recognized

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by MDR pumps.[15] In this regard, it was shown before that a nanocombination of PTX/TPGS could reverse drug resistance in ovarian breast cancer and showed beneficial therapeutic effects over the mixture of PTX and TPGS.[16] Moreover, nanoemulsion forms of PTX–TPGS[17] and PTX–curcumin[18] appeared useful to solve drug resistance problems in breast and ovarian cancer cells, respectively.

One of the best strategies to analyze cell functions is to examine their electrical behavior when they are exposed to an electric field. Using analytical techniques based on electrochemical properties in biology has attracted a significant amount of attention due to their low-cost, real-time, fast, and label-free procedures.[19] It was shown previously that the invasive properties of cancer cells could be determined *via* these methods, and a comparison between metastatic and non-metastatic breast cancer cells revealed that each cell line has a specific electrical signature which could be a great tool in pathology to determine the cancer stage.[20] Several methods in biology are employed to evaluate the drug effect and efficiency on cells, including MTT, flow cytometry, and immunoblotting; however, these techniques are time-consuming and expensive. That is why the use of electrical-based assessments is growing so fast in the field of biology. Electrical-based techniques can also be used to determine cell viability in the presence of different drugs with high sensitivity and precision. As a case in point, the toxic effects of doxorubicin on the cell viability of lung cancer cells have been proved using electrochemical methods.[19] Moreover, the efficiency of electric biosensors for the detection of the sensitivity of leukemia K562 cells toward anticancer drugs has been established, and the obtained results were confirmed by conventional CCK assay.[21] Among these methods, cell-based impedance spectroscopy (ECIS) is considered as a suitable label-free non-invasive strategy to analyze cell characteristics, including cell proliferation and efficacy of anticancer reagents.[22,23] The increased cell proliferation on ECIS electrodes would make a cellular barrier which results in impedance augmentation. Therefore, antiproliferative drugs such as PTX are expected to decrease the impedance of cancer cells compared to untreated ones.[24] For the current study, we have employed a nanoemulsion formulation of PTX fabrication with the essential oil of the *Satureja khuzestanica* plant to bypass drug resistance and to induce apoptosis in the MDA-MB-231 cell line. Moreover, we have used impedance sensing biosensors to determine the exact time that PTX-NE induced apoptosis in cancer cells.

Methods

Materials

Paclitaxel (>97%) was acquired from Sobhan Oncology Pharmaceutical Company (Iran), Tween 80 and Span 80 surfactants were obtained from Merck Millipore (Germany), and MTT was purchased from Sigma-Aldrich (USA). The essential oil of *Satureja khuzestanica* was a gift from Khorraman Pharmaceutical Company (Iran). TPGS was obtained from Shahid Beheshti University. MDA-MB-231 breast cancer cells were a gift from Dr Houri Sepehri.

Nanoemulsion preparation

A high energy emulsification method was used for the fabrication of the paclitaxel-loaded nanoemulsion (PTX-NE). The mixture containing 3% oil and 9% surfactants (Tween 80 plus Span 80) was mixed using a high-speed homogenizer. 375 μM paclitaxel was dissolved in the essential oil, and to achieve a stable nanoemulsion, suitable values of the hydrophilic–lipophilic ratio were calculated as reported previously.[25] The combination of Tween 80 and deionized water was added to the oil phase comprising the essential oil, paclitaxel, and Span 80. Next, 5 mL of the primary emulsion was mixed using a high-shear mixer (SilentCrusher M, Heidolph, Germany) for 5 min at 15 000 rpm with a dispersion tool.

Characterization of PTX-NE

The average particle size of the established PTX-NE was obtained with Dynamic Light Scattering (DLS) (Nanophox Sympatec GmbH, Germany). Transmission electron microscopy (TEM) was used to evaluate the structure of PTX-NE.

Nanoemulsion release

We employed the MTT assay to evaluate the drug release in a 120 h period. For this purpose, a 500 nM nanoemulsion solution was centrifuged at 14 000 rpm to isolate the supernatant containing the released drug from the heavier nanoemulsion particles. This process was carried out regularly after the nanoemulsion was allowed to stand for 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 36 h, 48 h, 60 h, 72 h, 96 h, and 120 h. Next, the cultured cells were subjected to the supernatant solutions, and their viability was normalized to the viability of the cells exposed to 250 nM PTX-NE.

Cell culture

Triple-negative MDA-MB-231 breast cancer cells were cultured in RPMI medium enriched with 10% FBS and 1% Pen/Strep. The cells were maintained in 5% CO_2 at 37 °C.

