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Nanomedicine, a new therapeutic strategy in breast cancer treatment

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ABSTRACT

Background: As cancers, especially breast cancer, have become the most lethal and concerning subject, new methods to promote therapies and achieve better results are strongly essential. Nanotechnology has offered a new approach to advocate the strategies being used and to vanquish their impediments. This article provides a review of the nanomaterials used most recently, mainly in breast cancer, for more effective and specific treatment.

Methods: Documents were found in PubMed and Google Scholar using "nanomaterials" and "breast cancer" as the main keywords. Additionally, each individual nanomaterial with "liposomes", "polymeric NPs", "dendrimers", "quantum dots", "virus like nanoparticles" and "magnetic NPs" keywords were searched and selected after assessing publishers, journals impact and their relativities to the subject of the review.

Results: Six frequently used nanoparticles in breast cancer treatment including liposomes, polymeric NPs, dendrimers, VLPs, quantum dots, and magnetic NPs were selected to be discussed in this review. They all showed correlative results such as promoting drug maintenance, hydrophilicity, and accumulation in the tumor site by their specific cell targeting system and high cellular uptake. Each of these NPs has unique properties and disadvantages and therefore many in vitro and in vivo experiments have been carried out.

Conclusion: Extensive research in nanotechnology in medicine, especially in cancer, suggests that nanotechnology could be the dawn of a new era in cancer treatment and imaging.

Introduction

Cancers are diseases originating from abnormal cell proliferation, which have the potential to spread to considerably remote regions of the body through lymphatic system, a phenomenon called "metastasis."[1] According to the World Health Organization (WHO) reports, 13% of all global death reports have been directly caused by cancer. Regretfully, due to the presence of hundred types of cancer or more, it is not very unlikely to reach a rate of 70%, causing about 13 million deaths by 2030.[2,3] Despite recent technological advances made in medicine, cancer research has not reached ultimate solution and the disease is still considered a major challenge for public health.[4]

Breast cancer is the one of the life-threatening malignancy and globally concerning health issue amongst women affecting millions worldwide.[2,5,6] After lung cancer, breast cancer has the highest death rates among women of 60 years old and above.[7,8] More than 1.1 million females are diagnosed with this malignancy around the world every year.[9] The role of breast cancer among all cancers and deaths caused by cancer has been estimated at 25% and 15%, respectively. The

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odds of developing a tumor are 10-12.8% throughout a woman's lifespan. Unfortunately, these values have had an increasing trend since 1990 which had 1.5% increase rate of breast cancer development per year.[7,10,11] Characteristics such as mutated genes (BRCA1, BRCA2 and p53), an abnormal endocrine system, metabolism and environmental agents; exhibit cancers specially breast cancer a unique life-threatening system.[9,12-15] Different receptors are expressed on breast cancer cells that distinguish them from one another, such as the progesterone receptor (PR), estrogen receptor (ER) and human epidermal growth factor receptor 2, also known as HER-2/neu receptor.[12] In test results, triple negative breast cancer (TNBC) indicates a deficient amount of the above three receptors on breast cells.[7]

Despite the fact that breast cancer is a heterogeneous disease, the therapeutic modalities are almost the same in many patients. Surgery is Principle treatment and could be used along with other therapeutic modalities. It includes mastectomy, defined as the removal of the whole breast, and lumpectomy in which part of the breast is removed.[8,10,13]

Radiotherapy with the use of intense radiation, chemotherapy by exploiting highly toxic drugs, endocrine therapy, also recognized as hormone therapy, to alter the cell cycle or immune system, immunotherapy, and finally combination therapy are listed as the most common therapeutic strategies implemented for breast cancer treatment.[7,10,13]

Although different technologies have been developed and several investigations have been carried out to help better comprehend cancer etiology and desirable treatment outcome[16], their limitations could not be overlooked. All the above methods, collectively known as conventional therapy, have their specific limitations that make them less efficient. The highly hydrophobic nature and lack of solubility make chemical drugs unstable with inadequate bioavailability.[10] Furthermore, their toxicity causes serious side effects such as hair loss, vomiting, nausea and diarrhea.[9,11,14,17] On top of all the major drawbacks of these methods is that they could not differentiate between normal and cancerous cells, resulting in nonspecific delivery of drugs. Therefore, there is a need for an appropriate therapeutic strategy to overcome these issues.

Nanotechnology is a field of science that uses material within the size of 1 to around 100 nm, providing new and unprecedented properties for materials not available in their bulk form. Nanoparticles could be used in many therapeutic methods not only to facilitate and improve these techniques, but also to overcome their limitations such as imaging and defense against cancer.[18] By combining nanotechnology and medicine, a redemptive science called "nanomedicine" was born. Nanotechnology could be employed as a new technology and indeed a new versatile instrument to vanquish the problematic drugs used for treatment of cancers such as breast cancer and other malignancies threatening the precious human lives. They could be applicable by enhancing the solubility and stability, reducing drug toxicity and more importantly, bringing targeted drug delivery strategies for better accumulation of drugs nanotechnology.[16,19,20]

The main goal of this study was to review the most contemporary drugs provided by nanomedicine used for breast cancer treatment (Figure 1), their process, and results.

Methods

The main portals used for this particular review were PubMed and Google Scholar. "Nanomaterials" and "breast cancer" were the main two keywords of the review article search within the last 5 years and it presented 90 results. After initial screening of titles and abstracts, 42 papers were selected. Due to references and journal eligibility investigation, it was limited to 20 papers. As this review focused on different nanomaterials, each particular nanomaterial was searched individually including "Breast cancer", "liposomes", "polymeric NPs", "dendrimers", "quantum dots", "virus like nanoparticles" and "magnetic NPs" main keywords and with the same evaluation methods, and finally 41 articles were reviewed.

