International Research Journal of Multidisciplinary Scope (IRJMS), 2026;7(3):753-764 IRJMS

Original Article | ISSN (O): 2582-631X DOI: 10.47857/irjms.2026.v07i03.010181

Innovative Nano-particle Engineered Targeted Drug Delivery on Triple Negative MDA-MB-231 Breast Cancer Cells

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Abstract

Breast cancer amidst one of the principal roots of tumour-related mortality in women globally that necessitating the development of targeted and less toxic therapeutic strategies. Human dental pulp mesenchymal stem cells derived extracellular vesicles (hDPSC-EVs) or exosomes are emerging as promising novelty in targeting cells to deliver novel or conventional drugs. In this study, we explored the anticancer potential of small extracellular vehicles (EVs) derived from human dental pulp, primed with Doxorubicin, a widely used chemotherapeutic agent. Human dental pulp was collected and processed to isolate and culture mesenchymal stem cells, which were subsequently characterized through immunophenotyping and gene expression analysis to confirm their stemness and mesenchymal lineage. The EVs were isolated following standard operating procedures. Doxorubicin was successfully loaded into the EVs to formulate a targeted nano-carrier system. The bioengineered Doxorubicin-EVs were then evaluated for their *in vitro* anticancer activity using cytotoxicity assays and wound healing assay on triple negative MDA-MB-231 breast cancer cells. The cytotoxicity assay demonstrated significant dose-dependent effect on breast tumour cell viability. Additionally, wound healing assay revealed marked inhibition of cancer cell proliferation, supported by quantitative ImageJ analysis. These findings highlighted the potential of Doxorubicin-primed hDPSC-derived EVs as a novel, cell-free and biocompatible nano-therapeutic strategy for the therapy of breast cancer, offering enhanced drug delivery efficiency and reduced systemic toxicity.

Keywords: Doxorubicin, Exosomes, Extracellular Vesicles, Nano-carrier System, Scratch Assay, Triple Negative MDA-MB-231 Breast Cancer Cells.

Introduction

One major global cause on mortality among women is cancer of breast. Young Indian women are regularly found in advanced stage in breast tumours and breast tumour linked death more than women from other nations around the world. Unexpectedly, breast tumour among urban population is significantly elevated due to life style modification (1). We can categorize tumours of breast into three. They are HR positive, HER2 and TNBC. HR positive and HER2 (triple positive) breast cancers have survival rates higher than TNBC. The global occurrence of TNBC is of 10% - 20% but is a violent type of cancer. The basic principle of targeting a hormone receptor for therapy related strategy is not applicable for TNBC and it does not contain any of the three hormonal receptors and so chemotherapy comes as the prime option (2). India is placed highest in terms of prevalence of TNBC when comparing with western world. In India women in 40's are very much prone to this aggressive type of cancer and most patients due to lack of awareness, come to know the condition in a very later stage, including metastasis.

Doxorubicin, the "Red Devil" is a conventional potent chemotherapy drug used to treat a wide variety of cancers like breast, ovarian, lung, bladder and stomach cancer as the drug slow down the growth and multiplication of cancer cells by either damaging the DNA or disrupting topoisomerase-II mediated DNA repair or by generating free radicals that damages the cell membrane or mitochondria (3-5). The disoriented DNA can't replicate a cancer cell anymore and the

(Received 19th January 2026; Accepted 02nd June 2026; Published 13th July 2026)

damaged cell membrane permanently damages the cancer cell. The damaged cancer cells thus formed will be engulfed by macrophages.

Despite advancements in chemotherapy, Doxorubicin often suffer from limitations with TNBC including systemic toxicity - as it is entering into cells by passive diffusion, drug resistance and poor tumor specificity in direct IV (Intravenous) or CV (Central venous) administration as it is non-targeted (6). Systemic toxicity like cardiotoxicity is non-reversible, as it involves with the reduction in the number and quality of progenitor cells and so reduction in the rate of renewal of cardiomyocytes and enhance apoptosis. These challenges have driven the need for novel, targeted and biocompatible drug delivery strategies to enhance therapeutic outcomes while minimizing adverse effects.

Recent research says, mesenchymal stem cells (MSCs), particularly those derived from human dental pulp (DPSCs), have emerged as promising candidates for regenerative medicine, due to their high proliferative capacity, immunomodulatory properties and ease of non- invasive collection and they are potent candidates to differentiate into multiple lineages (7, 8). Even it can be collected from the same person to get biocompatible MSC's without any major procedure.

A recent study had highlighted the role of MSC-derived small extracellular vesicles (sEVs), such as exosomes, in intercellular communication and their potential as natural nanocarriers for drug delivery (9). EVs offer distinct advantages, including biocompatibility, low immunogenicity and inherent tumor- targeting ability. All the three qualities are very important in targeting a cancer cell with any drug, also drugs can be easily incorporated with hDPSC-derived EVs. According to researchers, EVs are safe in-vivo and will not be interfered by the immune system (10-13).

EVs are potent candidates for TNBC cells as they are highly compatible to all, easily available, highly specific in identifying the tumor cells especially TNBC, very stable and keep the drug stable and soluble and can dodge the surveillance of the person's immune system (14). They can very well sense the tumor microenvironment as they involve in the cell-to-cell communication of tumor cells. The MSC-derived EVs are safe drug carriers and have inherent ability to track cancer cells by paracrine signaling (15).

