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PEG-PLA Nanoformulation for Breast Cancer Therapy

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Worldwide, breast cancer is the second most common type of cancer among women next to lung cancer. Recurrence is seen in 30% patients treated in their early stages of breast cancer due to therapeutic inefficiency. Breast cancer accounts for 25% of the women cancer patients. The major drawback of drugs are their side effects due to systemic delivery and non-targeted drug uptake. Nano formulations based drug delivery systems have the potential to ameliorate the problems associated with non-targeted delivery of potent drugs to the cancer cells thereby overcoming the side effects which at many times deprive the breast cancer patients the much-needed quality of life. Polymer nano formulations present advantageous properties as drug delivery systems when compared to conventional therapy. PEG-PLA nano formulations are biocompatible and biodegradable. The optimal size of nanoformulation is 10-200 nm. Polylactic acid (PLA) is a biodegradable polymer and in aqueous environments it is metabolized into water and carbon dioxide. Polyethylene glycol (PEG) presents outstanding properties like flexibility, biocompatibility, tailorable properties and good hydrophilicity. The copolymerised PEG-PLA has a great potential to be used for drug delivery systems as a nano carrier. In PEG-PLA composition, PLA is hydrophobic and PEG is hydrophilic. Wide spectrum of drug molecules like anastrozole (ANS), methotrexate (MTX), bortezomib (BTZ), thioridazine (Thio) and doxorubicin (Dox) are loaded very effectively thereby increasing their efficacy. The main aim of the polymeric nanoformulation is rate controlled and tissue targeted release of specific drugs. PEG-PLA nanoparticles can be used for their biodegradability and amphiphilic characteristics. The drug release of PEG-PLA nano formulations will be discussed in particular for their modifiable characteristics, chemico-mechanical properties and their therapeutic efficacy against breast cancer. We are delivering the drug using nano formulations to kill the cells; along with the drugs can we send anything else for targeted delivery thereby increasing the drug payload onto the breast cancer tumor surface.

Introduction

Biocompatible materials for controlled release to be used for drug delivery systems will be celebrating 50 years of technology development in 2026. Robert Langer the Edison of medicine, a chemical engineer by training holding the highest number of citations by an engineer found this concept of using biocompatible polymeric systems to be used for controlled drug release. Ethylene-vinyl acetate copolymer and hydroxy ethyl methacrylate popularly known as Hydron-S were the incipient biocompatible polymers to be reported for controlled drug release [1]. Many factors play crucial role in the drug release compartment namely, the raw material either natural or synthetic (polymer itself), crystallinity, amorphousness, porosity, surface area and so on. Embedding the drug to the polymer carrier depends on [2] binding affinity, reaction kinetics of the drug with the polymeric carrier, hydrophilic and hydrophobic nature of the drug; as these factors play a very critical role in the success of the drug delivery systems. Nanoformulation basically defined as a combination of a drug or multiple drugs with the corresponding carrier at the nano level in the dimension of 50-150 nm and administered as an emulsion to the patients through intravenous mode.

Polymeric drug delivery systems profoundly work on the principle

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of controlled drug release in the action site, more precisely onto the tumor surface in a controlled manner rather than an instant high drug load release leading to systemic toxicity. Cancer therapeutics are very challenging not only in terms of financials but also in terms of therapy. In most cases toxicity overweighs therapeutic efficacy and efficiency. Nanoformulation is one of the solution providers to combat systemic toxicity wherein the drug load is delivered to the target site of the tumor with very minimal toxic effects to the local, regional or distant cells, tissue or organs.