MTT assay

MTT assay was utilized for the measurement of cell viability. First, 5×10^3 cells in each well of 96-well plates were cultured in a final volume of 200 μL. After 24 h, the cells were treated with different concentrations of the drug for 24 h, 48 h, and 72 h. Then, 10 μL of the MTT solution (5 mg ml^{-1}) was added into each well, and the cells were incubated for 4 hours. Next, the MTT solution was discarded and 100 μL of DMSO was used to dissolve the insoluble formazan crystals. Lastly, the absorbance was measured at 570 nm using a microplate reader (BioLegend, USA), and the results were expressed as the percentage of cell growth relative to the untreated cells.

Annexin/PI and cell cycle assays

To detect apoptosis, MDA-MB-231 cells were cultured with PTX and PTX-NE for a defined time period. Then, the cells were washed and detached with trypsin and were incubated with the annexin/PI mixture (Sigma-Aldrich, USA) for 20 min. Then, the percentage of apoptosis was determined using a BD FACSCalibur™ flow cytometer (Biosciences, USA). For the analysis of the cell cycle, the fixation of the harvested cells was performed with 70% ethanol for 20 min. In the next step, a mixture of 1 mg mL^{-1} PI and 10 mg mL^{-1} RNase in PBS was added to the cells and incubated for half an hour in the dark. Flow cytometry was employed to evaluate the percentage of cell populations in different phases.

Measurement of impedance

The Au patterned ECIS (electrical cell impedance sensor) was provided by the Nanobioelectronic Devices Lab of the Faculty of Engineering. Using an impedance meter (Compactstat, IVIUM), a voltage of 100 mV and frequencies in the range of 100 Hz to 100 kHz were employed to measure the impedance. The cell behavior was assessed on a real-time basis between 4 h (completing the cell adhesion) to 52 h after cell seeding on Au electrodes; the measuring process was carried out 24 times for each sample.

Statistical analysis

All experiments were performed in three biological replicates, and graphs were prepared using Prism 5.0 software (GraphPad Software, USA).

Results

Paclitaxel effect on MDA-MB-231 breast cancer cells

First, the MTT assay was performed to evaluate the effect of paclitaxel on breast cancer cells (MDA-MB-231). The cells were subjected to different concentrations of paclitaxel (10 nM, 50 nM, 100 nM, 200 nM, 500 nM, 1 μM, and 10 μM) for 24, 48, and 72 hours. Our results demonstrated that none of these treatments were strong enough to reach the IC_{50} value for paclitaxel even after three days (Fig. 1A). However, it should be noted that high concentrations of this reagent (1 and 10 μM) reduced the cell viability near to 50% and were able to change the cell morphology (Fig. 1B). It is worth mentioning that lower concentrations of paclitaxel did not alter the cell morphology due to the treatment.

Effect of TPGS on MDA-MB-231 breast cancer cells

Since MDA-MB-231 cells did not respond effectively to paclitaxel, we concluded that these cells acquired drug resistance capability to this chemoreagent. Therefore, to confirm this hypothesis, for the next step, we employed a multidrug resistance inhibitor named TPGS in combination with paclitaxel. For this purpose, we used different concentrations of TPGS on MDA-MB-231 cells to obtain the non-toxic concentration of TPGS. Based on the MTT data (Fig. 2A), we chose 20 μM concentration of this reagent as the nontoxic concentration as it did not reduce the cell viability by more than 10%. As a result, the cells were treated with paclitaxel with the above-mentioned concentrations combined with 20 μM TPGS to overcome drug resistance. The obtained results showed that at 500 nM concentration of paclitaxel, the cell viability reduced to 59% (Fig. 2B), while the cell viability with the same concentration of free paclitaxel was 76%. The obtained result proved that TPGS could partially obviate drug resistance to paclitaxel.