This study aims to harness the therapeutic potential of hDPSC-derived EVs as a delivery system for Doxorubicin in targeting experimental *in vitro* triple negative breast cancer MDA-MB-231 cells. The test well with different concentrations of Doxorubicin, control and blanks were teste in triplets as directed by standard operating procedure followed by the laboratory. Different concentrations of doxorubicin were tested to find the optimum working dosage with MTT assay and the optimum dosage was used in scratch assay. Needed range of controls and blanks with or without MDA-MB-231 breast cancer cells and tests wells of unloaded and loaded EVs were tested for comparison.

By loading Doxorubicin into these exosomes, we seek to enhance its anticancer efficacy while reducing systemic side effects. The potency of the doxorubicin loaded exosomes were compared with the wells without doxorubicin, with free doxorubicin and with MDA-MB-231 breast cancer cells without any addition. Statistical analysis, graphs and scatter plots were drawn to compare and to analyze the doxorubicin loaded EVs against control groups. The engineered EVs were also characterized and assessed for their *in vitro* cytotoxicity and anti-migratory or proliferatory effects against triple negative breast cancer cells, provided insight into their potential as a cell-free, targeted nanotherapeutic platform.

Materials and Methods

Materials

Triple negative breast cancer cells - MDA-MB-231 - AU-KBC Research Centre, MIT, Chennai, Doxorubicin – Sigma Aldrich, 96-Well Tissue culture plates, 12-well culture plate, Biosafety cabinet (Class II – A2) – Clean Air, Inverted phase contrast microscope – Olympus, $C0_2$ Incubator – Thermo Scientific, Refrigerated Centrifuge – Sorvall ST 16R Thermo Scientific, Elisa Reader, Micropipette (100 to 1000uL, 20 - 200uL and 0.5 - 10uL), Trypan Blue dye, Cell counting: Neubauer chamber, Excel, Microscope Imaging software – Mag cam MU2A, Image J software for analysis.

Collection of hDPSC-EVs

The approval for the total experimental design was given by Institutional Human Ethical Committee, Sri Lakshmi Narayana Institute of Medical Sciences (IEC/C-P/4/2025), Committee for the Purpose of

Control and Supervision of Experiments on Human. Human dental pulp was collected from Vivi Dental and Cosmetic Clinic Chromepet upon submitting the ethical clearance. AcaDiCell - Innovations International - Pvt. Ltd., with 10,000 clean room facility and industry standard equipment, permitted us to carry out the stem cell bench work. The preserved dental pulp was processed to obtain the MSCs and cultured up to a confluence of 80% in a complete media, in standard laboratory condition. The Passage 3 or P3 cells were harvested and confirmed as MSCs by observing under a phase contrast microscope.

A condition media was prepared and the harvested P3 cells were treated with the media to stimulate the MSCs to release the EVs. The MSCs in condition media along with the EVs were then filtered in an Amicon unit, in order to collect the EVs and were confirmed with Nanoparticle Tracking Analysis.

Triple Negative - MDA-MB-231 – cultured Breast Cancer Cells

The MDA-MB-231 Triple negative breast cancer cells were bought from AU-KBC Research Centre, MIT, Chennai, India. After procurement the cells line was cultured in a 60mm tissue culture dish to maximize the proliferation of cells till they reach 70 to 80% confluency.

Doxorubicin (DOX)

Doxorubicin (Doxorubicin hydrochloride injection-10mg/5mL) was purchased from Sigma Aldrich Pvt. Ltd. and used as such. It is a conventional potent chemotherapy drug used to destroy a wide variety of cancer cells (cytotoxic) (16).

In Vitro Cytotoxicity Analysis (MTT Assay)

The *in vitro* cytotoxic efficacy of doxorubicin in different concentration on MDA-MB-231 triple negative breast cancer cells was analysed by MTT assay (17). This test is to prove the cytotoxic effect of the drug and to find the optimum cytotoxic dosage to be loaded in the EVs.

The principle behind MTT assay is that only viable cells have active metabolism and can convert MTT to purple formazan, which has an absorbance maximum at 570nm. The amount of formazan produced can be measured using a spectrophotometer or an ELISA reader and is directly proportional to the number of viable cells.

Seven groups of triplet culture wells were used in this test including a blank, control and 5 test groups. The blank had only culture media (No Cells & drug). The control wells had DMEM+FBS + MDAMB 231 cells. The test wells had DMEM, FBS, MDA MB 231 cells and different concentration of doxorubicin. Test well 1 received 0.6 uL (2μM) of doxorubicin, Test well 2 received 1.3 uL (4μM) of doxorubicin, Test well 3 received 2uL (6μM) of doxorubicin, Test well 4 received 2.7 uL (8μM) of doxorubicin and Test well 5 received 3.3 uL (10μM) of doxorubicin, all in triplicates.