Heart disease is the leading cause of death for women globally, lung cancer being the top killer cancer disease among women. Breast cancer is the second most fatal disease to the womenfolk. 45,000 women die of breast cancer annually and in the year 2020, 270,000 new cases of invasive breast cancer have been reported in the United States of America. In 2017, there were an estimated 3,577,264 women living with female breast cancer in the United States of America. Breast cancer occurs in 1 in 4 cancerous women and is the leading cause of death in women with around 600,000 deaths worldwide in 2018 [3]. Conventional chemotherapy has many side effects as it affects normal cells too. Thus, there is a need for effective therapies for breast cancer to reduce the mortality rate. Chemotherapeutic drugs loaded in biomaterial carriers popularly known as nanoformulations are more efficient than conventional chemotherapy as it results in higher drug accumulation within the tumor, reduced side effects and increased prognosis. The overall death rate from breast cancer decreased by 1.3% per year from 2013 to 2017, current overall death rate needs to be worked upon which can project the success trajectory of the existing drugs for breast cancer therapeutics. The incidence of breast cancer due to maternal inherited mutation is 15% whereas 85% of the breast cancer incidence is without a family history of breast cancer. There are around 40 drugs for the cure and treatment of breast cancer. The new drug approved (2019) by FDA namely, Atezolizumab joins the breast cancer therapeutics repository.

Two main types of Nano formulations which categorized as Organic and Inorganic. Micelles, dendrimers, liposomes, hybrid and compact polymeric nanoformulations comes under Organic category. Fullerenes, quantum dots, silica and gold NPs come under inorganic category. This report primarily deals with PEG-PLA nanoformulation for breast cancer therapy which will give more insight on the synthesis, drug release into the release medium, in-vitro testing using breast cancer cell lines and finally in-vivo testing using laboratory animal models.

PEG-PLA Nanoformulations

PEG-PLA based nanoformulations can be synthesized using different methods like spray drying, nanoprecipitation, single or double emulsion-solvent evaporation and emulsification solvent diffusion method [4,5]. Based on the method of preparation, the chemotherapeutic drug is either entrapped within the core of the co-block nanocapsule as well as adsorbed on or entrapped in the surface of the co-block matrix nanosphere [6]. Single or double emulsion-solvent evaporation method and nanoprecipitation method are the most commonly employed PEG-PLA based nanoformulations preparation methods. Emulsion-solvent evaporation method is generally preferred for PEG-PLA and other polymeric nanoformulations as it is non-toxic, requires only mild conditions like constant stirring and ambient temperature and rapid in reaction rate. Whereas, nanoprecipitation method is a single step process which is simple and has a minimal energy requirement and good reproducibility.

Single Emulsion-solvent Evaporation Method

Single emulsion-solvent evaporation method is the first method proposed for the preparation of polymeric nanoparticles [7]. It is commonly employed for the encapsulation of hydrophobic drugs [8]. The single-phase solution is first prepared by dissolving PLA alone or with other polymers such as PEG, PEI to form a co-polymer in an organic solvent. Dichloromethane and chloroform are the commonly used organic solvents; however, ethyl acetate has been recently preferred due to its enhanced biocompatibility. The chemotherapeutic drug is added to the single-phase solution to create dispersion and then emulsified into a large amount of water with emulsifier at the required stirring rate and temperature. The most commonly used emulsifier is PVA. This method results in oil /water (O/W) emulsion. Through evaporation of the organic solvent, the oil droplet is hardened [9],[10]. Evaporation can occur either under pressure or by continuous stirring by using a high-speed sonicator or homogenizer [5]. The nanoformulations are usually recovered by ultracentrifugation and they are washed with distilled water to remove excess free drug and emulsifier [7].

In order to conjugate the nanoparticles with specific ligands like Trastuzumab (HER2 antibody), the nanoparticles can be incubated with trastuzumab in phosphate-buffered saline (PBS) overnight so that it can cover the surface of the nanoparticle. The unconjugated trastuzumab is removed by centrifuging twice [11]. The carbodiimide-mediated method can also be used for HER2 antibody conjugation [12].

