Multi-Functionalized Nanocarriers for Breast Cancer Therapy

Subjects: Nanoscience & Nanotechnology

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Breast cancer (BC) is one of the most prevalent cancers in women. This type of malignancy often starts from ductal hyper-proliferation with its expansion into benign tumors or metastatic carcinomas resulting from exposure to carcinogenic agents. This disease exhibits not only a great deal of heterogeneity but also a great deal of variation in its occurrence, treatment response, progression, and even location of metastasis. Multifunctional nanocarriers include polymeric nanoparticles (NPs), self-nanoemulsifying drug delivery systems (SNEDDS), liposomes, and mesoporous inorganic NPs.

Keywords: breast cancer ; nanoparticles ; nanotechnology

1. Ligand-Based Core–Shell NPs

Cancers of the breast are the world's deadliest diseases, with a higher death rate among women. BC treatment is often associated with using oral chemotherapeutics in terms of radiation therapy, chemotherapy, hormonal therapy, and multi-functionalized ligand-mediated therapy [1]. Additionally, physical barriers to drug diffusion and insufficient delivery of drug concentration to the tumor are also major challenges [2]. The heterogeneity of tumor mass allows these approaches to transform, and it is necessary to target tumors more selectively [3]. In this connection, Dávid Kovács and co-workers also described the fact in detail that metallic-based core–shell NPs endorsed the efficacy in treatment against breast tumor metastasis. Therefore, they developed hybrid metallic NPs by adjunction of gold and silver NPs in the form of core–shell nanostructures. Hybrid NPs were synthesized by chemical reduction via using sodium borohydride. The results showed that targeted delivery of hybrid metallic core–shell NPs in the TME leads to the weakening of progressive tumor behavior of cancer-associated fibroblasts (CAFs) [4]. Darfarin and co-workers developed gold-silicon oxide shell-core NPs by conjugating gold NPs with silica NPs via amination and thiol-functionalization. The study concluded that gold-silicon core–shell NPs impart great efficacy towards MCF-7 BC cells via mega-voltage irradiation.

For the development of NPs, Zhang and associates [5] used electrostatic deposition and antisolvent precipitation. For the purification of Honokiol-free samples, centrifugation was performed. The synthesized nanostructures were analyzed via TEM, particle size determination, PDI and encapsulation efficiency, dissolution assay, as well as advanced cellular studies, in vitro Western blotting analysis, inhibitory effect, in vivo tissue distribution of NPs by living to image, in vivo therapeutic efficacy and toxicity studies. The results concluded that manufactured NPs resulted in 210 nm size, having a negative charge with improved anti-proliferative and pro-apoptotic changes against 4T1 cells. The mechanistic approach of HA-Zein-HNK was downregulation of the Vimentin expressions and upregulation of the E-cadherin expressions. In conclusion, it can be believed that HA-Zein NPs can serve as a promising approach in HNK delivery for metastatic BC therapy [5].

2. Dual pH-Responsive Polymeric NPs

L. Palanikumar and the research group developed biodegradable pH-responsive NPs conjugated with a polymeric system comprising poly(lactic-co-glycolic acid (PLGA). This polymeric system was then coated with bovine serum albumin (BSA). The important feature of this formulation was the inhibition of the macrophages, which causes the inhibition of recognition targets. Moreover, after the uptake of NPs, intracellular microenvironment conditions lead to degradation of NPs and proficient anti-cancer activity against BC cell lines [6].

Similarly, Zhihao Guo and co-workers developed a tailor-made 2,3-dimethyl maleic-anhydride-poly(ethylene glycol)-*ε*-poly-l-lysine-DOX/lapatinib polymeric nanoplatform for encapsulation of anti-cancer drug lapatinib for its conversion into switchable charge based dual pH-responsive NPs. Advanced physicochemical properties of novel NP conjugates lead to

stability in the circulation of physiological conditions. However, charge switching capability from negative to positive charge leads to high-level sensitivity in the slightly acidic TME, facilitating strong muco-penetration [7].