Results

Liposomes

With their globular lipid bilayer made of a variety of phospholipids and cholesterols, they can encapsulate a large number of molecules such as drugs and biological agents.[20,21] Liposomes have several
features such as enzyme degradation immunity, high circulation time, weak immunogenicity, and high biocompatibility due to PEGylation both in vivo and in vitro.[22,23] They could be delivered to the tumor site either by taking advantage of enhanced permeability and retention (EPR effect) or by coating ligands on their surface to target overexpressed receptors by abnormal cells.[20,24]

Etoposide was encapsulated in liposomal NPs with 99.1± 2.8 % efficiency to investigate its cytotoxic effects on MCF-7 and T-47D cells. Both non-encapsulated drug and liposome encapsulated Etoposide showed cytotoxicity in a concentration dependent manner. However, MTT assay showed that the drug loaded into liposomes have displayed higher cytotoxic efficiency than the free drug itself in vitro (Figure 2a).[25]

A study of the effect of pH-responsive liposomes encapsulated with Cisplatin on MDA-MB-231 and MDA-MB-468 metastatic breast cancer cell lines was carried out and the results were compared with non-

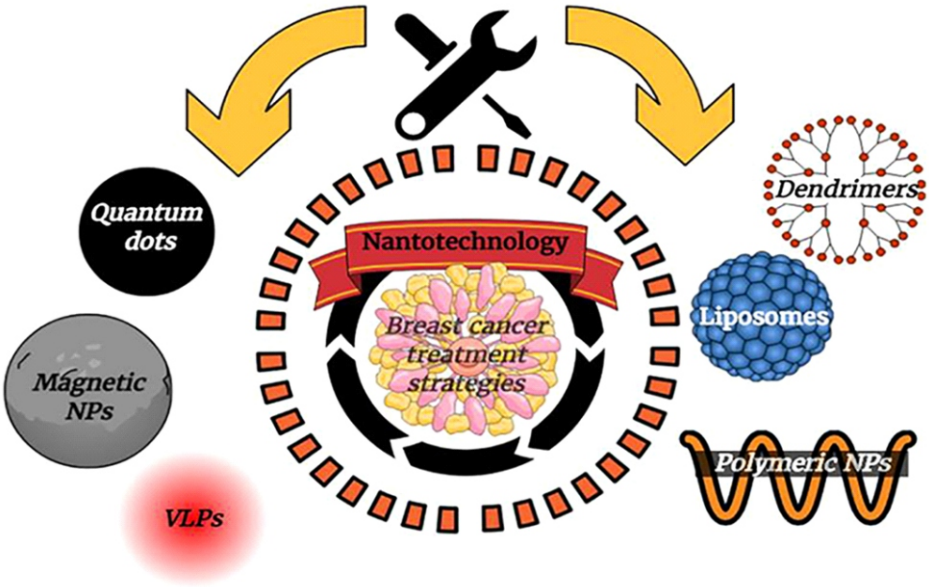

Figure 1. Nanomaterials discussed in this study

sensitive liposomal Cisplatin and also free Cisplatin in different pH ranges. By altering pH from a normal range of 7.4 to 6.5 and 6, although the retention rate of pH sensitive liposomes in both cell lines reduced and the drug release ratio increased, a weak cellular uptake was observed. However, pH appeared to be insignificantly effective for non- pH-responsive liposomes and its releasing efficiency. An exclusive increase in the incubation time (24 hours of incubation) resulted in stimulation of the releasing rate in non-pH-responsive liposomes. Free Cisplatin also showed no dependency on pH (Figure 2b).[26] The use of liposomes as carriers for immunological agents has its own advantages. In this particular research, DOX (Doxorubicin) was loaded into different NPs coated with CD44, αIL-6R Ab-PE (PE conjugated antibody) and αIL-6R Ab-PE-CD44. Referring to the sensitivity of liposomes to pH, releasing potency of encapsulated drugs and agents were increased by reducing pH levels. Active targeted delivery of liposomal Dox and anti-IL6R Ab-PE with CD44 was applied to mice with 4T1 triple negative metastatic breast cancer cells and showed more than 6 –fold anti-IL6R and 4-fold Dox cellular accumulation than a non-targeted liposome that was used as a control. The use of discussed nanoparticles in MMTV-PyMT mice by targeting strategy of anti-IL6R Ab-PE showed enhanced accumulation in the tumor site that was about 11 times larger than the control cell line; therefore, the accumulation of liposomal drug in unnecessary organs such as the liver, lungs, spleen, kidneys, and intestines reduced significantly. In addition, this method was also used with CD44 and showed more promising results than free drugs. Furthermore, all experiments carried out in this study showed significant antitumor results in vivo.[27]

As liposomes are very modifiable, they have been moderated by PEGylation using hyaluronic acid (HA) for delivering GGCT (γ-glutamylcyclotrasferase) Si-RNA to drug resistance MCF7 breast cancer cell lines. In vitro studies have demonstrated cellular uptake enhancement by assessing with Cy5-labeled siRNA. Moreover, gene silencing effect was detected by western blotting assay which gives strong evidence of desirable cellular uptake and internalization of synthetized liposomes (G-PEG-HA-NP). MTT assay approved the cytotoxic effect of G-PEG-HA-NP with increasing the siRNA concentration to 100 or 200 nM in vitro by decreasing cell viability. Also, FITC-Annexin V/PI showed an increased ratio of apoptosis and necrosis of G-PEG-HA in comparison to control formation. The western blotting technique revealed downregulation of GGCT in tumors treated with G-PEG-HA-NP compared to other control structures in vivo.[28]