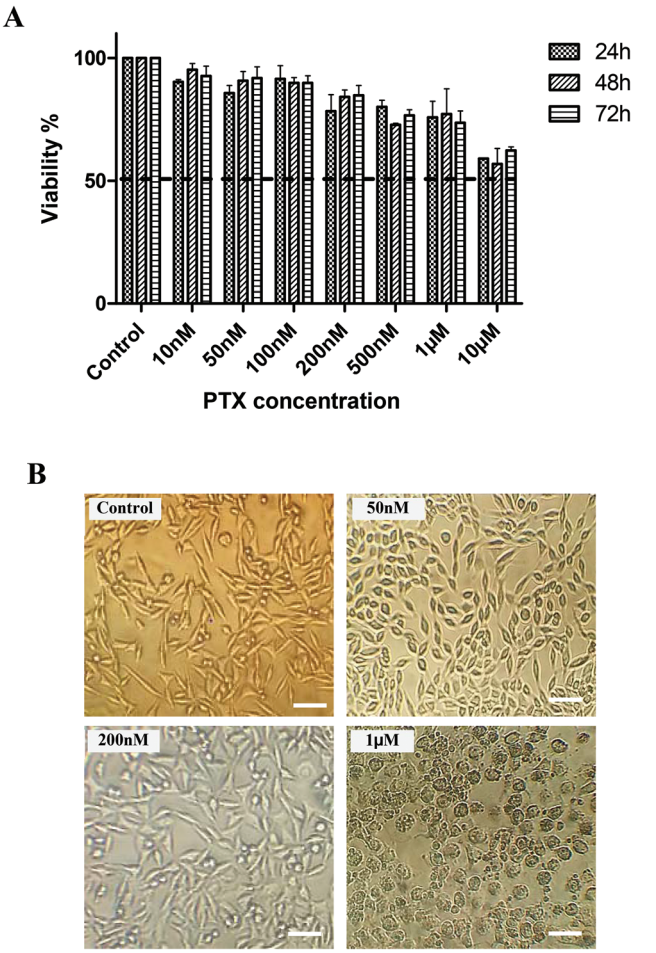


Fig. 1 Effect of PTX on the growth of MDA-MB-231 cells. (A) Viability of human breast cancer MDA-MB-231 cells after 24, 48, and 72 hours of treatment with different concentrations of PTX. The experiments were at least performed in three replicates, and the data are presented as mean ± SD. (B) Phase-contrast microscopy images of MDA-MB-231 cells treated with PTX (scale bar = 100 μm).

Nanoemulsion form of paclitaxel to overcome drug resistance

Next, we wondered whether the nanoemulsion form of paclitaxel could be effective enough to eradicate the resistance. For this, the nanoemulsion was fabricated employing the essential oil of the *Satureja khuzestanica* plant by a high-speed homogenization method. In order to employ the non-effective concentration of nanoemulsion for its fabrication with paclitaxel,

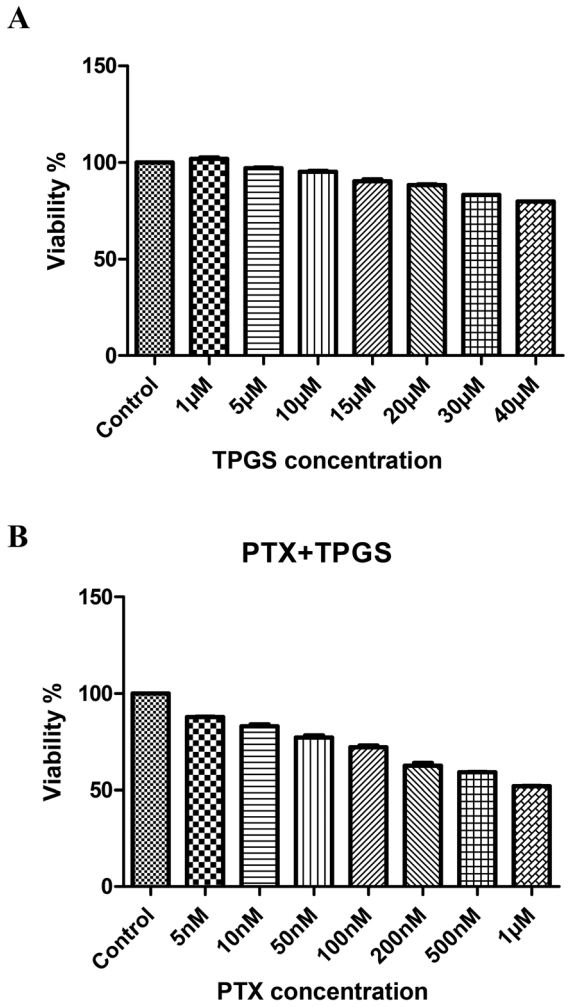


Fig. 2 (A) Viability of human breast cancer MDA-MB-231 cells after 24 hours of treatment with different concentrations of TPGS. (B) Viability of MDA-MB-231 cells after 24 hours of treatment with different concentrations of PTX plus 20 μM TPGS.

first, we treated MDA-MB-231 cells with different concentrations of the nanoemulsion (5, 10, 20, 30, 40, 50, and 60 μg ml^{-1}). The MTT results indicated that this nanocarrier was not toxic at low concentrations (Fig. 3A). Therefore, we selected 40 μg ml^{-1} dose of this nanoemulsion in which the cell viability was 90%. Then, paclitaxel was loaded, and the nanoemulsion was functionalized. The obtained paclitaxel nanoemulsion, called PTX-NE, was characterized by DLS and TEM methods. As can be seen in Fig. 3B and C, the average size of the achieved PTX-NE is 100 nm, which confirms its nanostructure identity and the TEM images verified the round and uniform shape of the droplets. Afterward, MDA-MB-231 cells were treated with four concentrations of PTX-NE, containing 62.5 nM, 125 nM, 250 nM, and 500 nM paclitaxel for 24, 48, and 72 hours. Our data showed that after 72 hours, at 250 nM concentration, we could finally achieve a 50% reduction in cell viability (Fig. 4A).