The cytotoxic efficacy of doxorubicin was analyzed by calculating the cell Survival Rate (%), as in Equation [1].

$$\text{Cell Survival Rate (\%)} = \text{OD Sample} - \text{OD Blank} / \text{OD Control} - \text{OD Blank} \times 100\% \quad [1]$$

Procedure

We conducted MTT test in a labelled 96-well culture plate. 100μL (about 5,000 cells) of cell suspension was taken per well except blank and were incubated at 37°C, 5% CO2 incubator overnight for cell adherence. The next day specific concentration of doxorubicin was added as shown in Figure 1, in the test wells and again incubated for 24 hrs in the CO2 incubator.

The well plates were removed from the incubator after incubation and 10% to the total volume of MTT reagent was added as final concentration. Aluminium foil was used to wrap the well plates to avoid the light exposure till they were placed in the incubator for 2 hrs incubation period. After incubation period 100μl of solubilisation solution was added to each well and the solution was mixed well with the help of a micropipette to dissolve the formed crystals.

The culture plates were again incubated overnight after adding the solubilisation solution followed by dissolution of crystals and the absorbance values were read on an ELISA reader at 570nm and tabulated.

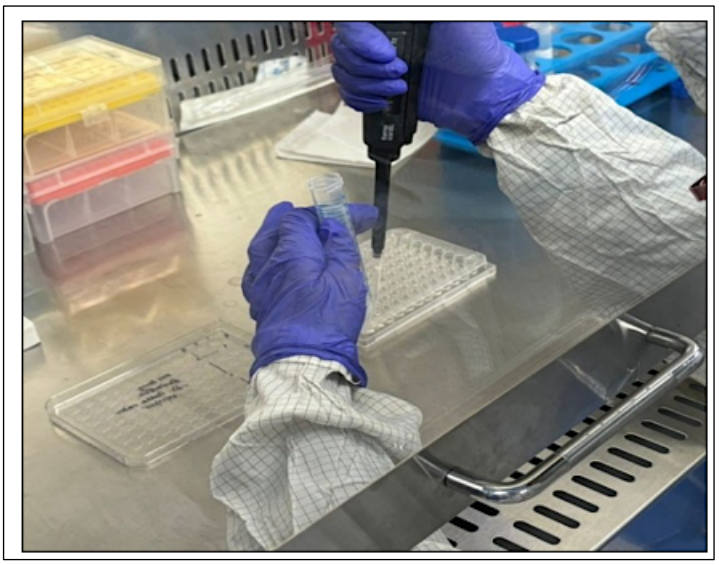

Figure 1: Representative Image showing MDA MB 231 Cell Seeding for MTT Assay and Addition of Doxorubicin in Different Concentrations in 96 Well Plate

In vitro Scratch Assay in MDA MB 231 Cell Lines

The scratch-wound assay is a simple, reproducible assay commonly used to measure basic cell migration parameters such as speed, persistence and polarity (18). The MDA MB - 231 cells were grown to confluence and a thin "wound" was introduced by scratching the cells in the middle with the tip of a 1 mL pipette. Cells at the wound edge polarise and migrate into the wound space and try to close it. The efficacy of the bioengineered drug will be analysed by analysing the percentage of wound closure.

Bioengineering The Small EV's Using Doxorubicin - Doxorubicin was loaded into small extracellular vesicles (sEVs) using a microwave-assisted simple technique but a lot more techniques are available like freeze-thaw cycles to load the drug into exosomes (19).

a) Purified sEVs were mixed with doxorubicin at 1:1 ratio and incubated at room temperature for 60 minutes.
b) The mixture was then exposed to microwave radiation at ~400–600 W for 5 seconds to enhance membrane permeability and promote active drug loading. The drug loading efficiency was found to be around 65% by analysing the residual doxorubicin.
c) Doxorubicin loaded small EVs were then used for further studies

The MDA MB - 231 cells were grown in 12 well culture plates with 1-2 mL of 10% culture media (DMEM + 10%FBS), all in triplets as follows for the test and control wells.

Well 1: Test 1 Culture media + MDA-MB - 231 + 2 µL EVs (8.0×10^{14} particles/mL).

Well 2: Test 2 Culture media + MDA-MB - 231 + 2µL Doxorubicin (DOX)

Well 3: Test 3 Culture media + MDA-MB - 231 + 2µL DOX loaded EVs

Well 4: Positive Control – Culture media (With FBS) + MDA-MB -231

Well 5: Negative Control – DMEM (Without FBS) + MDA-MB – 231

Each well of 12-well culture plate has about 3.5 cm^2 of growth area.

For MDA-MB-231 cells, recommended plating is 14k to 18k cells in each well of a 12-well plate to get confluence by the next day. The layer of cells on the middle of the plate was then scratched to introduce a wound at a confluence of 80 to 90% of cells by using the tip of a 1 mL pipette, as shown in Figure 2. When making scratch, tip needs to maintain contact with bottom of well to remove the cell layer, but pressure should not be excessive.