Another study investigated the use of liposomes as a multidrug targeted delivery system. The liposomes were synthetized at a size of 140-160 nm. However, coating mAbs and loading drugs may cause size increase. According to measurements, Doxorubicin (DOX) and Bevacizumab (Avastin) were encapsulated to liposomes with 80% and 37% encapsulation efficiencies respectively. Although the release rate was high in the first 24 hours, it has achieved a steady release rate through the next 48 hours. In vitro cellular uptake analysis of immunoliposomal DOX in BT474/MDR showed targeted delivery and internalization, while free DOX used as control was not able to pass the cell drug resistance barriers. Moreover, in vivo studies started when the tumor size was 100 mm3 and the effects of different forms of encapsulated drug (Free DOX, liposomal DOX, immunoliposomal DOX, liposomal bevacizumab and immunoliposomal DOX+ liposomal bevacizumab)

were investigated during 60 days in BT474/multidrug resistance bearing nude mice in which a combination of immunoliposomal DOX and liposomal bevacizumab had the highest tumor growth inhibitory strength.[29]

In one study, liposomes with a mean size of 101.50±0.44 nm were synthesized and Epirubicin and Quinine were loaded onto them with 95.0±1.3% and 94.5±1.3% of encapsulation efficiency and 1.12± 0.16% and 1.51± 0.19% of releasing flux, respectively. Free drugs at different dosages were supplemented to MCF-7 cells which were sensitive to Epirubicin and the results were compared to their liposomal forms, indicating a lower survival rate of cancerous cells. As for the MCF-7/ADR cells, free Epirubicin presented no sensitivity while a combination of free Epirubicin and quinine showed cytotoxic effects. However, liposomal drugs had less cytotoxic effects on normal cells. Further details are shown in Figure 2c. Confocal laser scanning microscopy approved accumulation of functional Epirubicin liposomes, specifically in the mitochondria, to induce apoptosis, slow release, and internalization of the drug into the cells. Confocal laser scanning microscopy approved accumulation of functional Epirubicin liposomes, specifically in the mitochondria, to induce apoptosis, slow release, and internalization of the drug into the cells. In vivo imaging findings in MCF-7/ADR cells in nude mice also explained localization of functional liposomes at the tumor site and promoted drug retention time as it was observed.[30]

In table 1, the most ultimate drugs loaded to liposomal NPs for breast cancer therapy has been indicated.