Releasing pattern of PTX-NE

The obtained release profile of PTX-NE demonstrated the gradual release of PTX from the nanoemulsion over time (Fig. 4B) with the time intervals as shown in Fig. 4C, in which 72 h treatment shows 60% drug release and the highest release amount (~75%) can be observed after five days. This result confirms the delayed release of the drug in the nanoemulsion system, which leads to its better efficacy compared to free PTX.

Apoptosis induction of PTX-NE on breast cancer cells

To study the mechanism underlying the anti-proliferative effect of PTX-NE on breast cancer cells, annexin/PI and cell cycle assays were employed. The cells treated with 250 nM PTX and PTX-NE were examined with the annexin/PI assay after 12, 36, and 72 hours post-treatment. The data showed that after 12 and 36 hours, no apparent apoptosis was observed in both treatment groups compared to the control cells. However, after 72 hours, an obvious increase in the number of apoptotic cells in the PTX-NE treated group could be detected compared to the control and PTX groups (Fig. 5A & B). To confirm the apoptotic effect of PTX-NE, we have also used the cell cycle assay, in which after 72 hours the population of the sub-G1 phase in the PTX-NE treated cells equaled 76% compared to 12% and 16% in the control and PTX treated cells, respectively (Fig. 5A & C).

Determining the efficacy of PTX-NE on cancer cells with impedance measurement

The electrodes of the biosensor were nano-roughened, which primed the surface for the optimum attachment of cells without employing any extra adhesion molecules. In order to enhance the cell–electrode surface in our study, the Au electrode ECIS was employed (Fig. 6A). In the next step, we measured the impedance of MDA-MB-231 cells treated with 250 nM free and nanoemulsion forms of PTX. The alterations in the impedance were measured 4 hours after cell culture on the chip, which was considered time 0, and the impedance was measured in 4-hour time intervals (0, 4, 8 up to 52 h). As can be seen in Fig. 6B, the mean impedances of the control and free PTX treated cells show the same behavior. However, a significant decrease in the mean impedance of the PTX-NE treated cells can be observed after about 42 hours. Moreover, the impedance phase of the PTX-NE treated cells decreased with a steeper slope after 39 hours compared to the control and free PTX groups (Fig. 6C).

Discussion

Paclitaxel is an efficient chemotherapeutic reagent, although some cancer lines have intrinsic resistance to this drug, and several cancers, including triple-negative breast cancer cells, obtain resistance to this drug with time.[6] Drug resistance is one of the main challenges in cancer chemotherapy, and chemoresistant cells use different strategies, including evading

