A Study of Cellular Toxicity of some Nano Cationic Polymers in BT, MCF7 and SKBR3 Breast Cancer Cell Lines

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Abstract
Background:
Aim: This study conducted aiming to develop antitumor system.
Materials and methods: In this work, some cationic polymers which are namely; poly ethylene glycol (PEG), poly vinyl alcohol (PVA), poly vinyl pyrrolidone (PVP), and polylactide (PLA) were converted to nanoparticles in size by subjecting them to the sonication method. They were evaluated as anticancer potency towards human breast carcinoma cells. The cancer cells were monitored under a fluorescence microscope after treatment with propidium iodide.
Results: MTT assay revealed the higher cytotoxic efficacy of cationic polymers NPs than non-treated cell lines. In addition, results also suggest that PLA NPs exposure the cell viability decreased highly significant than other types of NPs polymers. **Conclusion:** PLA NPs demonstrated strong antitumor activity *in vitro* by reducing cell viability.
Keywords: MTT, cellular toxicity, cationic polymers, SEM, cell viability, nanoparticles

Introduction

Polymers are materials that are suitable for all medical applications because of their wide and varied properties. The science and engineering of polymers are of great strategic and technological importance to be used in a variety of areas and uses, the most important being their use as vital alternatives [1,2]. They were first used in medical applications in 1960 [3].

Natural and synthetic polymers have been used as a promising tool for nanoscale drug carrier systems, especially in the oral administration of poorly absorbed therapeutic drugs [4,5]; the advances in nanotechnology and Bioengineering are supporting tremendous efforts in optimizing the methods for genomic, epigenomic and proteomic profiling [98], especially the cationic polymers are among the materials that have been studied for gene delivery given their ability to electrostatically bind and condense nucleic acids to form nanoparticles, such as poly-l-lysine (PLL), polyethylenimine (PEI), chitosan, and poly(amino-co-ester)s. (PAEs) are particularly promising due to their facile synthesis, transfection efficiency, and degradability. Such polymers have already shown potential to deliver DNA in a variety of *in vitro* and *in vivo* [6,7].

Polymeric nanoparticles systems are useful in biomedical applications, for many important scientific reasons, which are: they're easy to prepare from well-understood polymers and have high stability in biological fluids as well as during storage. And the most used in the field of tumors as an anticancer treatment [8]. Nanoparticles can bind DNA fragments, drugs, and proteins, thus exerting the function of transportation and targeted therapy. At present, numerous surface-modified compounds are available, including polyethylene glycol (PEG), polyethylenimine (PEI), polyvinyl alcohol (PVA), etc. [9].

Synthetic polymers are able to improve control properties and modifications freely in order to identify desired properties; their biological compatibility can be improved [10]. Therefore, this study aims to use some biodegradable cationic polymers with nanometer dimensions and evaluate their anticancer potency of the prepared NPs in human breast carcinoma cells. Thus this study conducted aiming to develop antitumor system based on some cationic polymer nanoparticles to improve their bioavailability and anticancer activity in antitumor treatments, and this can be achieved firstly by examining their cellular toxicity on several types of human breast cancer cell lines as in vitro model, these types including BT cells, MCF-7 cells, and SKBR-3 cells.

Materials

Different polymers were purchased from Sigma-Aldrich, and they are poly (ethylene glycol), PEG, poly (vinyl alcohol), PVA, poly (vinyl pyrrolidone), PVP, and polylactide, PLA. Cationic polymer nanoparticles were prepared by dissolving 25mg of each polymer in 25mL of the suitable solvent, and all solutions were left stirring and heating at 50ºC for an overnight, then the mixture was dispersed by subjection to the ultrasonic instrument for 5min at 50W with a pulse of 5sec and 10sec between pulses, to perform the nanoparticles of the polymer [11].

Proliferation Assay

A 3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to determine cell proliferation. Briefly, MCF-7, BT, and SKBR-3 human breast cancer cell lines were plated individually in 96-well plates. After 24-hour incubation, the medium was removed from the wells and replaced with filter sterilized complete medium containing the polymer at concentrations 1mg/ml (100 µl/ well) for indicating times, then MTT (20 µl of 5 mg/ml in PBS) was added to each well of the plates for all incubation times. Plates were incubated for a further 3.5 h., and covered with tinfoil, agitate cells was done on an orbital

shaker for 15 min. Then the medium was removed and DMSO (100 µl) added before a further incubation of 30 min at 37 ºC.

Finally, the absorbance at 550 nm with a reference filter of 620 nm of the plates was read by the Tecan plate reader. Absorbance values were blanked against DMSO only, and the absorbance of cells exposed to medium only (no polymer added) was taken as 100 % cell viability (the control [12,13].