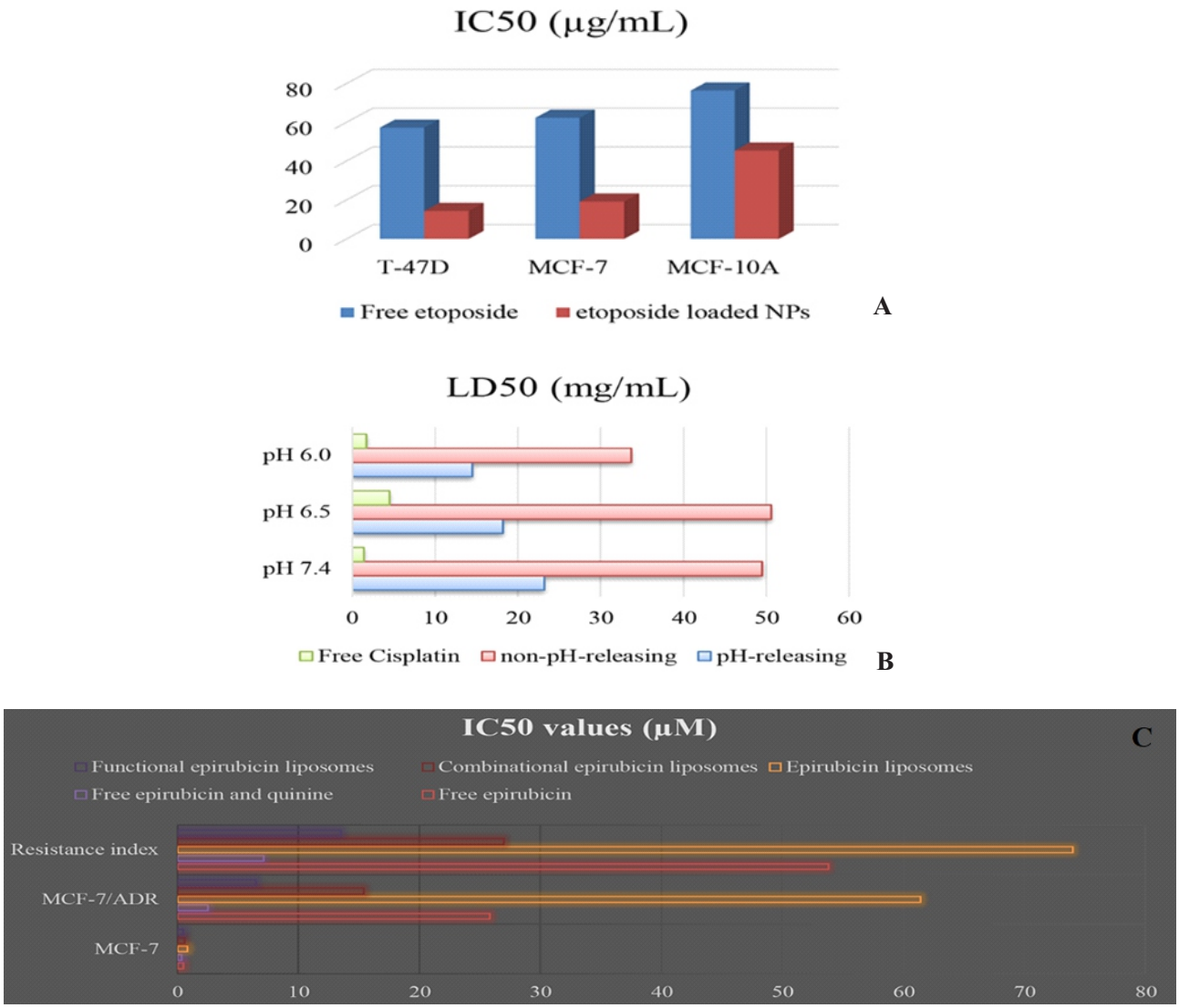

Figure 2. Calculated IC50 and LD50 of liposomal NPs in different cell lines and pH ranges. **A)** IC50 (µg/mL) of etoposide liposomal NPs was compared with that of free etoposide in T-47D, MCF-7 and MCF-10A cells. **B**) LD50 (mg/mL) values were compared within free cisplatin, non-pH-releasing and pH-releasing liposomes in pH 6.0, 6.5 and 7.4. **C)** Different forms of Epirubicin, quinine and their resistance index IC50 were assessed in MCF-7/ADR and MCF-7 cells.

Table 1. Most recent liposomal nanomedicines used in breast cancer and their characteristics

Therapy approach	Inclusive name	Nanomaterial	Loaded drug	Cancer type	condition
Non-targeted chemotherapy[29]	Liposomal DOX (Myocet)	Liposome	Doxorubicin	Metastatic breast cancer	Canada and Europe approved
Non-targeted chemotherapy[30-33]	Liposomal paclitaxel (EndoTAG-1)	Liposome	Paclitaxel	Pancreatic, Liver metastases and HER2-negative and triple negative breast cancer	Phase II
Targeted chemotherapy[34]	MM-302	HER-2 targeting liposome	Doxorubicin	HER2-positive breast cancer	Phase II/III
Immunotherapy[35]	Dher2+AS15	Liposome	Recombinant HER2(d- HER2) antigen and As15 adjuvant	Metastatic breast cancer	Phase I/II

Polymeric-based nano structures

ISL-loaded hybrid NPs composed of a polymeric PLGA core coated by a layer of lipids and PEG were prepared, and iRGD peptides were modified on the surface of NPs. ISL-iRGD NPs with an average size of 137.2±2.6 nm and zeta potential of - 34.21±1.23 mV were used to deliver loaded Isoliquiritigenin (ISL) to breast cancer cells. The nano formed drug showed more anticancer effects on MCF-7, MDA-MB231 and 4T1 cells than its unstrained form. In addition, drug loaded NPs presented 40% higher apoptotic effects in vitro. Better internalization of drug loaded NPs due to their smaller size (137.2 nm) and both passive and active targeting systems was confirmed in MDA-MB-231. In vivo discoveries in bearing nude-mouse 4T1 cells exhibited tumor shrinkage (474 mm3), augmented mitotic body, decreased effective dosage and eventually no toxicity in none targeted organs such as the lungs, liver, and kidneys.[38]

PLGA as a core, lectin as a shell, and polyethylene glycol (PEG) as a modifier were used to manufacture hybrid NPs to deliver Salinomycin (Sali) to breast cancer cells with 55% and >8% encapsulation and loading efficiency, respectively. Flow cytometry results showed that CFPE-Sali-NPs-HER2 had the highest accumulation in MDA-MB-361 ADH+, ADH-, and BT-474 cells. It also showed more drug release (80%) than the free drug and NPs without HER2 targeting system. CCK-8 assay confirmed that Salinomycin loaded to NPs and coated with HER2 had the highest anti proliferative efficiency among other forms of the drug (Figure 3). Tumorsphere studies revealed that Sali-NP-HER2 caused a significant decrease in MDA-MB-361 and BT-474 tumorsphere quantities. Furthermore, in vivo investigations demonstrated a 79% decrease in the tumor volume, lessen in tumor mass and therefore reduction in breast cancer stem cells.[38]

DOX (D) and redox sensitive indocyanine green (ICG or I) with strengths of 98.54±0.2% and 96.54±0.03% were loaded onto polycaprolactone (PCL)-poly ethylene glycol (PEG) NPs with folate (FA) on the surface (159.93±8.08 nm). Enhanced thermal responses at 43°C and drug release of I-NPs and FA-DINPs were confirmed by infrared thermal imaging camera and TEM. Glutathione and laser irradiation were used to reach 82.2% release in 24h. Moreover, NPs were taken by cells with receptor-mediated endocytosis (RME) and promoted uptake of FA-DINPs was observed by laser irradiation. Interestingly, FA-DINPs neutralized 75.86% of EMT-6 cells at a concentration of 20µg/ml in comparison to non-toxic black NPs. NIR imaging confirmed the highest accumulation and intercellular retention of FA-DINPs. Furthermore, drug accrual in unassociated organs such as the kidneys, lungs, spleen and liver was scarcely observed in vivo.[39]

Table 2 presents the most recent strategies being carried out for breast cancer therapy using polymeric Nps.