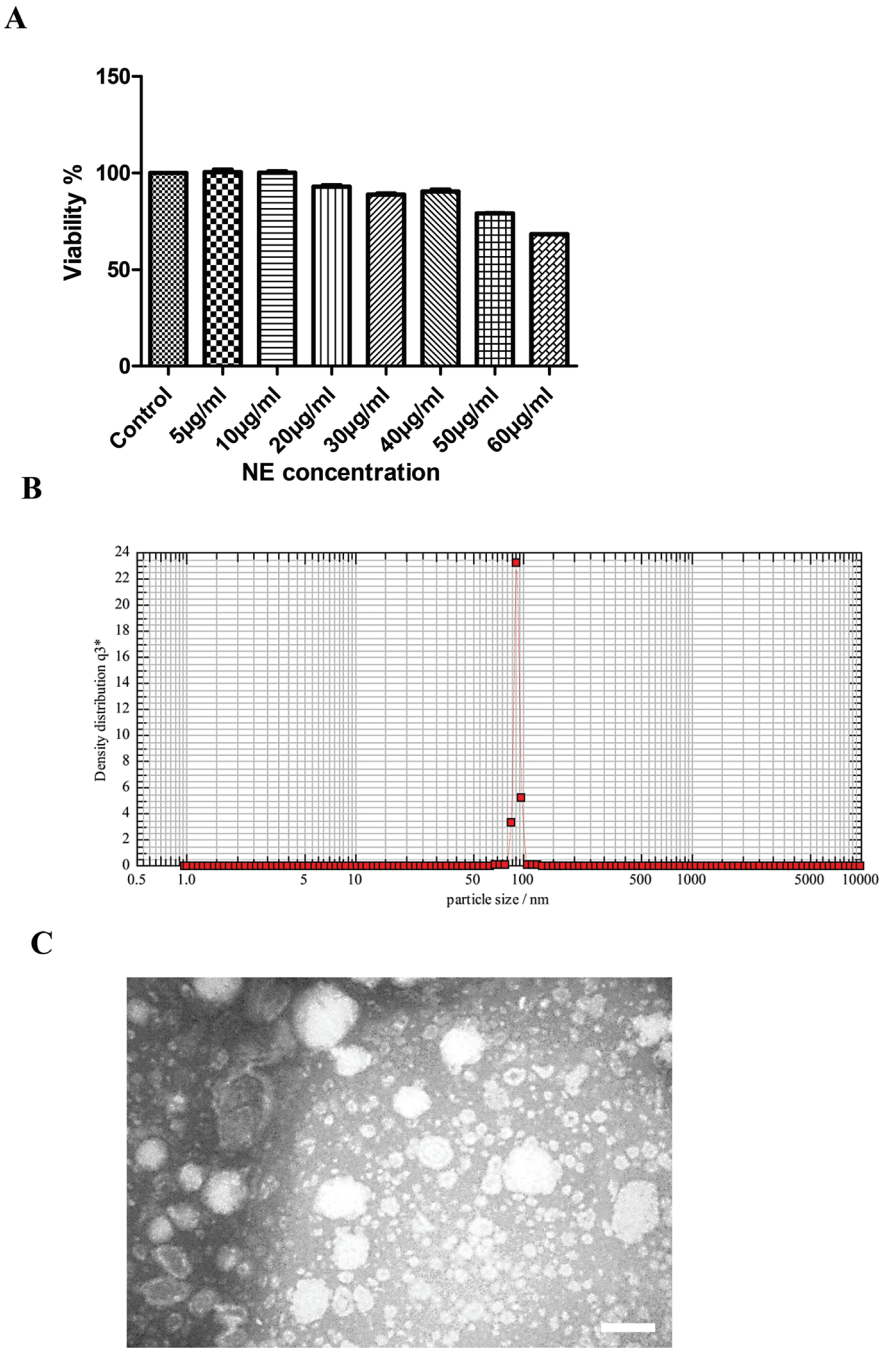
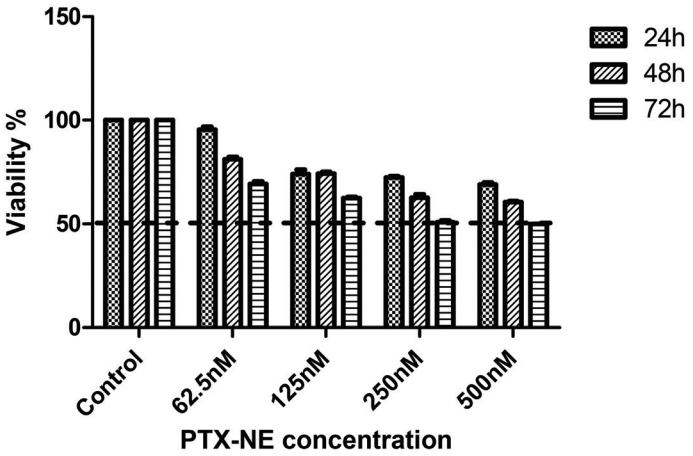


Fig. 3 (A) Viability of human breast cancer MDA-MB-231 cells after 24 hours of treatment with different concentrations of the nanoemulsion. (B) The size distribution of PTX-NE assessed by DLS. (C) TEM image of PTX-NE (scale bar = 100 nm).

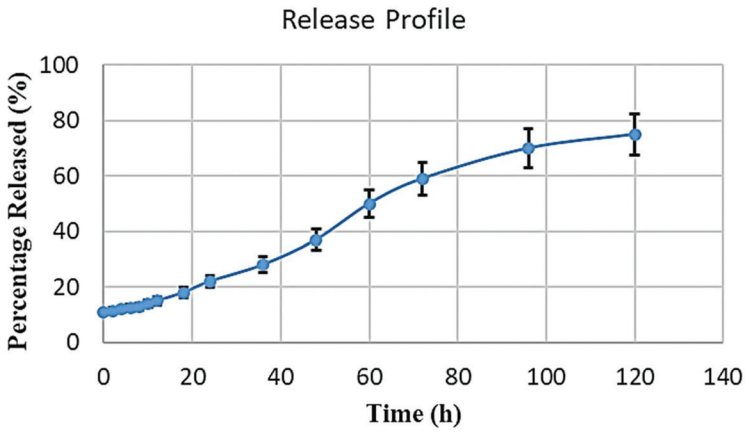
apoptosis and effluxing reagents *via* efflux pumps. Breast cancer patients who receive taxans as chemoreagents mostly face resistance over time, and whenever taxan resistance occurs, the remaining treatment options are very narrow.[26]