Double Emulsion-solvent Evaporation Method

Double emulsion solvent-evaporation method is also known as an emulsion of emulsion [13]. Nanoformulations synthesized using this method are in water/oil/water (W/O/W) emulsion form. Double emulsion-solvent evaporation method follows the same procedure of the single emulsion-solvent evaporation method until the oil/water (O/W) emulsion form is reached. This oil/water (O/W) emulsion is further added to an aqueous solution containing emulsifier to obtain a water/oil/water (W/O/W) emulsion form [4]. The nanoformulations are obtained by the evaporation of the organic solvent. This method is commonly used to encapsulate hydrophilic drugs such as proteins, peptides and vaccines [5,6].

Nanoprecipitation Method

Nanoprecipitation method is also known as the solvent displacement method or interfacial deposition method [5,6]. PLA and other polymers along with chemotherapeutic drugs are dissolved in an organic solvent such as ethanol, acetone, acetonitrile, or methanol. This solution is added dropwise into another aqueous solution which contains the surfactant. After continuous stirring, the solvent is evaporated completely [4-6,9]. This method was initially used to encapsulate hydrophobic drugs, but it has recently been modified to encapsulate hydrophilic drugs. Encapsulation of hydrophilic drugs by using the standard nanoprecipitation method results in poor encapsulation [14]. Thus, encapsulation of hydrophilic drugs can be performed by adapting certain modifications by choosing suitable solvents based on polarity, non-solvents based on final drug loading, and surfactants [15,16].

Anti Breast Cancer Drug Loaded PEG-PLA Nanoformulation Synthesis

ANS based nanoformulation has been produced by the well-established double emulsion method. The multi block nano carrier PLA-PEG-PLA powder becomes a slimy emulsion in the oil phase

with ANS which is pre-dissolved in DMSO [17]. For stabilization of the nanoformulation conventional sonication process is used; for further in-vivo testing as a breast cancer therapeutic agent.

For the preparation of Curcumin-NPs conventional emulsion method involving the initial solidification process was carried out [18]. The product of the oil in water emulsification (NPs) was dispersed by utilizing a tip-based sonicator.

The drugs to be embedded namely DOX and DTX were made into a nanoformulation using mPEG-PLA co-block polymer chain. Double emulsion method was carried out. Initially the hydrophilic and hydrophobic polymers mPEG and PLA were added to the solvent dichloromethane (DCM) resulting in an emulsified colloidal solution. To this, the aqueous solvent polyvinyl alcohol (PVA, 2% (w/v)) was added resulting in a solution due to the double emulsification process [19]. The resultant solution was sonicated thereby producing nanoparticles with dual drugs (DOX & DTX) embedded onto the PEG-PLA nanocarrier; to be used as an efficient nanoformulation drug delivery system for breast cancer therapy. By this process of double emulsification and ultrasonication, DTX being hydrophobic sets out to be embedded onto the intermediary hydrophobic oil phase, while the other hydrophilic drug namely, DOX gets embedded into the interior hydrophilic core entity.

Methotrexate (MTX) based nanoformulation preparation was formulated using the conventional double emulsification method. MTX, an established drug for breast cancer therapy forms a basic emulsion mixture to which sonication process as a batch mode was carried out resulting in a very inconsistent emulsion mixture. To be used as a nanoformulation drug for breast cancer therapy, the final commercial formulation needs to be homogeneous and devoid of agglomeration [20]. PVA addition to the emulsion mixture conciliates this hurdle of agglomeration, resulting in a homogeneous solution to be administered for in-vivo breast cancer therapeutic testing. The physico-chemical phenomenon of the interaction between hydroxyl group with DMSO and the vinyl group with chloroform is attributed for the resultant homogeneity of the nanoformulation. This final nanoformulation is very stable and can be used as an in-vivo breast cancer therapeutics.

Thio/mPEG-PLA nanoformulation was fabricated using DCM under elevated temperature (60° C) using a rotary evaporator under reduced pressure wherein a film was produced. Rehydration of the film in phosphate-buffered saline (PBS; pH =7.4) lead to self-assembly ending up in Thio/MPEG-PLA NPs synthesis [21]. Dropwise addition of DOX in aqueous solution under gentle reaction conditions ends up in DOX-Thio/MPEG-PLA NPs. This nanoformulation will be stored at refrigerated conditions for further experimentation.