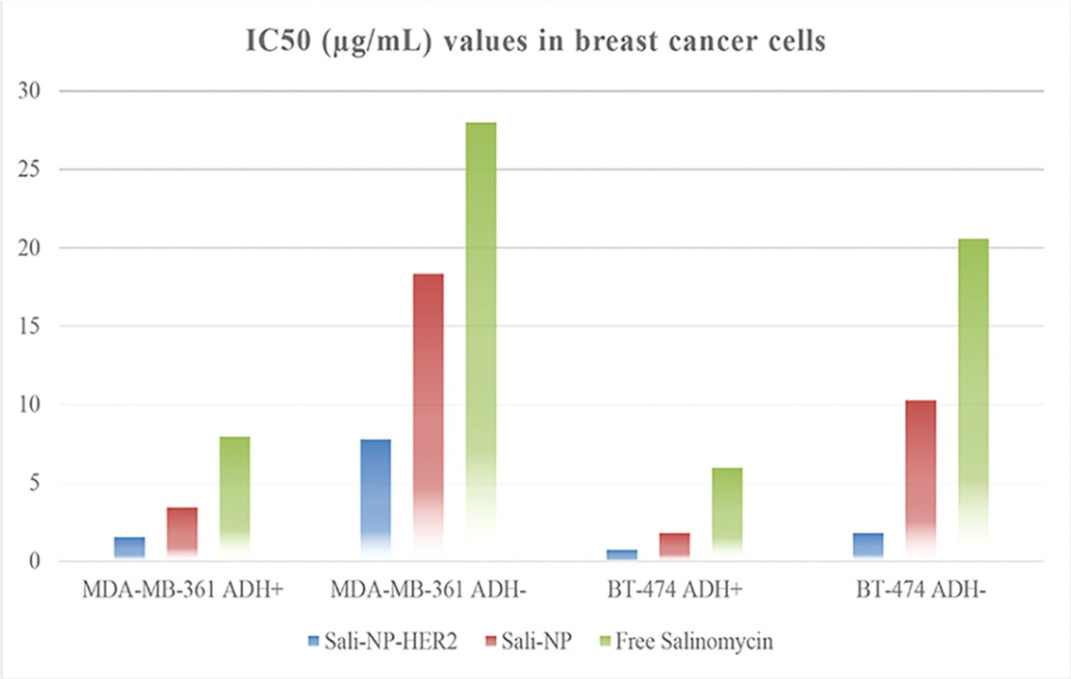

Figure 3. Polymeric nanoparticles in different forms such as free Salinomycin, Salinomycin loaded NPs-HER2 receptor and none targeted Sali-NPs IC50 (µg/mL) in MDA-MB-361 and BT-474 with negative and positive ADH.

VLPs (Virus like Particles)

Nanoparticles were derived from Nicotiana glutinosa plants, identified as PVX NPs. Herceptin (HER) was coated on NPs as an active targeting agent and receptor blocker, approved by western blot and ELISA sandwich technique.In cytotoxicity studies of NPs on SK-OV-3 and SK-BR-3 cell lines,Herceptin linked Nps showed more promising outcomes than the free form of Herceptin (Figure 4).

In constitution of VLPs, potato virus X (PVX) from N. benthamiana plants was used and DOX was selected as the cargo for delivery in breast cancer cases. Neutralizing activity studies in MDA-MB-231 cells showed elevated IC50 values for DOX-PVX (0.94µM) compared to free DOX (0.13µM).

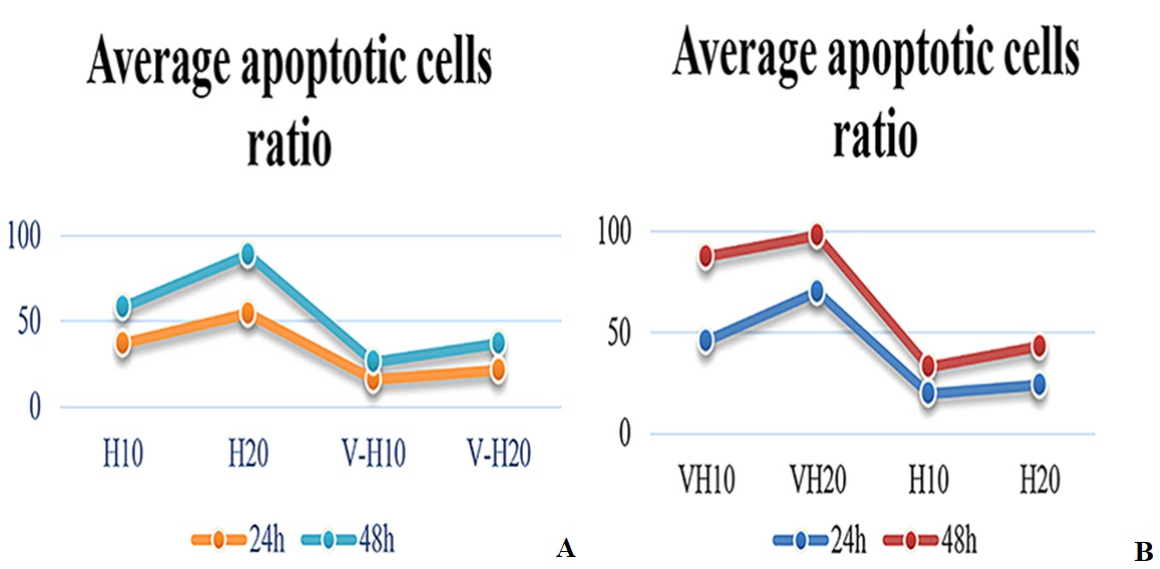

Figure 4. Effects of VLPs conjugated with Herceptin (HER) and free HER at concentrations of 10 and 20 µg on SKOV-3 and SKBR-3 cell lines **A)** apoptotic values in SK-OV-3 cell line in 10 µg and 20 µg of free Herceptin and virus coated Herceptin and **B)** the same experiment in SK-BR-3 cell line.