Liu and coworkers [8] developed dual pH-responsive multi-functionalized nanocarriers via combining immunotherapy and chemotherapy based on poly(L-histidine) and HA for co-loading R848 (immune-regulator) and DOX via different encapsulation modes. Therefore, HA was initially reacted with succinic dihydrazide (SDH) by adding EDC and NHS as reaction activators to synthesize HA-SDH conjugate. The conjugate mixture was then dialyzed against deionized water, followed by lyophilization. To obtain a red cotton wool-like product, DOX was further conjugated with HA-SDH via an acid-cleavable hydrazine bond. Furthermore, PHIS and R848 nano-cores were formed by the nanoprecipitation method to obtain a slightly milky solution. The unloaded R848 was removed via ultrafiltration. The synthesized nano-cores were analyzed and characterized via infrared (IR) spectroscopy, proton nuclear magnetic resonance (1H NMR), drug loading, dissolution mechanistic, evaluation of maturation of DC2.4 cells, assessment of activation of advanced BC cell lines by flow cytometry, cellular uptakes, and intracellular locations, cell viability assay, in vivo pharmacokinetics, as well as biodistribution studies. Results concluded that the above-discussed combination is a promising novel therapy for cancer.

Moreover, the ionization of PHIS in the TME converts its hydrophobicity into hydrophilicity, which is very helpful in exerting immune regulation. Hydrazone bond breakage at endosomal pH accelerated the release of DOX, exerting cytotoxic effects. HA-DOX was overexpressed in BC cells and resulted in the internalization and inhibition of cell growth. In summary, this multi-functionalized nano-core system could deliver precisely in the TME and BC cells to achieve synergistic effects for excellent tumor-targeting ability against BC [8].

3. Mesoporous Carbon NPs

Carbon-based NPs can be synthesized via different approaches based on their application [9]. Therefore, Fan and coworkers [10] developed Resveratrol (RES)-loaded mesoporous carbon NPs as a promising approach against metastatic BC. However, successfully synthesized mesoporous oxidized carbon NPs (oMCNs) using mild oxidation techniques enabled the RES to be loaded with high efficiency. Synthesized RES-oMCNs polymer composites were characterized based on particle size determination, poly dispersity index, zeta potential, FTIR, XRD, SEM, and TEM. Then, the formulation was characterized in terms of some parameters, including dissolution, solubility studies, cell culture, cellular uptake, in vitro cytotoxicity assay, as well as cell apoptosis studies, including flow cytometry analysis and Western blot assay. This study found that oMCNs had a size below 200 nm, excellent water dispersion, and good encapsulation. OMCNs exhibited biocompatibility and excellent cellular uptake because of the preferential uptake of NPs owing to increased solubility of oMCNs-RES compared to the pure RES. Sustained drug release referred to the drug release only at lysosomal pH to improve the targeting capability of the therapeutic moiety. Moreover, in vitro cytotoxicity and apoptosis analysis showed caspase-3 protein cleavage in TNBC cell lines, respectively, thus showing greater interest in inducing anti-cancer activity against various metastatic cancer cell lines [10].

Additionally, Abid Hussain and Shengrong Guo investigated a new controlled system to release drugs from mesoporous carbon NPs (MCNs). The surface of the NPs was decorated with natural sophorolipids (SLPD). The pores were developed on the MSNs for inducing photothermal activity and encapsulating a chemotherapeutic drug (DOX). This advanced nanoparticulate system acts as a checkpoint for trapping DOX inside the pores of mesoporous carbon NPs and triggering its release in the TME via NIR irradiation. As a result, they demonstrated that this system is highly effective against BC cell lines [11].