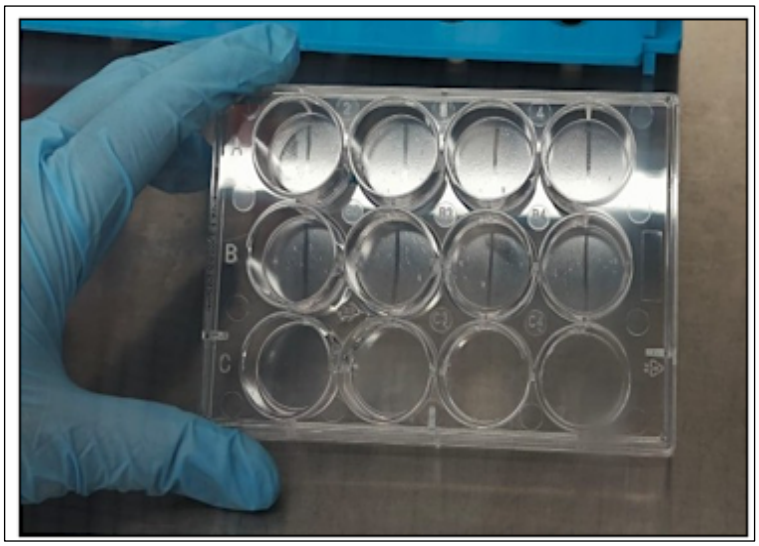

Figure 2: Representative Image showing Scratches Made over the MDA-MB - 231 Monolayer in the 96 Well Plates Using 1ml Pipette

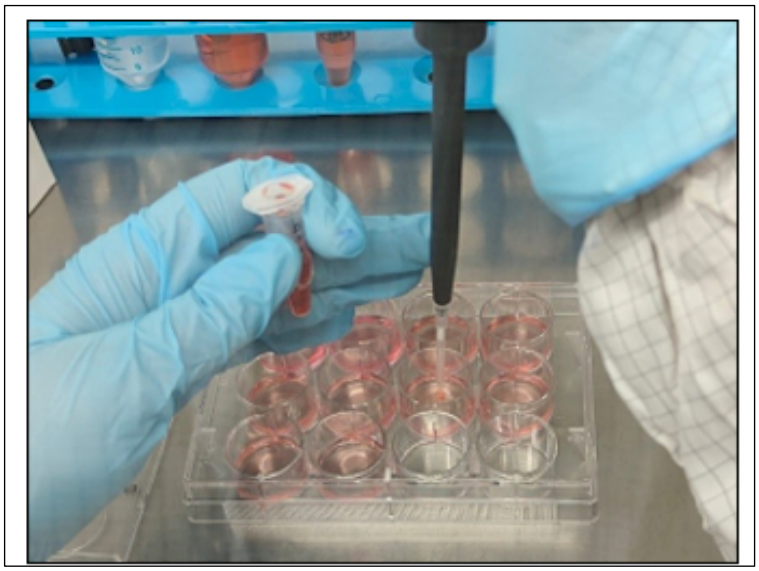

Figure 3: Representative Image for Addition of Doxorubicin Over the Scratched MDA-MB-231 Monolayer in the 96 Well Plates

After the scratch, the cell monolayer was gently washed to remove the detached cells using DPBS. Fresh culture media was added in the respective wells in specific concentrations of doxorubicin with the test and also the control wells as shown in Figure 3.

Images were taken using a phase contrast inverted microscope on 4x magnification at 0hr, make a note of where images were taken and to take the next image at the same spot at 24 hours.

Using image J software, the measurement of scratch distance on 0^{th} and 24 hrs were taken and the percentage of wound closure was calculated.

Results

In vitro Cytotoxicity Analysis

The cytotoxic potential of doxorubicin was assessed by MTT assay after 24 hours of treatment, by using Equation [1], across a concentration range of 2 µM –10 µM, as shown in Figures 1 and 2. The microscopic picture of well plates of blank and control in 0 hrs and 24 hrs were shown Figure 4 (A-D).

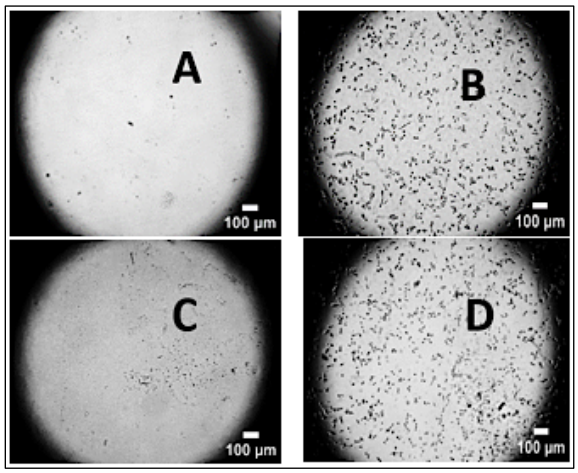

Figure 4: Images of Cells in the Control and Blank Wells after 24 hrs of procedure under 4X. (A) Photograph of Blank Well Plate at 0 hrs, (B) Photograph of Control Well Plate at 0 hrs, (C) Photograph of Blank Well Plate at 24 hrs, (D) Photograph of Control Well Plate at 24 hrs