Microscopic Estimation

The microscopic estimation of cell viability was done in equivalent cultures after staining the cell pellet with propidium iodide [14]. Subsequently, slide smears were made and the slides were examined under a fluorescence microscope. Dead cells (orange fluorescence) and viable cells (unstained) were counted randomly found in a microscopic image.

Statistical Analysis

All samples were detected in triplicates and were presented as (means ± SD). For statistical analysis using Graph pad prism 5, one-way analysis of variance (ANOVA) test was used to test for significance between the groups, highly significant if $P < 0.001$.

Results and Discussion

A comparative *in vitro* cell proliferation for three different types of human breast carcinoma cell lines, MCF-7, BT, and SKBR-3 was achieved applying to pure cationic polymers as nanoparticles shape PEG, PVA, PVP, and PLA NPs, using *in vitro* MTT assay. The mean population triplicate time (PTT) of % cell viability between non-treated control samples and polymers NPs treated cells for all the tested carcinoma cell lines were analysed in Table (1); $p<0.001$. These data clearly suggest that the polymers NPs were cytotoxic against breast carcinoma and have a highly significant consequence of the cellular proliferation of human breast carcinoma cell lines. The presence of the NPs (1mg/ml) has a strong effect on the growth and cell viability of human breast carcinoma cell lines in culture, as measured by MTT assay, at different times ranging from 24h to 72h.

Figure (1) illustrates cell proliferation for each type of examining cell lines, the results performed highly significant, ($p<0.001$), decreasing of the cell proliferation with increasing the time exposure to test polymers nanoparticles, in comparison with untreated cells (control samples). The mean population triplicate times (PTT) perform differ between non-treated control cells, polymers NPs treated cells for all the tested times, Table (1); these types of polymers NPs appeared significant decreases in cell viability with increases transfection times.

The cytotoxic behavior of each PEG, PVA and PVP NPs, were approximately having a near cytotoxic effect against all types of human breast cancer cell lines, which may be attributed to the presence OH functional groups in the structure of PEG, PVA and PVP NPs, in addition to the nanosize of the polymer which may enhance to use the polymers in medical applications; meanwhile, the PLA NPs had exclusively antitumor effect against MCF-7 type of cell lines with time intervals, which reach to 9.33±2.082, although the effect against BT and SKBR3 were 57.67±9.018 and 47.00±1.732, respectively. Otherwise, the decreasing in the cell viability of all types of cancer cell lines was highly significant when compared to the control samples, Table (1).

The data given in Figures (2-5) show that the MTT assay revealed the higher cytotoxic efficacy of PLA NPs than positive control cells, that may be attributed to the increased reactive oxygen species production that leads to loss of mitochondrial membrane and necrotic cell death due to apoptosis, as demonstrated by the

formation of apoptotic bodies. Furthermore, PLA NPs demonstrated strong antitumor activity *in vitro* by reducing cell viability, inducing cell necrosis, decreasing the negative surface charge and mitochondrial membrane potential, and fragmenting DNA [15].

As can be seen in Figure (2), the morphological examination of the cells was performed using a fluorescent microscope to tested nano-cationic polymers, the presence of nanopolymer 1mg\ml, in the breast carcinoma cultures led to an increase in the reduction of the viable cell after 24h of treatment, when compared with the control culture (without nano cationic polymers), Figure (6).

Conclusions

In summary, cationic nanoparticles polymers may be considered as a therapeutic target for treating different casing of breast cancer, by suppressing breast cancer proliferation. *In vitro* Cytotoxicity (MTT assay) and fluorescent images results revealed that all the tested polymer nanoparticles demonstrated highly significant antitumor effect; ($P<0.001$), compared with the control samples, against several types BT, MCF-7, and SKBR3 of human breast cancer cell lines at different times of treatment. Furthermore, PLA nanoparticles appear highly antitumor effect in comparison with others; this finding enhances using PLA as a therapeutic target for treating breast cancer.