Table 2. Polymeric nanomedicines and their characteristics

Therapy strategy	Inclusive name	Nanomaterial	Loaded drug	Cancer type	condition
Non-targeted chemotherapy[29]	Polymeric micelle paclitaxel (Genexol-PM)	Polymeric micelle	paclitaxel	Breast cancer and NSCLC	Korea approved
Non-targeted chemotherapy[39]	NK-105	Polymeric micelle	Paclitaxel	Metastatic or recurrent breast cancer	Phase III
Combination of chemotherapy and anti-drug resistance[40]	-	Polymeric micelles or Nps	DOX and Disulfiram	Drug resistance breast cancer	Unknown
Combination of chemotherapy and RNAi therapy (targeting SNAIL and TWIST)[41]	-	Polymeric Nps	Paclitaxel and siRNAs	Breast cancer	Unknown
Combination of chemotherapy and gene therapy using IL-12 encoded plasmid[42]	-	Polymeric Nps	Paclitaxel and DNA	Breast cancer	Unknown
Antisense therapies against miRNA miR-10b and miR-21[43-45]	-	Polymeric Nps	Antisense oligonucleotides	Triple negative breast cancer	Unknown

Although the DOX-PVX showed less toxicity, it resulted in more therapeutic activity and drug retention than free DOX.Thus, the NPs were PEGylated and evaluated in vivo. PEGylated Nps showed better biocompatibility, enhanced distribution and interestingly 1.2 times higher tumor shrinkage contrasted to free DOX.[47]

Another investigation carried out with PVX-HER and free HER by ELISA, western blot and RT-PCR resulted no pathogenicity of fabricated NPs.[48]

As PVX has gained enormous attention in breast cancer treatment, it was conjugated with HER to investigate its cytotoxicity influences on various cell lines. SKBR3, SKOV3, MCF-7, MDA-MB-23 and MCF-12A were treated with 10 and 20 µg of PVX-HER and free HER.

After 24 hours, no significant toxicity was seen in MCF-7, MDA-MB231 and MCF-12A but cell viability reduced when SKBR3 and SKOV3 were treated with PVX-HER and Free-HER at both 10 and 20 µg dosages.

As equally consequential, fluorescence microscopy findings explained higher values of nuclei accumulation in cells treated by PVX-HER compared to free-HER.[49]

Dendrimers

As for delivering DOX and [(Cyclo) (D-Tyr-Arg-Arg-L-3-(2-naphthyl) alanine-Gly (FC13)] as CXCR4 antagonist to breast cancer cells, dendrimers (D) were used to convey the agents. Linear FC131-DOX-D4 (LFC131-DOX-D4) and DOX-D4 also presented 97.25%±0.04% and 92.37%±1.03% encapsulation efficiency and the agents were loaded with a strength of 57.96% on average. Furthermore, drug release measurements resulted in higher tax at a pH 5.5 in comparison with a normal pH range in vitro. BT-549 and T47D cells were selected for this study. Fluorescent microscopy confirmed that LFC131-DOX-D4 (4th grade dendrimers) were taken by cells with a greater internalization in comparison to the none antagonist supplemented drug (Figure 5).[50]

Toxicity assessments on both cell lines explained that LFC131-DOX-D4 have a greater killing efficiency comparing to DOX-D4, LFC131-D4, D4 and free DOX itself. LFC131-DOX-D4 presented IC50 of 25.2 and 124.4 µg/ml in both BT-549 and T47D cells after 120h in respect. LFC131-D4 showed the highest value of the migration inhibition index at 0.5mg/ml in both cell lines.[50]

Polyamidoamine (PAMAM) with unparalleled molecular uniformity was used to form dendrimers

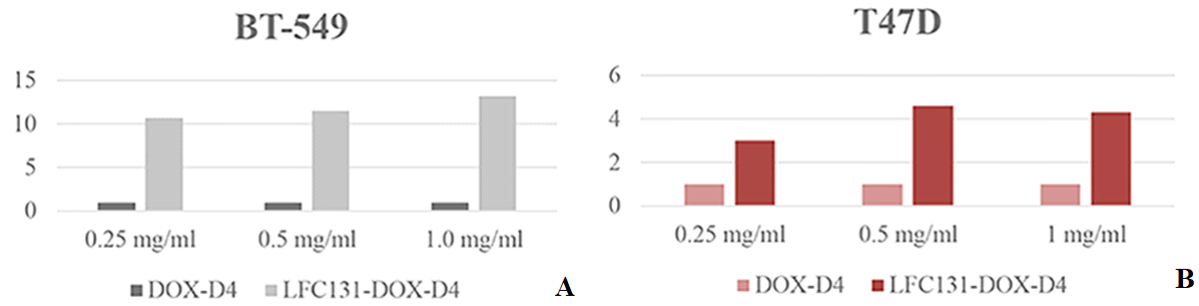

Figure 5. Impact of dendrimers on cellular uptake when loaded with DOX, LFC131and their combination **A)** and **B)** cellular uptake intensity of DOX-D4 and LFC131-DOX-D4 intensity in BT-549 and T47D cells.

(D) at a size of 31.6±2.1nm. Maleimide PEG NHS (NHS-PEG-MAL) was conjugated to dendrimers for stability and biocompatibility improvement. In addition, Trastuzumab (TZ), also known as Herceptin, was grafted to the structure for better active targeting of 216.4±2.79 µg/ml loaded Docetaxel (DTX). Eventually, 71.84% and 93.5% drug release rate in 24 and 48 hours confirmed continuous release of drug. Hemolysis activity was assessed and resulted in 1.5% of TZ-D hemolytic cytotoxicity in comparison with dendrimer alone. Toxicity estimations showed 36.2% and 60.9% cell viability in TZ-D-DTX treated MDA-MB-453 and MDA-MB-231 cells, respectively. Figure 6 presents IC50 values of D-DTX and TZ-D-DTX.[51]