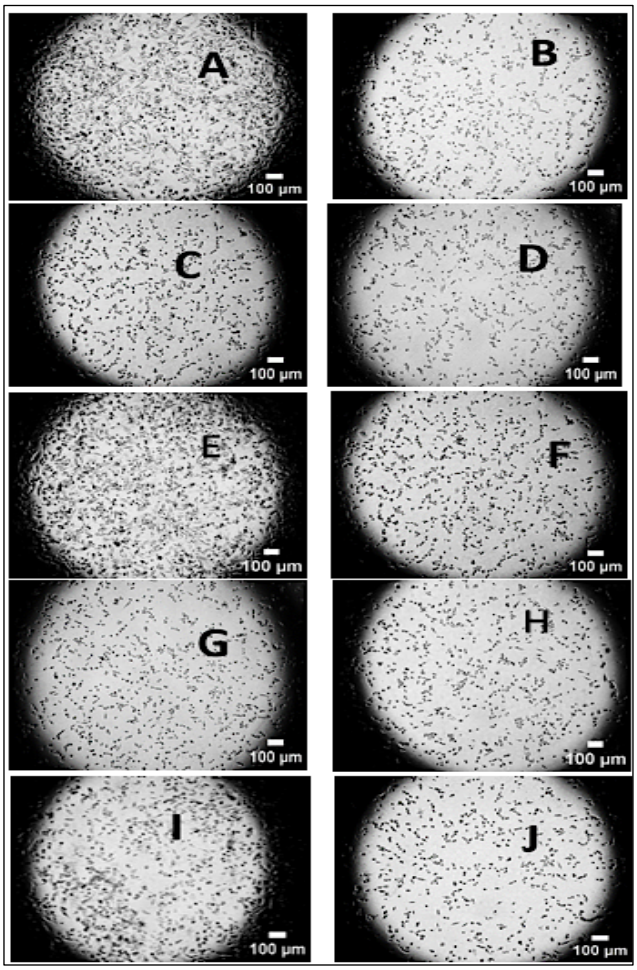

Figure 5: Images o f Doxorubicin Cytotoxicity in MDA MB 231 cells at Different Concentrations after 24 hrs Treatment Under 4X. (A) Photograph of 2 µM Doxorubicin treated well plate at 0 hrs, (B) Photograph of 2 µM Doxorubicin treated well plate at 24 hrs, (C) Photograph of 4 µM Doxorubicin treated well plate at 0 hrs (D) Photograph of 4 µM Doxorubicin treated well plate at 24 hrs, (E) Photograph of 6 µM Doxorubicin treated well plate at 0 hrs, (F) Photograph of 6 µM Doxorubicin treated well plate at 24 hrs, (G) Photograph of 8 µM Doxorubicin treated well plate at 0 hrs, (H) Photograph of 8 µM Doxorubicin treated well plate at 24 hrs, (I) Photograph of 10 µM Doxorubicin treated well plate at 0 hrs and (J) Photograph of 10 µM Doxorubicin treated well plate at 24 hrs.

The well plates of blank has no cells. The control well plates have cells but no doxorubicin. OD values were registered both in 0 hr and 24 hrs for comparison. Figure 5 (A-J) holds the pictures of different concentrations of doxorubicin treated well plates in 0 and 24hrs. OD values were recorded from all the wells, and the values were tabulated as follows in Table 1.

The OD values of the MTT assay were assessed and tabulated for easy observation as shown in Table 1. A clear dose-dependent cytotoxicity was observed, with cell viability decreasing from 67.59% at 2 µM to 39.15% at 10 µM. 6uM exhibited a significant cytotoxicity range. Cytotoxicity is increasing from 32.41% for 2µM to 60.853 for 10 µM concentration of doxorubicin as stated early by researchers (20).

Table 1: O D Values and % of Cytotoxicity on Different Concentrations of Doxorubicin by MTT Assay after 24 hrs

	Cytotoxicity of Doxorubicin in MDA-MB-231 for 24 hrs						
	Test wells OD (S)					**Control (C)**	**Blank (B)**
	2uM	**4µM**	**6µM**	**8µM**	**10µM**		
1	0.54	0.501	0.488	0.435	0.442	0.6908	0.241
2	0.555	0.517	0.491	0.429	0.426	0.666	0.282
3	0.549	0.529	0.443	0.454	0.427	0.686	0.291
Avg.	0.548	0.516	0.474	0.439	0.432	0.681	0.271
Standard Deviation (SD)	0.008	0.014	0.027	0.014	0.009		
Test (S) - B	0.277	0.244	0.203	0.168	0.16		
C - B	0.409	0.409	0.409	0.409	0.409		
S - B/C - B	0.676	0.596	0.495	0.41	0.391		
% live cells	67.59	59.632	49.463	40.971	39.147		
Cytotoxicity%	32.41	40.368	50.537	59.029	60.853		