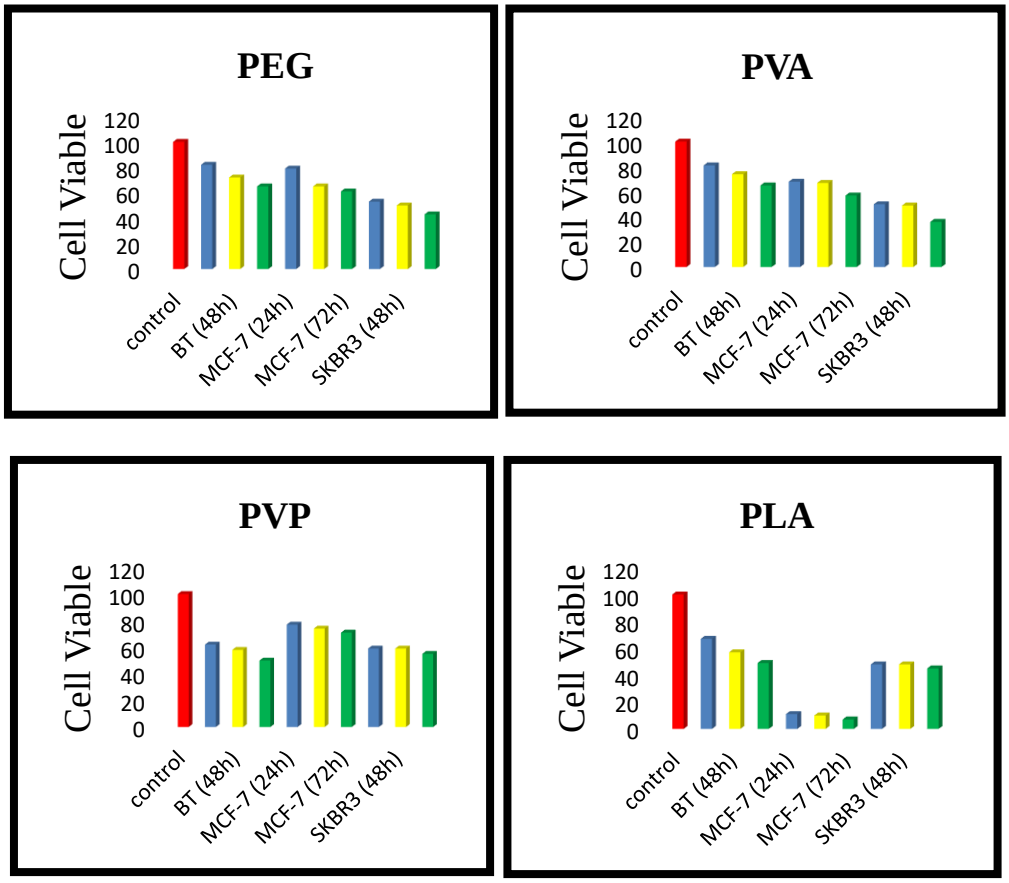
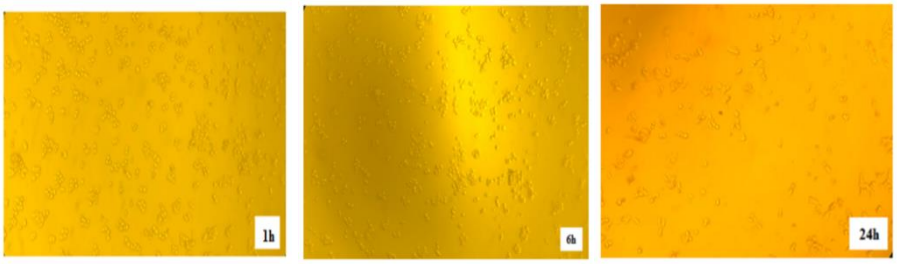


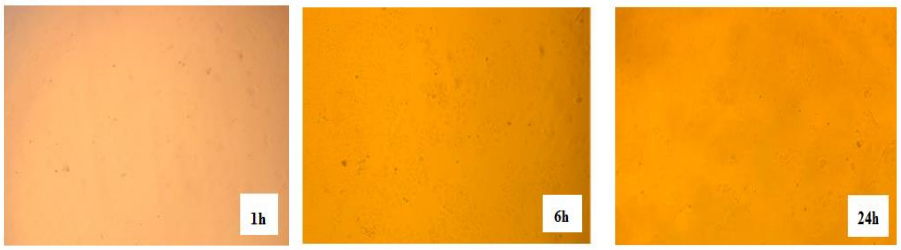
Figure (1): Cell viability percentages of human breast cancer cell lines (BT cells, MCF7 cells, and SKBR3 cells) affected with PEG, PVA, PVP, and PLA NPs, at different times for 24h, 48h and 72.

Table (1): The mean population triplicate time (PTT) ±standard deviation (SD) values as the antitumor effect of prepared polymer nanoparticles against the proliferation of human breast cancer cell lines BT cells, MCF7 cells, and SKBR3, with highly significantly affected (P<0.001).