Drug Release Profiles for Nanoencapsulated Breast Cancer Agents

Drug release profiles for ANS – NPs were carried out using PBS (pH = 7.4) and for a duration of 144 hours. Test sample of 2 mL was taken and replaced by fresh release media namely, PBS (pH = 7.4) at regular time intervals [17]. ANS drug release was measured using the absorption value at ëmax of 360 nm. Release profile (figure 1) depicts an initial burst of 60% within 18 hours. A slow and controlled release for next 120 hours has been identified for further 20% of the drug. In an overall reaction time of 144 hours, 80% of the total drug gets released.

Curcumin is highly unstable under physiological conditions and can act as a very good molecule for bioabsorption. Curcumin is known for ages for its antibiotic and antioxidant activities. Curcumin is extracted from *Curcuma longa* and is been used for its antioxidant and anti-inflammatory activities. Curcumin is a clinically useful tool which has anti tumoral activity against cancer cell lines and is highly aggressive towards some cancers. Curcumin is the major ingredient in turmeric which is used on a daily basis in several countries. Curc-PEG-PLA drug release experiments were carried out. PBS in methanol (55% (v/v)) was used as the release medium [18]. The release profile projected a biphasic reaction kinetics. Initial burst release of 40% of curcumin was shown within 24 hours. Curcumin release of 53% was exhibited in the next 100 hours. Summing it up, 93% of curcumin was released within 120 hours, basically 5 days.

Simulating the cellular environment is challenging, as they vary not only in molecular compartments but also in pH. The intracellular and extracellular pH varies drastically when considering the cellular microenvironment of the tumor. The release medium used for these studies was basically PBS but in two different pH, namely 7.4 and 5.4 [19]. DOX-DTX-PEG-PLA NPs were the nano-formulation used for drug release testing. The experiments were carried out for a duration of 96 hours in the acidic (5.4) extracellular microenvironment; 98% of both the drugs, DOX and DTX gets released within 48 hrs. In terms of the neural intracellular microenvironment pH of 7.4, at 48 hrs only 55% of both the drugs gets released and takes 98 hrs for the entire drugs to get released. Nanocarriers' properties play a very critical role in the release profiles; the extracellular microenvironment being acidic, the nanocarriers gets degraded quickly than in the neural intracellular microenvironment.

BSA was used as the coating agent for MTX-PLA-PEG-PLA nanoformualation resulting in BSA-(MTX-PLA-PEG-PLA) for better controlled and sustained release. Even upon coating with BSA, burst release could not be avoided, as BSA-(MTX-PLA-PEG-PLA) showed an initial burst within very short duration. Controlled drug release was achieved for next 100 hours [20]. The experiments were carried out for a total duration of 168 hours. MTX release was measured at λmax of 310 nm.

Thioridazine and doxorubicin were worked upon to co-deliver for breast cancer therapeutics. Thioridazine trade names are: Mellaril and Melleril, Adramyicn as well as Rubex are the trade names for Doxorubicin. Thio-DOX-mPEG-PLA nanoformulation was tested for the release studies using PBS as the release medium. Two