4. Self-Nano Emulsifying Drug Delivery System (SNEDDS)

In 2019, Batool and collaborators synthesized SNEDDS of tamoxifen (Tmx) for targeting BC [12]. Following the previous method, thiolated hyaluronic acid (THA) was prepared with a few modifications [13]. Furthermore, papain (Pap), HA, and lithocholic acid (LCA) conjugate was prepared via chemically grafting lithocholic acid on papain-modified THA via the amide bond formation [14]. However, the novel polymer was initially characterized based on swelling, disulfide bond formation, and conjugation. However, this polymer conjugate was further linked with Tmxto form Tmx-PAP-HA-ss-LCA SNEDDS. Other characterization techniques included FTIR, XRD, DSC, TGA, solubility studies, preliminary screening of surfactants, preliminary screening of co-surfactants, construction of pseudo ternary phase diagram, percentage transmittance, dispersibility test, saturation solubility, robustness to dilution, cloud point measurement, physicochemical tests, and drug content determination. However, ex vivo characterization includes mucoadhesion, permeation study and P-gp efflux pump analysis, biocompatibility studies, and histological analysis. In addition, stability was measured over a six-month period in order to evaluate any discrepancies. Furthermore, SNEDDS showed stabilized encapsulation of the polymer. In vitro dissolution directed around 85% drug release within 48 h. The permeation study showed enhanced

permeation of SNEDDS compared to standard drugs. Therefore, SNEDDS was muco-penetrating and showed anti-proliferative activity against BC cell lines [12].

Moreover, solid SNEDDS can improve patient compliance and tackle the limitations associated with liquid SNEDDS capsules. However, the selection of SNEDDS components is heavily influenced by their physicochemical features, drug solubility, and physiological fate.

5. Biodegradable Boron Nitride NPs

In 2019, Le and coworkers developed the advanced and on-demand technique of biodegradable boron nitride NPs (BNNPs) for specified targeting against negative BC cells via neutron capture therapy of boron [15]. For the NPs synthesis, researchers grounded melamine and boric acid (H_3BO_3) at a molar ratio of 1:6 to convert them into a powdered form. These precursors were then heated under airflow in the horizontal furnace. The resulting crude samples were then centrifuged to remove impurities, followed by scattering in the presence of 25 mL of H_2O_2 and then transferred to a Teflon-lined autoclave for thermal treatment at 120 °C for 24 h. Furthermore, the dispersions were cooled at room temperature and subsequently centrifuged at 4000 rpm for 10 min, followed by vacuum drying overnight, resulting in the synthesis of BNNPs. These NPs have been characterized via vitamin C triggered degradation, cellular uptake, PET imaging, biodistribution in tumor-bearing mice, degradation behavior in vitro, degradation behavior in vivo, tumor-bearing animal models, radiolabeling, in vivo Boron Neutron Capture Therapy (BNCT), and histological studies. Results concluded that in PET imaging, the coated BNNPs exhibited high tumor boron accumulation while maintaining an excellent tumor to non-tumor ratio, and BNNPs were found to be rapidly cleared from major organs according to ex vivo ICP-OES analysis. Furthermore, when compared with the control group, animals treated with BNNPs showed tumor growth suppression with negligible side effects. This novel strategy not only employed the high boron content of BNNPs but also efficaciously completed an on-demand degradation of BNNPs to avoid the potential toxicity caused by the long-term accumulation of NPs [15].

6. Polymeric Nanogels

Overexpression of estrogen hormone is linked to BC, and anti-estrogen therapies involving tamoxifen are the most commonly prescribed methods of treatment for it [16][17][18]. Nevertheless, breast tumors have been associated with resistance and non-targeted therapies, resulting in metastasis. However, using polyoxometalates (POMs) can provide a very effective treatment method for this condition [19]. POMs are discrete anionic metal-oxo clusters formed with early transition metals in the highest oxidation states, and over the past decades, POMs can be recognized for treating different types of cancers and infectious diseases [20]. In this regard, Pérez-Álvarez and coworkers [21] documented that low molecular weight chitosan was cross-linked in the inverse phase microemulsion medium forming nanogels. Characterization was performed via ^{1}H-NMR and ^{31}P-NMR spectroscopy, DLS, zeta potential, TEM, and atomic emission spectroscopy. Based on the findings of this research, synthesized nanocarriers are potentially useful in future treatments for BC [21]. Yi Zhang and co-workers developed dual-sensitive nanogels by inducing redox reactions. Dual sensitive nanogels were manufactured with the capability of rapidly dislodging the anti-cancer drug (DOX) for dual sensitization. These studies led to the development of drugs with extended-release times and rapid onset of action [22].