Cellular uptake inspection by FITC demonstrated more TZ-D-DTX (23.5%) cellular uptake than DTX-D (11.4%) after 1h. As the time passed, it increased to 57.9% and 34.2% in MDA-MB-453 cells, respectively. There was no consequential distinction between D-DTX and TZ-D-DTX in MDA-MB-231 cells. Competition assay disclosed more efficient uptake of TZ-D-DTX in MDA-MB453 cells. In apoptotic efficacy evaluation with acridine orange and ethidium bromide, the lowest cell viability was seen in MDA-MB-453 cells treated by TZ-D-DTX. In addition, Annexin V FTIC/PI assay in an similar cell line showed 54.35% cell viability, which was the lowest rate among control and other forms.[51]

In another case of using PAMAM dendrimers (D) for drug delivery, Pluronic F68 (PF68) was conjugated to the fabricated structure to reduce the hemolytic effect of the dendrimer. Moreover, cytotoxicity reports elucidated that DOX loaded to D-PF68 diminished the tumor spheroid volume, its protein content and cell viability in HEK293 and MCF-7/ADR cells. DOX was encapsulated to PAMAM-n2 PF68 (second-degree conjugation) with an efficiency of 60.6% DOX per macromolecule. In addition, drug release was sensitive to pH and the highest releasing rate was observed at pH 5.5. Furthermore, Annexin V-FITC/PI and Hoechst 33342 stain confirmed DOX loaded grafted PAMAM, especially the second grade, had the highest apoptotic and necrotic activity (31.0±13.5%). In vivo studies of the distribution using ICG revealed that drug loaded grafted dendrimers were accumulated desirably in the tumor site with markedly reduced cardio cytotoxicity. In vivo tumor inhibition test by histological and TUNEL assay showed tumor volume and density shrinkage of MCF-7/adr after treating with DOX-D-PF68.[52]

Quantum Dots

Hybrid NPs were assembled by combining quantum dots with liposomes to produce quantum dots liposomes (QLs). Subsequently, the structure was loaded with siRNA and anti-EFGR (Cetuximab). Two times of PEGylation (for QDs and receptor binding) were coated on the surface, resulting in a size of 175.5±9.0 nm and potential of -1.9±0.7 mV. The mean fluorescent intensity showed that Cetuximab coated QLs (called immuno QLs) had a promising targeting efficacy in MDA-MB-453 and MDA-MB-231 cells. Furthermore, confocal microscopy revealed the great strength of immuno-QLs in QD delivery in the same cell lines. Clathrin assistance, receptor mediated endocytosis,, and endosomal escape of siRNA were confirmed as cellular uptake activities during 7 hours of observation. Inhibition of protein kinase C (PKC), cell migration, tumor growth, and induction of cell apoptosis and autophagy were observed when siRNA was delivered.[53]

In spite of valuable in vitro findings, encouraging in vivo results are still needed. Hence, MDA-MB-231 xenograft mice were selected. Subsequently, upon intravenous (IV) injection of immuno QLs, even though a large accumulation of NPs was seen at the tumor site, NPs were still observed in the liver and lungs; however, as the time passed, the density of NPs in non-targeted organs began to decrease. Anti-tumor assessments of immuno-QLs showed 44.89±2.87% apoptotic cells. In addition, pathological and histological investigations revealed no specific toxicity and non-targeting accumulation in other organs.[53]

Graphene, rhodamine, β-Cylodextrin (β-CD) as a

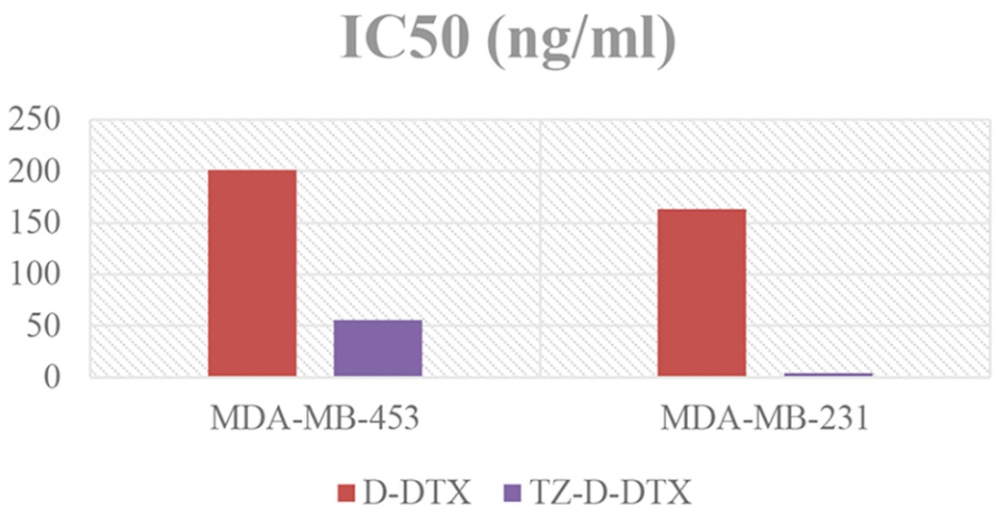

Figure 6. IIC50 value of Trastuzumab grafted DTX-Dendrimers and its none grafted form in two different cell lines.

modifier, HER2 as a targeting ligand, and DOX as the therapeutic agent formed graphene quantum dots (GQDs) with a size of 222nm.. CCK-8 assay in MCF-7 and BT-474 cells treated with prepared GQDs resulted in a high cell viability, which explains the great targeting efficacy in HER2+ breast cancer. Drug release in this particular system was designed to be temperature and pH sensitive. DOX releasing ratio in 37 °C and at 5.5 pH was 60% which was the highest value compared to other temperatures and pHs. Confocal laser scanning microscopy using quantum dots light-emitting properties and fluorescence confirmed desirable cellular uptake and internalization of DOX loaded GQDs in BT474 cells. Finally, apoptosis assessments in vitro showed that DOX loaded GQDs could decrease cell viability to no more than 30% compared to unexposed GQDs.[54]