Sample	Cell Lines		
	BT	MCF-7	SKBR 3
PEG	73.00±8.544	68.33±9.452	48.67±5.132
PVA	71.33±5.508	64.00±6.083	49.33±17.388
PVP	56.67±6.110	74.00±3.000	57.67±2.309
PLA	57.67±9.018	9.33±2.082	47.00±1.732



PEG- BT

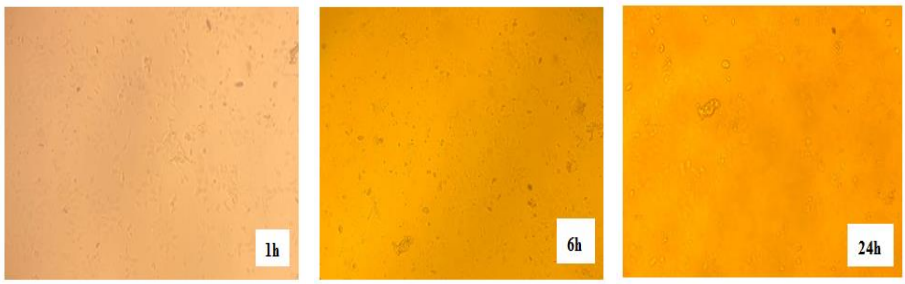


PEG- MCF7

PEG- SKBR3

Figure (2): Fluorescence Microscopy Images of BT cells treated with PEG Nano cationic polymers at different times of treatment.

PVA- BT



PVA- MCF7

PVA- SKBR3

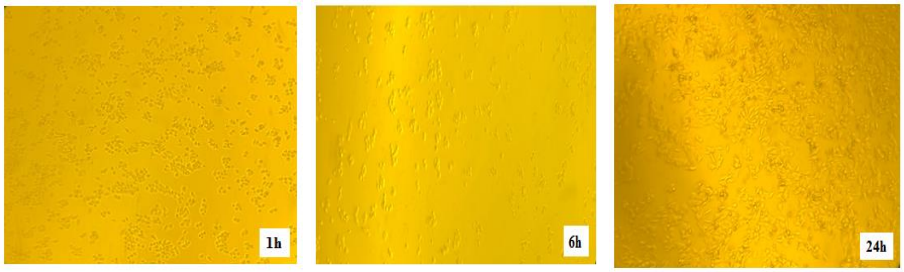
Figure (3): Fluorescence Microscopy Images of MCF-7 cells treated with PVA Nano cationic polymers at different times of treatment.

PVP- BT

PVP- MCF-7

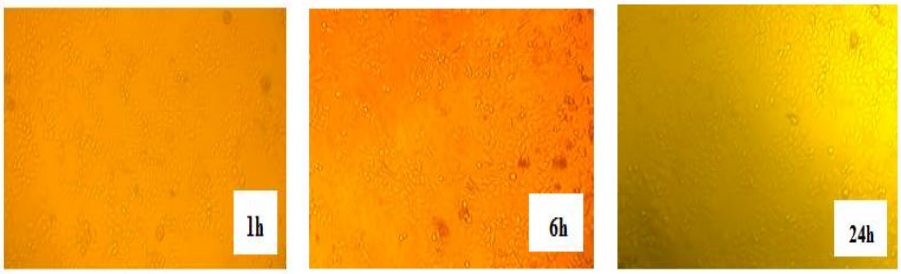
PVP- SKBR3

Figure (4): Fluorescence Microscopy Images of SKBR3 cells treated with PVP Nano cationic polymers at different times of treatment.



PLA- BT

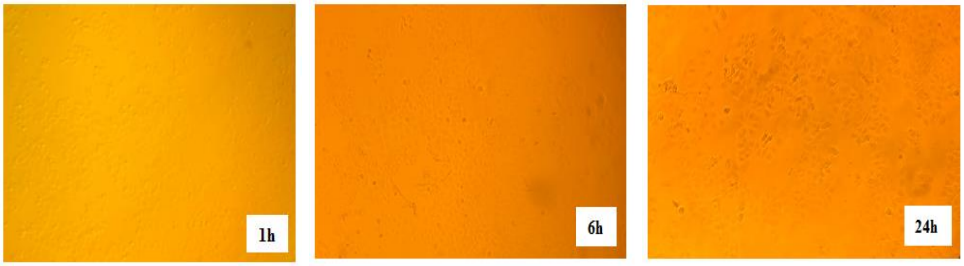
PLA- MCF-7



PLA- SKBR3

Figure (5): Fluorescence Microscopy Images of SKBR3 cells treated with PLA Nano cationic polymers at different times of treatments.

Control – BT



Control – MCF-7

Control –SKBR3

Figure (6): Fluorescence Microscopy Images of Various control of BT cells, MCF-7 cells, and SKBR3 cells with no Nano cationic polymers at different times of treatments.

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