Our data have indicated that the MDA-MB-231 cells did not respond to PTX even after 72-hour treatments; thus, it raised the possibility of drug resistance obtained in this cell. To assess this possibility, we have employed TPGS as an inhibitor of MDR pumps combined with PTX to investigate their combined effect on cell viability. With the same approach, it was shown previously that PTX resistance was due to P-glycoprotein overexpression since verapamil as a P-gp inhibitor could obviate PTX resistance.[27] Interestingly our results showed that TPGS could partially remove the resistance to PTX, and MDA-MB-231 cells responded to PTX at 500 nM concentration. It should be noted that before this step, MDA-MB-231 cells were subjected to different concentrations

A



B



C

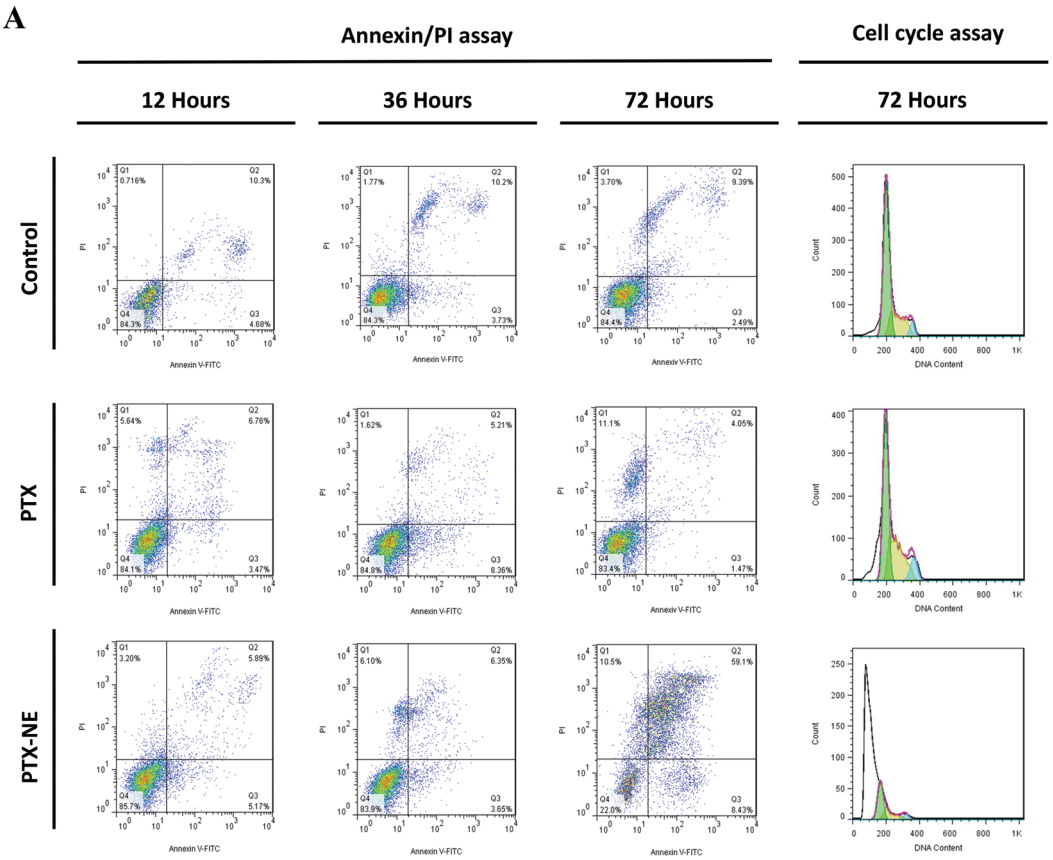
0hrs	2hrs	4hrs	6hrs	8hrs
10hrs	12hrs	18hrs	24hrs	36hrs
48hrs	60hrs	72hrs	96hrs	120hrs

Fig. 4 Effect of PTX-NE on the growth of MDA-MB-231 cells. (A) Viability of human breast cancer MDA-MB-231 cells after 24, 48, and 72 hours of treatment with different concentrations of PTX-NE. (B) The release profile of PTX from PTX-NE in a 120 h period. (C) Time intervals used for the release profile.

of TPGS and the non-effective concentration of TPGS, which preserved cell viability up to 90%, was utilized. There are numerous pieces of evidence in support of the inhibitory role of TPGS on MDR pumps. TPGS is a water-soluble derivative of vitamin E which can directly interact and block P-gps.[9] For instance, a TPGS–doxorubicin combination was used previously to inhibit P-gp pumps and to resensitize hepatocarcinoma cells to doxorubicin to improve its efficacy.[10]

It is well known that drug delivery based on nanostructures can reverse drug resistance due to the efficient intracellular delivery of the therapeutic reagent through the endocytosis pathway. For instance, PTX/TPGS nanocrystals *in vitro* have