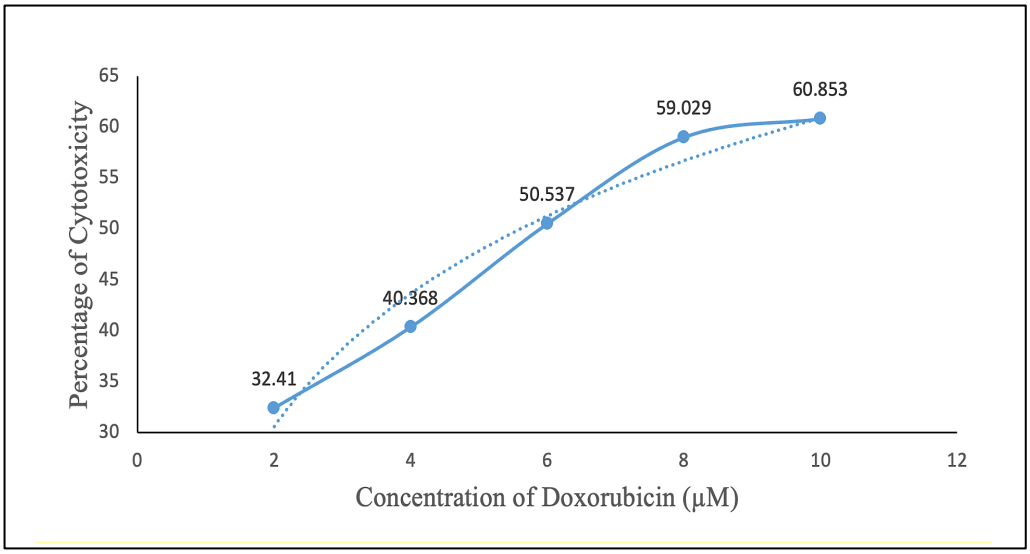

Figure 6: Scatter Plot for Cytotoxicity of Doxorubicin at Different Range of Concentrations Against MDA-MB-231 Cell Lines

A scatter plot with a logarithmic trend line was drawn to compare the cytotoxicity of different concentrations of doxorubicin as shown in Figure 6 and to find the effective cytotoxic dosage EC50 was taken as 6 µM, based on the point where the curve hits 50% cytotoxicity.

In vitro Scratch Assay in MDA-MB-231 Cell Lines

The images of both 0 hr and 24hrs scratch assay tests were analysed as shown in Figures 7 (A-D) using image J software and the percentage of wound closure for control and test wells were calculated.

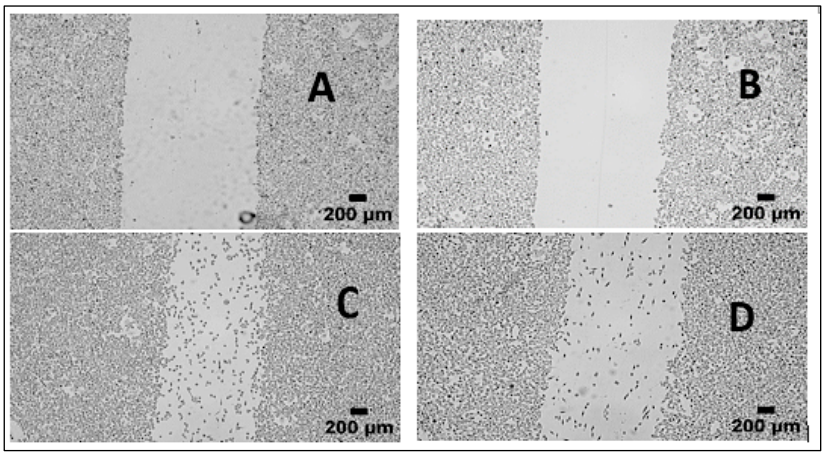

Figure 7: Migration of MDA-MB-231 Cell Lines in the Negative Control (NC) and Positive Control (PC) wells 0 and 24 hrs after Scratch Assay Under Inverted Microscope in 4X. (A) Image of Scratch Wound in the Negative Control Well Plate in 0hr, (B)Image of Scratch Wound in the Positive Control Well Plate in 0hr, (C) Image of Scratch Wound in the Negative Control Well Plate in 24hr, (D) Image of Scratch Wound in the Positive Control Well Plate in 24hr

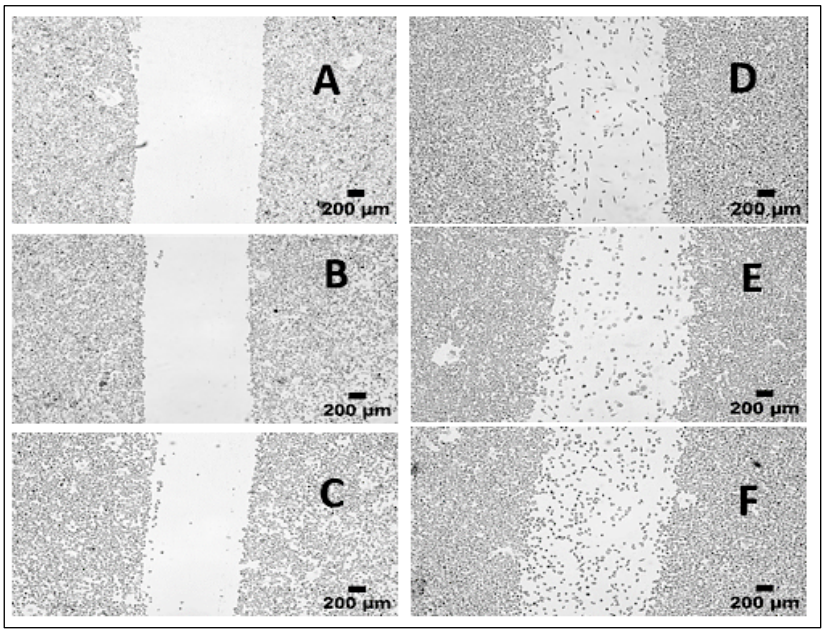

Figure 8: Migration of MDA-MB-231 Cell Lines in the Test Group with Extracellular Vehicles (Evs), Doxorubicin (DOX) and Extracellular Vesicles Doxorubicin (EV+DOX) At 0 And 24 Hrs After Scratch Assay. (A) Image of Scratch Wound in Evs Treated Well Plate in 0hr, (B) Image of Scratch Wound in DOX Treated Well Plate in 0hr, (C) Image of Scratch Wound in EV+DOX Treated Well Plate in 0hr, (D) Image of Scratch Wound in Evs Treated Well Plate in 24hr, (E) Image of Scratch Wound in DOX Treated Well Plate in 24hr, (F) Image of Scratch Wound in EV+DOX Treated Well Plate in 24hr