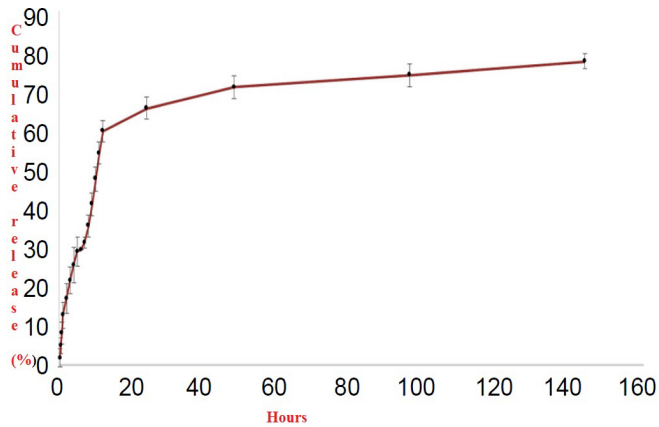

Figure 1: Cumulative release of ANS from ANS-PLA-PEG-PLA nanoformulation [reproduced from ref 17, under Creative Commons Attribution-Noncommercial License]

different release medium were used varying in pH namely, 5.5 and 7.4. Acidic pH and neutral pH as mentioned before represents plasma environment and solid tumor surface environmental compartments. Thio gets released in very controlled fashion at pH 5.5 as well as at pH 7.4 [21]. Only 30% of the drug gets released by 100 hours. At both acidic (pH 5.5) representing the solid tumor surface environment as well at neural pH (pH 7.4) representing the plasma compartment. On the other testing, DOX release kinetics varied with pH. DOX gets released by 30% in 100 hours at neutral pH (7.4), whereas at acidic pH (5.5), more than double the release quantum was observed. The release % was around 65% for 100 hours kinetics release studies. These study results prove systemic toxicological effects of the breast cancer therapeutic drugs can be very much reduced by administering them as nanofomulation rather than the conventional free drug.

In vitro Analysis Using Breast Cancer Cell Lines

Cancer cell lines are conventionally used for research purposes and in fact they are a great model for decoding the mechanism in cancer. Human breast cancer cell lines like MCF-7, MCF-7/ADR, SKBr-3, MDA-MB-231 and mice breast cancer cell lines like 4T1 were used [22]. MCF-7, MCF-7/ADR, SKBr-3, MDA-MB-231 and 4T1. Cell line name, Tissue, Cell type, Cell line origin, Mutated gene, Genes expressed, Application. PEG-PLA has the capability to encapsulate both hydrophilic and hydrophobic drugs. The block polymer can be loaded with various drugs like Anastrozole, Dodecanol, Platinum (II), Paclitaxel and Doxorubicin. Drugs like Doxorubicin and paclitaxel are some of the commonly used in chemotherapy. For cancer cells to grow they need this enzyme topoisomerase 2 and Doxorubicin blocks this enzyme. DOX drug is inserted between base pairs of DNA/RNA causing changes in its structure and in turn leading to arrest of cancer cell growth [19]. Also, a combination of drugs like Docetaxel (DOX) - Doxorubicin (DOX), Lapatinib (LPT) - Paclitaxel (PTX), Doxorubicin (DOX) - Curcumin (CUR) is encapsulated inside the nanoformulation. Generally, cells get resistant to paclitaxel due to excessive expression of HER-2. Xinguo Jiang *et al.* proved that LPT-PTX combination is synergistic. LPT not only interrupts epidermal growth factor receptor (EGFR) pathway and HER-2 but also sensitizes PTX resistant cells to PTX. Curcumin- Bortezomib (curc- BTZ) are hydrophobic anti-cancer drugs with synergistic effects [23,31].

Nanoformulations can interact with the cell membranes and enter the cell via endocytosis or diffusion. To transport inside the membrane the drugs enter the cells via endocytosis, a process with various stages where the extracellular components react and form invaginations interacting with the cellular membranes. Endosomes are formed after the invaginations get pinched and then are delivered inside the cell to target compartments. Endosomal escape happens and the nanoformulation releases the drugs from the endosome. Now apoptosis / inhibition of cell growth occurs [22,32]. DTX impedes the microtubule assembly and blocks the G2/M phase of the cell cycle. Y.Wang used folic acid (FA) as a ligand and the ligand's cellular uptake is controlled by receptor-mediated endocytosis. Folic acid is expressed excessively in various breast cancer cells [5,6]. Paclitaxel (PTX)-Lapatinib (LPT) increased the apoptosis and blocked the G2/M and S phase. Crizotinib has anti-angiogenic potential and can also induce apoptosis through caspase-3. Sildenafil impedes the breast cancer resisting protein (BRCP) and multi drug resistance-associated protein (BCRP) in breast cancer cells [19,23-25]. IGF-1R gene is over expressed and is also an apoptosis protein in various types of cancer Cationic lipid polymer hybrid nanoparticles (HNPs) encapsulate siRNA which targets the IGF-1R gene [28].