7. Ultrasound-Triggered Liposomes

The functionalization of liposomes with monoclonal antibodies is a potential strategy for increasing the targeting capability of the HER2, overexpressed in HER2-positive BC cells [23][24][25]. Therefore, calcein and DOX-loaded immuno-liposomes were functionalized by Elamir and co-workers with the monoclonal antibody trastuzumab (TRA) [26]. Liposomes were characterized for their size, phospholipid content and antibody conjugation, estimation of phospholipid content, and other advanced imaging techniques. It was concluded that combining immuno-liposomes and LFUS is a hopeful technique for targeted drug delivery in reducing the cytotoxicity of antineoplastic drugs [26].

Anticancer medications can be delivered to the body via liposomes to either lessen the harmful effects of the drugs when administered on their own or lengthen the circulation time and effectiveness of the treatments [27]. According to the evidence given below, it is possible to direct aqueous contrast-enhancing chemicals that are loaded in liposomal carriers to the breast tissue and then use computed tomography to differentiate between normal and tumorous breast tissue. In this regard, Yonghong Song and co-workers developed emodin-based liposomal NPs. As it is reported in the literature that emodin possesses strong anti-cancer activity, but its efficacy is somehow compromised owing to the poor solubility and non-targeted delivery. Therefore, the specificity of the emodin was improved by incorporating it into the liposome's lipid bilayer. Furthermore, the hydrophobic layer of the liposomes was concurrently loaded with high-performance

ferromagnetic iron oxide nanocubes. Results concluded that magnetic field targeting leads to the efficacious and sensitive targeted killing of BC cell lines [28]. Yuko Okamoto et al. developed paclitaxel-based liposomes for the reason that paclitaxel is an insoluble anti-cancer drug and has issues of low solubilization and poor pharmacokinetics. Therefore, a liposomal formulation of paclitaxel within its aqueous core without adding organic solvents finally showed potent activity against BC cell lines [29]. Similarly, Snehal K. Shukla and her research team developed metformin hydrochloride (Met) encapsulated within liposomal vesicles via thin-film hydration technique by active and passive loading of drug-loaded lipid film. The metformin liposomal formulation leads to advanced therapeutic efficacy, i.e., an increased therapeutic outcome at the minimized dosage and profound apoptosis-induced activity in BC cells [30].

Various preclinical studies regarding NPs against BC involve methotrexate and beta carotene conjugated lipid polymer hybrid NPs for inducing BC by A Jain and co-workers [31]. Moreover, zein NPs coated with beta carotene can improve the anti-cancer activity and abolish the toxicity of methotrexate by the same group [32]. M. Khoobchandani's research group utilized a new approach for targeting BC via green nanotechnology by conjugating NPs with the ayurvedic system for pilot human clinical investigations [33]. The advantages and disadvantages of different strategies against BC are shown in **Table 1**.

Table 1. Advantages and disadvantages of different strategies against BC.

Nanocarrier	Advantages	Disadvantages
Ligand-based core–shell NPs	**A promising approach in honokiol delivery for metastatic BC therapy**	**Low mechanical resistance**
Dual pH-responsive multifunctional NPs	**Dual pH-responsive multifunctional NPs for synergistic therapy against BC.**	**Economical burden**
Mesoporous carbon NPs	**MCNs can be an encouraging approach against metastatic TNBC.**	**Anaphylactic reactions**
HA-based SNEDDS	**SNEDDS formulation was muco-penetrative as well as anti-proliferative towards BC cell lines.**	**Stability issues**
Biodegradable Boron Nitride NPs	**On-demand technique for NPs can be specified for targeting TNBCs through neutron capture therapy of boron**	**Non-broad-spectrum activity**
Nanogels	**Nanogels were carriers for the polyoxometalates against metastatic BC**	**Less encapsulation**
Liposomes	**Favorable for targeted drug delivery in reducing the cytotoxicity of antineoplastic drugs.**	**Costly, Difficult industrial scaling**

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