The area of wound closure, as in Figure 8 (A-F), on EVs, DOX and EV+DOX well plates were also calculated in both 0-hrs and in 24 hrs using image J software and the results were tabulated. The tabulated results were used to draw a graph for better understanding, a graph with trend line, for comparison as shown in Table 2 and Figure 9. 35% of wound closure was observed with PC well plates in contrast with 3.6% wound closure with EV+Dox well plates, as shown in Table 2. That shows the combinations of EV+Dox shows cytotoxicity rather wound closure and this property is important to treat the cancer cells.

Table 2: Comparison in the Percentage of Wound Closure between Different Groups, 24 hrs after Scratch Assay

Well Plates	Initial Wound Area	Final Wound Area	Wound Closure	Wound Closure %
NC well plates	759281.33	654477.33	0.138030524	13.80305236
PC well plates	738362.67	475386.67	0.356161019	35.61610193
EV well plates	686533.33	613276	0.106706157	10.67061565
Dox well plates	728059.11	655397.33	0.099802031	9.980203073
EV+Dox well plates	761186.6667	733061.3333	0.036949325	3.694932474

Note: NC = Negative Control, PC = Positive Control, EV = Extracellular Vesicles, Dox = Doxorubicin, EV+Dox = Extracellular Vesicles + Doxorubicin.

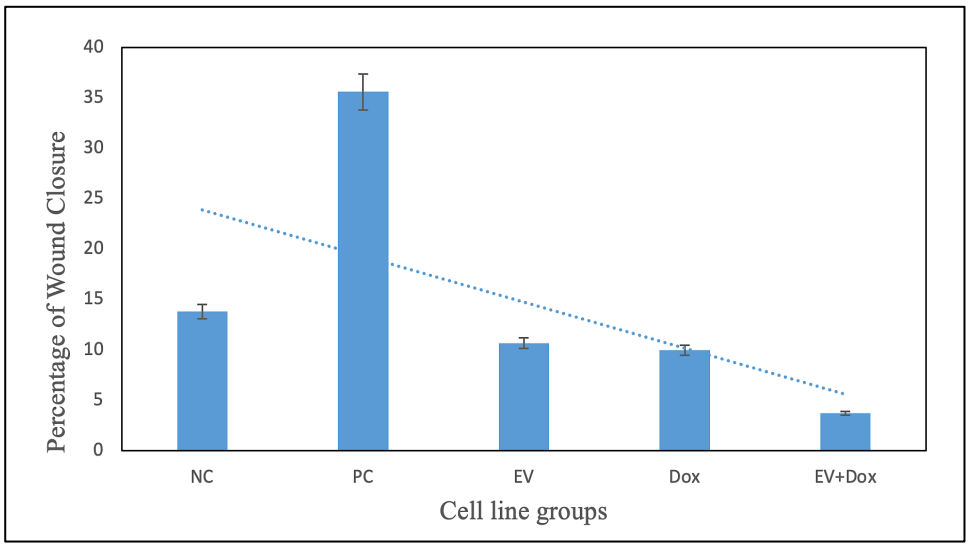

Figure 9: Percentage of Wound Closure between Different Groups, 24 hrs after Scratch Assay

Discussion

In vitro Cytotoxicity Analysis

Cytotoxicity of doxorubicin was analysed by MTT assay, a standard assay in research on tissue engineering. On tabulating the data, a clear dose dependent cytotoxicity was observed in triple negative breast cancer cells from doxorubicin 2uM to 10µM concentration. The percentage of viable cells on calculation was seen decreasing from 67.59% with 2uM concentration of doxorubicin to 39.15% at 10µM concentration and with 49.463% of viable cells with 6uM concentration. The calculated cytotoxicity based on the above values were 32% for 2uM, 51% for 6uM and 61% for 10uM. Optimum increase in dose dependent cytotoxicity or decrease in cell viability was observed in 6uM concentration of doxorubicin, later concentrations shown a decrease in the increment of cytotoxicity and so 6uM concentration of doxorubicin was considered as optimal cytotoxic dosage.

The mesenchymal derived EVs have the potency to sense the micro environment, around tumor cells the homing property and also, they are richly provided with integrin that facilities the ready up-take of these EVs by tumor cells, all belong to their inherent targeting ability to tumor cells (21).

According to a recent study, doxorubicin is cytotoxic to MDA-MB-231 breast cancer cells and a steep increase in cytotoxicity was observed with 2µM, 4µM and 6µM of doxorubicin but with higher concentrations the increase in cytotoxicity was not appreciable (22). So based on MTT assay data and the bar chart on the data, a concentration of 6 µM doxorubicin corresponding to ~49.46% cell viability or 51% cytotoxicity was considered optimum and was selected to assess the synergistic anti-cancer effect when combined with small EVs. This concentration permits sufficient cell viability to evaluate functional parameters such as cell proliferation, while still reflecting pharmacological activity. It also aligns with therapeutic strategies aiming to lower chemotherapeutic toxicity by selectively reduce the tumor cells and as high dosage involves with systemic toxicity, while improving the efficacy through EV-mediated delivery or support (23, 24).