Anti-cancer drugs after reaching the target site should be able to kill the cancerous cells without disturbing the normal cells [33]. MCF-7 cell lines were used and the efficacy of the drug is assessed with the cell's viability. After 48h of exposure to MPEG-PLA-DOX-DTX the percentage of viable cells were less (33%) when compared to just free delivery of DOX-DTX (43%). The drug release efficiency was at 98% in the time frame of 96 h at pH 7.4 [19]. With a sustained release of 144 hours, PLA-PEG-PLA encapsulating ANS shows cytotoxicity against MCF-7 cells by alteration in the gene expression of MAPK3, c-MYC, MCL-1 cells [17]. PEG-PLA micelles loaded with Crizotinib and Sildenafil showed decrease in viable cells. The co-delivery of drugs showed up to 37% decrease in viable cells when compared to delivering the drugs separately (14%). So, the codelivery of these drugs has a 2.7fold decrease in viable cells [24]. PLA-PEG-PLA encapsulating siRNA showed 70% ±4 decrease in IGF-1R expression when compared to free delivery of siRNA. PEG-BHETE-PLA loaded with DOX and Ce6 is a copolymer with a red-light sensitive part - (1,2-bis(2-hydroxyethyl thio)ethylene, BHETE). PEG-BHETE-PLA-DOX-Ce6 was more effective and showed decrease in metabolic activity of MCF-7 cells when exposed to light rather than not having been exposed to light. The cells exposed to light showed more than 25% decrease in viable cells compared cells that are not exposed to light. On the contrary, when PEG-BHETE-PLA was treated on MCF-7 cells they showed nil variation in their metabolic activity [28],[29]. SKBr-3 cell lines were used to test drug loaded PEG-PLA micelles(PPM). PPM was encapsulated with lapatinib (PPM - LPT), Paclitaxel (PPM - PTX) and together (PPM - LP) for combinational approach. PPM-LP escapes into the cytoplasm from the endosomes easily and blocks the G2/M and G1/S stages in SKBr-3 cell lines. SKBr-3 cell lines' apoptosis explains that PPM-LP is more effective than PPM-LPT and PPM-PTX [23]. PLA-PEG-PLA is encapsulated with Dodecanol (Dol) in which amine group and folate were added to the PEG layer producing Dol-PLA-PEG-PLA-FA. CCK-8 assay explained that the folate on the nanoformulation increased the targeting to MCF-7 cells. Hence, it is a drug which is safe and has precise tumor targeting capacity. Its cellular uptake is controlled by receptor - mediated endocytosis [25]. Poly (L-lactide-co-2-methyl-2-carboxyl-propylene carbonate/platinum (mPEG-b-P(LA-co-MCC/Pt) and folic acid-poly (ethylene glycol)-block-poly(L-lactide) (FA-PEG- PLA) was chosen in which FA is in the outer structure of the nanoformulation to increase the targeting capacity. The biodistribution studies shows that FA-M(Pt) is more effective than M(Pt) and oxaliplatin [26]. On SKBr-3 (HER-2 positive) the cytotoxicity was higher when PPM - LP was used. The IC50 values of PPM-LP were lower, which explains that LPT and PTX have synergistic drug interactions [23]. MCF-7 and MCF-7/ADR cells are used for testing PEG-PLA micelles encapsulated with Doxorubicin (DOX) - Curcumin (CUR). At pH 7.4, release rates of DOX from the DOX - Micelles and (DOX-CUR) - Micelles were 78.23% and 84.45% [30]. 4T1 cells were used for assessing the in vitro efficacy of PEG-PLA micelles (PMs). PMs was loaded with paclitaxel (PTX) and a HE-CPP sequence (polyglycine linker ((HE)10G5R6 or HE-CPP) was added forming PTX/PMs-HE-CPP. The IC50 values of PTX/PMs -HE-CPP has almost 1.4fold increase at pH 7.4 (1.456 μg/mL) than at pH 6.5 (1.268 μg/mL). PMs-HE-CPP exhibits wider cytoplasmic release, productive endosomal escape and targeting efficacy than PTX/NPMs [27].