Celecoxib (CXB) and Hydroxynorketamine (HNK) loaded, Lactoferrin (LF) coated Nanocapsules (NCs) were congregated with cadmium telluride (CdTe) derived mercaptopropionic acid (MPAs) modified QDs to form a theranostic system. Promoted cytotoxicity in MCF-7 and MDA-MB-231 and reduced IC50 levels (20.04 and 28.16 µg/mL respectively) were also detected in comparison to free drug, blank NPs, CS-NCs and LF-CS-NCs. Moreover, more extravagant cellular uptake compared to free QDs was observed in the MCF-7 cell line after 24h.Interestingly, size shrinkage and protein corona were observed as in vitro findings. In vivo studies have shed light on LF-QDs-CS-NCs cyclooxygenase-2 (COX-2) inhibitory activity, antiangiogenic and apoptotic inducer activity, and protein kinase B (p-AKT) reduction. Moreover, no immunogenicity and unassociated drug accumulation in organs like the kidneys and liver were detected in treated mice.[55]

Magnetic nanoparticles

Chitosan as a modification for super paramagnetic material has fabricated magnetic nanoparticle (MNP) with special characteristic such as mesoporous structure. As a drug delivery system, DOX was loaded onto chitosan coated mesoporous magnetic nanoparticles (CMMNs) with about 19% strength and more than 90% entrapment effect. The final size of the DOX-CMMN was about 120nm using SEM imaging. In vitro studies confirmed pH sensitivity of CMMNs. In addition, enhanced rate of drug release were observed (~55%) at 5.5 pH in 24h and after 48h nearly 100% of the drug were released.

By contrast, in a normal pH range, only about 35% and 60% release was reported after 24 and 48h, respectively. Furthermore, the results of MFC-7 cells treated with NPs showed that CMMNs loaded with DOX had the lowest cell viability at 1.25 µg/mL in comparison with free DOX. The cell death rates increased markedly from 40% to 90% when applied alternating current magnetic field (ACMF) was implemented.[56]

In one study, super paramagnetic iron oxide NPs, also known as SPIONs, were characterized and modified with a 12.5 nm layer of PEG for biocompatibility achievement. Paclitaxel (PTX) was loaded to SPIONs as a therapeutic agent. Assessments on MCF-7 cells has indicated that SPIONs loaded with PTX with hyperthermia ability cause a viability downgrade in both wild type (WT) and Taxol-resistant (TR) cells. Moreover, PTX loaded to SPIONs with a hyperthermia ability reduced the viability of both cell types (WT and TR) about 10% at 25nM of concentration. Elevated amounts of apoptotic and micro-nucleated cell were observed when WT and RT MCF-7 cells were treated with PTX-SPIONs-HT, which explains its apoptotic efficacy.[57]

In one study, 200 ng magnetic NPs and Lipofectamine as a lipid-based transfection agent were selected to induce CD95 (Fas), c-FLIP and procaspase-8 expression in MCF-7 breast cancer cells. Presence of Fas in transfected cells were confirmed

with fluorescence microscopy due to simultaneous expression of Fas and green fluorescence protein (GFP).Meanwhile, apoptosis assessments demonstrated that NPs loaded with gene and grafted with FasL (Fas ligand) could cause apoptosis to more than 50% of cells within 24h.[58]

Discussion

In this review, we explored the most recent and most commonly used nanomaterials in breast cancer treatment. It was found that nanomaterials used in medicine, especially in breast cancer, could enhance therapeutic drugs efficacies, promote circulation time in the body, enhance the drug retention time, enhance their solubility and hydrophilicity, reduce the product price, and most importantly, prevent toxicity in healthy cells by their active and passive targeting systems. Therefore, it can be concluded that breast cancer cells treated with drug loaded NPs have less cell viability, decreased tumor size, and increased drug accumulation in the tumor site compared to treatment with free drugs.

According to different studies, liposomes with their specific structure could be loaded by various numbers of drugs; however, hybrid nanoparticles such as quantum dots liposomes, magnetic polymeric NPs, and PLGA-Lectin NPs have a higher circulation time. Averagely in MCF-7 cells treatment, liposomes presented 9.855 µg/ml of IC50 while with polymeric NPs an 8 nM of IC50 could be observed. It could be comprehended that in this particular study loading drugs to polymeric NPs causes more influence on MCF-7 cells than liposomes.

In the assessment for MDA-MB-231 cells drug loaded to dendrimers dispensed significantly lower IC50 value than VLPs and quantum dots. Therefore, it explains drug loaded dendrimers more excessive therapeutic action. Nevertheless, this information is limited to this particular review and results of further investigations could be slightly different. Despite all advantages, there are some limitations for this new method. Cationic liposomes could show toxicity and induce mononuclear phagocyte system (MNP), deterioration in polymeric NPs, toxicity of quantum dots[14] and more importantly, establishment of a bimolecular layer on NPs recognized as protein corona that could alter NPs properties and interrupts drug delivery system.

For instance, studies on mesoporous silica NPs (MSN) and gold NPs presented sever protein absorption intensity which leads to protein corona development on the surface of the NPs.[59,60] Furthermore, some NPs such as TiO2, Au, Ag and SiO2 induce endothelial leakiness in the tumor site.[61] As nanotechnology is a new field of science being combined with medicine, further research is firmly required. However, there are many drug loaded nanomaterials in the process of earning approval from drug associations and it is likely to expect more development from nanomedicine science.

Conflict of Interest

None.

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