A



B

	12 hours		36 hours		72 hours	
Apoptosis	Early (%)	Late (%)	Early (%)	Late (%)	Early (%)	Late (%)
Control	4.7	10.3	3.7	10	2.5	9.4
PTX	3.5	6.8	8.4	5.2	1.5	4.1
PTX-NE	5.2	5.9	3.7	6.4	8.4	59.1

C

Cell Cycle Phases (After 72 hours)	Sub-G1 (%)	G1 (%)	S (%)	G2 (%)
Control	12.71	61.23	25.64	5.42
PTX	15.99	45.3	33.87	8.97
PTX-NE	76.36	13.58	7.98	2.25

Fig. 5 Apoptotic effect of PTX-NE on MDA-MB-231 cells. (A) Left panel: histograms of annexin/PI assay after 12, 36, and 72 hours of treatment with PTX and PTX-NE. Q3 and Q2 demonstrate the percentage of early and late apoptosis, respectively. Right panel: histograms of cell cycle analysis in the control, PTX, and PTX-NE treated cells after 72 hours. (B) The percentage of early and late apoptosis in the control, PTX, and PTX-NE groups. (C) The percentage of sub-G1, G1, S, and G2 phases in the control, PTX, and PTX-NE groups after 72 hours.

shown a continuous release profile of PTX after 72 hours, and importantly the early burst release was inhibited in this structure, while PTX in the Taxol form of the drug displayed a rapid 100% release in 22 h.[16]

Next in our study, we formulated PTX in a nanoemulsion form, and we employed the essential oil of the *Satureja khuzestanica* plant for the mentioned PTX-NE formulation.[25] Nanoemulsion particles range from 20 to 200 nm, and this system mostly works based on oil in water droplets in which the oil portion helps to solubilize the hydrophobic reagents such as PTX. Our results showed that after 72 hours, 250 nM PTX-NE was able to reduce the cell viability to 50%; however, the equivalent concentration of free PTX reduced the cell viability just to 78%. Since in our study, PTX-NE entered MDA-MB-231 cells *via* endocytosis, it resulted in a higher intracellular concentration of this reagent and increased its efficiency. In line with our results, a study used flaxseed oil to fabricate a PTX–curcumin nanoemulsion to overcome PTX re-

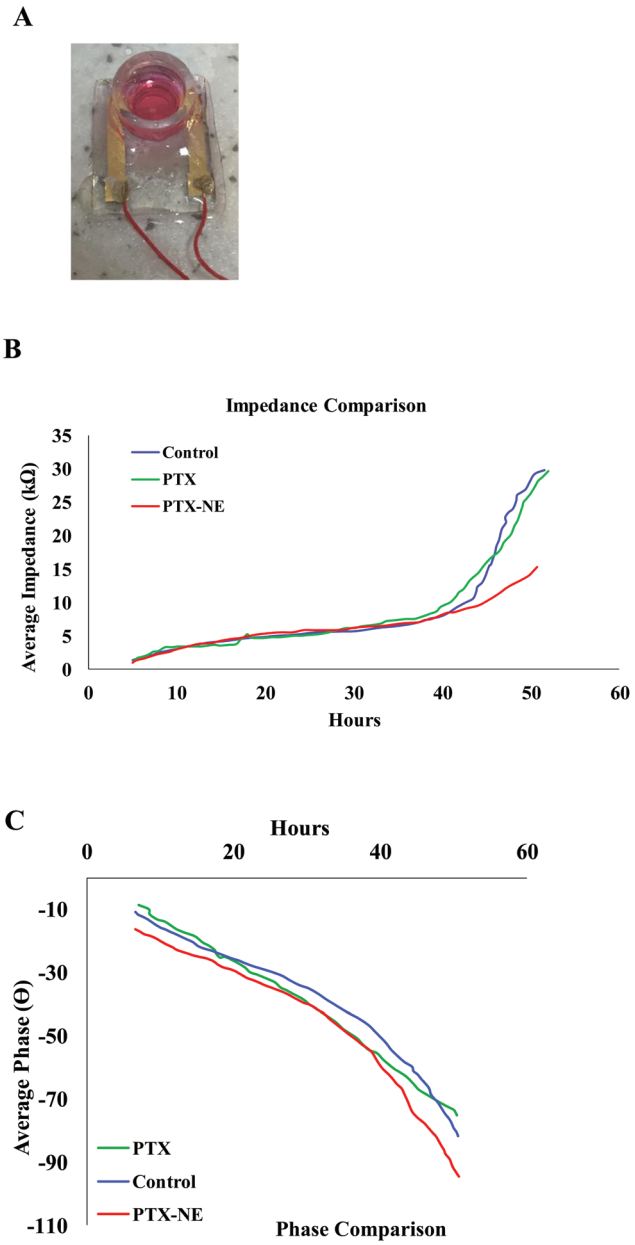


Fig. 6 (A) The Au electrode ECIS with MDA-MB-231 cells cultured on the chip. (B) Average impedance of MDA-MB-231 cells in the control, free PTX, and PTX-NE treated cells. (C) Impedance phase of MDA-MB-231 cells in the control, free PTX, and PTX-NE treated cells.