Studies on Breast Cancer Xenograft Model

Breast cancer cell lines like MCF-7, Ehrlich ascites tumor (EAT), MDA-MB-231, SUM149, BT549, MDA-MB-468, 4T1 cell lines are injected into BALB/c nude mice, swiss albino mice, SCID mice to induce breast cancer in mice. In order to study the effects of chemotherapeutic drugs encapsulated within PEG-PLA

nanoformulations in vivo, various breast cancer cell lines are either implanted into the mammary fat pad or injected through intravenous and intracardial routes in mice to induce breast cancer in the animal model. Common breast cancer cell lines used in the xenograft animal models are MCF-7, Ehrlich ascites tumor (EAT), MDA-MB-231, SUM149, BT549, MDA-MB-468, 4T1 are used to induce breast cancer. Different mice models like BALB/c nude mice, swiss albino mice, SCID mice are generally used for in vivo studies.

Pharmacokinetics

The pharmacokinetics following the injection of the free drugs and drugs encapsulated in PEG-PLA nanoformulations revealed that free drugs such as Taxol, Bortezomib, and Quercetin were rapidly eliminated from the blood. As free drugs are quickly cleared from the drug, minimal drug is delivered to the tumor site. On the contrary, F3 conjugated paclitaxel loaded PEG-PLA nanoparticles, K237 conjugated paclitaxel loaded aldehyde-PEG-PLA nanoparticles, Bortezomib encapsulated PEG-PLA nanoparticles, methoxy PEG-PLA nanoparticles encapsulating Quercetin had a dramatically longer blood circulation time which was marked by delayed blood clearance. Encapsulation of the drugs in PEG-PLA nanoparticles leads to longer blood circulation time as PEGylation reduces the binding of plasma proteins, hinders interactions of blood components, and prevents drug carrier interactions with opsonin [34-37].

Anti-tumor Activity

The anti-tumour activity of the free drug, drug encapsulated within PEG-PLA nanoparticle is studied to understand the effect of encapsulating the drug within PEG-PLA nanoparticles. F3 conjugated paclitaxel loaded PEG-PLA nanoparticle had a tumor inhibition rate of 57.3%, higher than paclitaxel alone; as the nanoparticle specifically binds to nucleolin expressed on the surface of tumor cells and increases the specificity of the nanoparticle [34]. Similarly, folic acid and trastuzumab functionalized PEG-s-s-PLA-s-s-PLA-s-s-PEG polymersomes also showed 90% tumor inhibition when compared to 38% inhibition by free doxorubicin [38].

Quercetin loaded Methoxy-PEG-PLA nanoparticles, bortezomib loaded PEG-PLA nanoparticles and paclitaxel encapsulated in HE-CPP coupled PEG-PLA polymer micelles, showed an increased reduction in tumor volume and weight compared to the free drugs. Furthermore, 8-fold higher dose of free quercetin was needed to obtain similar results of encapsulated quercetin. The heightened tumor suppression activity of the encapsulated drugs could be due to the increased accumulation of the drugs within the tumor [36], [27,37]. Combination therapy involving doxorubicin and thioridazine encapsulated into methoxy-PEG-PLA (MPEG-PLA) nanoparticles demonstrated higher anti-tumor effect when compared with free individual drugs and combined drugs [21].