In vitro Scratch Assay in MDA MB 231 Cell Lines

This study analysed the percentage of wound closure as a parameter to understand the efficacy of the doxorubicin loaded EVs, a potent anticancer drug that reduces the rate of cell growth and multiplication and so wound closure.

Analysis of wound closure was done on 24 hours after treatment. The PC well plates shown maximum (35.61%), wound closure as the wound in this group of well plates were not treated with doxorubicin but provided with growth media, so the MDA-MB-231 cells grown faster and tried to close the wound as fast as possible. With EV group, the well plates received only the EVs and no doxorubicin and so no cytotoxicity that allowed the cells grow naturally without any hindrance, lead to good wound closure of 10.67%, followed by Dox group. Dox group well were only provided with doxorubicin without EVs, so that the targeted drug delivery was not possible with the group, leads to a wound closure of 9.9%, more or less equal to Dox group. The % of wound closure with EV + Dox was observed to be only 3.69%, very less than any other group in this study that shows the drug was delivered into the cells in a targeted manner rather delivering it in the environment around that need not be taken inside effectively as the triple negative breast cancer cells lack the receptors to pick any molecules. Thus, it is proved that the EVs selectively deliver the doxorubicin effectively into MDA-MB-231 cells.

Based on *in vitro* scratch assay we can very well observe the impact of the extracellular vesicles combined with Doxorubicin in inhibiting breast cancer cell proliferation and migration in the wound area (25). While EVs facilitated 10.67% cell migration into the wound area, Doxorubicin exhibited a migration percentage of 9.98%. However, EVs+ Doxorubicin showed approximately 3.69% cell migration, which is relatively lower than the negative control (without serum supplement). The positive control shows better cell migration, where the EVs+ Doxorubicin inhibit cell growth for a particular duration of time (26). So negative wound closure is a positive action for doxorubicin loaded EVs, as described by present researchers.

Thus, the combined effect of EVs+ Doxorubicin have higher impact in inhibiting cell migration in a fast manner as that the effect was visible in 24hrs (27).

Conclusion

This study highlights the potency of doxorubicin-loaded small extracellular vehicles (EVs) derived from dental pulp mesenchymal stem cells (DPSCs) as a promising approach for triple negative breast cancer, aggressive among the types of breast cancer cells and so against all types of cancer therapy. The use of a microwave-assisted loading method enabled efficient drug encapsulation while maintaining vesicle stability. EVs are very small and flexible and has homing property that enhances the permeability of the EVs through the tumor vasculature and can be easily taken up by the tumour cells, so they can directly deliver the drug to the interior of the targeted cells without any wastage. Being a membrane bound structure EVs can enter the cells with ease. As the exosomes have the inherent ability to track the tumour cells by paracrine signalling, they can specifically target the tumour cells without causing systemic side effects unlike the direct infusion of doxorubicin through venous route, concept was thus cleared by the present study. The study has its own limitation, the future target, in explaining the homing property of the EVs in molecular level that will be cleared in future research in big picture. In this present study, the bioengineered EVs that are derived from dental pulp mesenchymal stem cells demonstrated, effective cytotoxicity and anti-proliferative effects at sub-toxic concentrations on triple negative MDA MB 231 cell, an aggressive form of breast cancer, thus supporting their role as a biocompatible and effective drug delivery system for cancer treatment.

Abbreviations

α- MEM: Alpha Minimum Essential Medium, DPBS: Dulbecco's Phosphate Buffered Saline, EVs: Extracellular vesicles, hDPSC-EVs: Human Dental pulp mesenchymal stem cells derived extracellular vesicles, MDA-MB-231 breast cancer cells: MD Anderson-Metastatic Breast -231 cancer cells, MSCs: Mesenchymal stem cells, MTT**: 3**-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, P4: Passage 4, PBS: Phosphate Buffered Saline.

Acknowledgement

We would like to thank the Health Sciences Research Centre (HSRC), Princess Nourah bint Abdulrahman University for their technical support.

Author Contributions

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The data are available from the corresponding author upon reasonable request.

Declaration of Artificial Intelligence (AI) Assistance

Generative AI was not used in the writing process. The authors must take full responsibility for the content's originality, interpretation and accuracy.

Ethics Approval

Institutional Human Ethical Committee, Committee for the Purpose of Control and Supervision of Experiments on Human, Sri Lakshmi Narayana Institute of Medical Sciences - (IEC/C-P/4/2025).

Funding

None.

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How to Cite: Shukla S, Praba MAA, Rashid NA, Cordero MAW, Ganesh K, El Hasnaoui-Saadani R, Chinnappan V, Pawar S. Innovative Nano-particle Engineered Targeted Drug Delivery on Triple Negative MDA-MB-231 Breast Cancer Cells. Int Res J Multidiscip Scope. 2026;7(3):753-764.
DOI: 10.47857/irjms.2026.v07i03.010181