sistance in the ovarian cancer cell line. In the mentioned study, the PTX nanoemulsion appeared to be very efficient, and the required concentration of PTX for cancer treatment showed a 1.8-fold reduction.[18] It is well known that when nanoemulsions are used for the delivery of lipophilic reagents, the properties of the formulation determine the bioavailability of the drug. Moreover, it was demonstrated previously that the IC_{50} values of PTX for sensitive MCF7 and PTX-resistant MCF7 cells were 3.9 and 101 μg mL^{-1}, respectively. However, when the resistant MCF7 cells were treated with the nanoemulsion formulation of PTX–TPGS, the IC_{50} value of PTX was reduced to 5.3 μg mL^{-1}.[17] Additionally, the evaluation of apoptosis in the PTX and PTX-NE treated cells indicated that PTX-NE was able to induce apoptosis after 72 hours post-treatment. Previous studies also confirmed that the nanoemulsion form of PTX was effective in overcoming drug resistance and inducing apoptosis in ovarian and glioblastoma cancer cells, respectively.[18,28]

To have a better look and find out the exact time that MDA-MB-231 cells responded to PTX-NE in comparison with free PTX, we utilized the impedance assay. Electrical cell substrate impedance sensing (ECIS) was used to assess the biophysical and electrical properties and behavior of cells *in vitro*. Several cellular properties, including cell toxicity and proliferation, can be evaluated using this method. The electrochemical based methods can monitor cell events under label-free and real-time conditions. Our results demonstrated that no difference in the average impedance of cells treated with 250 nM free PTX could be detected compared to the control cells; however, 40 hours after treatment, a significant decrease in the average impedance of the PTX-NE treated cells compared to the untreated and free PTX treated cells could be validated. It is well established that because of the dielectric nature of cells, their expansion on the ECIS electrodes can produce a barrier for electrical current, and subsequently, the impedance will increase.[29,30] Therefore, our data indicated that free PTX could not prevent cell proliferation in which no difference in the cell numbers of the control and PTX-treated groups was demonstrated. The augmentation of impedance in both PTX-treated and control cells was indicative of the increased cell index. However, in the PTX-NE treated cells, an obvious reduction in the average impedance compared to the untreated and free PTX groups was detectable. It was illustrated previously that the reduced cell numbers *via* PTX treatment resulted in impedance reduction compared to the untreated group.[24] This is in line with our data and confirms that PTX-NE was able to induce apoptosis and the subsequent impedance reduction in the MDA-MB-231 cells. In addition, our results confirmed the slow release of PTX after 40 hours within three days. Since we could detect a significant reduction in cell viability and increase of the apoptosis rate in 72 hours, it seemed that PTX-NE worked well as a nanoparticle delivery system, which inhibited burst release at the early stages of treatment.

Conclusions

To conclude, we have demonstrated here that the nanoemulsion form of PTX provided controlled release of this drug and also PTX-NE could circumvent resistance and induce cell apoptosis in PTX-resistant TNBC cells, which could be inferred from their impedance measurement. Here we have used the cellular TNBC model *in vitro*, and in the next step the *in vivo* effect of PTX-NE on triple negative or hormone responsive breast cancer will be evaluated on animal models. This formulation could be a suitable candidate for paclitaxel-resistant

patients if the *in vivo* and clinical trial studies confirm the results. Interestingly, the ECIS method could evaluate the antiproliferative and apoptotic effects of PTX-NE in a real-time manner; moreover, the method was easy and no staining was required. The ECIS method is the most common cell impedance sensing method, and its simplicity, uncomplicated analysis, ease of fabrication, and availability make it an attractive approach for researchers. By real-time measuring of impedance, the ECIS was able to monitor the impedance alterations which were directly related to deposited cell changes. However, real-time monitoring of the cells using this sensor will stimulate the cells and make them withstand the applied voltage that may cause some changes which would be the most challenging limitation of this sensor. The use of biocompatible nanostructures as electrodes to monitor the cell lines precisely with the least interaction to reduce the external stimulation will be the future trend to improve these measuring systems.

Abbreviations

PTX	Paclitaxel
NE	Nanoemulsion
TNBC	Triple-negative breast cancer
ECIS	Electrical cell impedance sensor

Availability of the data and material

All data generated or analyzed during this study are included in this published article.

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Conflicts of interest

The authors declare no competing financial interest.

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