H&E staining for histology analysis has revealed that the tumor section with the treatment of folic acid and trastuzumab functionalized PEG-s-s-PLA-s-s-PLA-s-s-PEG polymersomes, K237-PTXNP showed the presence of necrotic or apoptotic cells with very few viable cells. In contrast, the free drug showed the presence of necrotic or apoptotic cells along with patches of viable cells indicating partial tumor regression [35,38].

Biodistribution and Systemic Toxicity Studies

The tumor-targeting ability of PEG-PLA nanoparticles was compared with free drugs. Paclitaxel, CDK1 siRNA, Bortezomib, and doxorubicin encapsulated PEG-PLA nanoparticles and have demonstrated higher accumulation of drugs in the tumor tissue than free drugs [34,36,38,39]. Furthermore, active targeting nanoparticles like F3 conjugated paclitaxel loaded PEG-PLA nanoparticles had a 2-fold and 4-fold higher accumulation when compared to paclitaxel PEG-PLA nanoparticles and Taxol respectively [15]. Paclitaxel and doxorubicin loaded PEG-PLA nanoformulations also had decreased accumulation in the liver, spleen, kidney [34,38,40]. CDK-1 siRNA loaded PEG-PLA nanoparticles also had no substantial systemic toxicity, and they did not activate the innate immune response [39]. Mice treated with free doxorubicin also experienced a loss of body weight and adynamia suggesting acute toxicity of doxorubicin; whereas, folate modified pH-sensitive targeted polymeric micelle encapsulating doxorubicin group had stable and normal body weight and animal status indicating the biocompatibility and non-toxic properties of PEG-PLA nanoparticles [41]. Mice treated with free doxorubicin was found to have cardiotoxicity, hepatotoxicity, nephrotoxicity and pulmonary toxicity whereas mice treated with folic acid and trastuzumab functionalized PEG-s-s-PLA-s-s-PLA-s-s-PEG polymersomes, folate modified pH-sensitive targeted polymeric micelle, and doxorubicin-loaded PEG-b-PLGA nano-polymersomes showed no substantial organ toxicity [42].

Specific active targeting and enhanced permeation retention (EPR) mediated passive targeting of the nanoparticles results in high accumulation of the drugs in the tumor resulting in minimal exposure to other organs leading to low systemic toxicity.

Conclusion

Polyethylene glycol–polylactic acid (PEG–PLA) is a biodegradable block copolymer that has been approved by the food and drug administration (FDA) and european medicines agency (EMA). PEG-PLA based nanoformulations are an excellent drug delivery system for breast cancer as it has targeted delivery, controlled release of drugs, and very good biocompatibility. As a co-block polymer carrier, PEG-PLA has been loaded with various drugs like anastrozole, dodecanol, platinum (II), paclitaxel, and doxorubicin as a single drug or as a combination of two drugs like docetaxel (DOX) - doxorubicin (DOX), lapatinib (LPT) - paclitaxel (PTX), doxorubicin (DOX) - curcumin (CUR) for the treatment of breast cancer. Our focus is on the PEG-PLA based nanoformulations, mechanisms of action as a drug delivery system, different combinations of PEG-PLA nanoformulation, ingredients as a co-block polymeric system along with the drugs they deliver, and the various benefits of each combination for breast cancer therapy. To date, there are more than 15 organic-based nanoformulations approved by FDA for clinical and commercial use against different diseases. These fifteen organic-based commercial nanoformulations very much appreciably boost the translational research and technology development for breast cancer therapeutics involving convergence technology. Nanoformulations and nanobots as drug carriers are the future of breast cancer treatment by making personalized precision medicine possible with synergistic drug combinations and lower concentrations of the drugs that targets to kill specifically the cancerous cells of the breast and target tumor heterogeneity rapidly with high precision and with very minimal side effects.

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