BREAST CANCER THERAPY THROUGH LIPOSOMAL DRUG DELIVERY SYSTEM

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy
Brac University
November, 2022

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Approval

The project titled “Breast Cancer Therapy Through Liposomal Drug Delivery System” Nusrat Jahan Ome (18346024) of Spring, 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) in November, 2022.

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Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

Liposomes have been particularly effective among the several nanoparticle-based delivery systems, with various formulations making it into clinical applications. They are widely recognized and efficient gene and/or medication delivery systems that are often utilized in the treatment of cancer, especially breast cancer. We talk about liposome design in this review along with the targeting and triggering features. We also provide an overview of current developments in liposome-based breast cancer therapies, such as chemotherapy and gene therapy, since 2014. Finally, we have identified certain issues with developing liposomal technology for future clinical translation.

Keywords

Liposome, cancer, breast cancer, nanomedicine, drug delivery, recent advancement, pharmacokinetics, biodistribution.

Acknowledgement

First of all, my highest gratitude is for Almighty Allah SWT for giving me the opportunity, strength and patience to complete my project work properly. I am thankful to Dr.Sabrina Sharmin miss (Assistant Professor, School of Pharmacy, BRAC University), my respected project supervisor for her kind guidance regarding my work. Moreover, my special thanks to Dr. Eva Rahman Kabir, (Dean and Professor, School of Pharmacy, BRAC University) for giving me the opportunity to conduct my project work successfully. Lastly, my sincere gratitude is towards my parents, family and friends for their motivation and support throughout the journey.

Contents

List of Acronyms

DCIS Ductal Carcinoma In-situ

LCIS Infiltrating Lobular Carcinoma

ILC Infiltrating Lobular Carcinoma

IDC Infiltrating Ductal Carcinoma

BSE Breast Self-Exam

PC Phosphatidylcholine

PE Phosphatidylethanolamine

EPG Egg Phosphatidylglycerol

EPC Egg Phosphatidylcholine

MPS Mononuclear Phagocyte System

EPR Enhanced Permeability and Retention

PEG Polyethylene Glycol

NPL Non-PEGylated Liposome

HFS Hand-foot Syndrome

NPLD NPL Doxorubicin

LTSL Lysolipid Thermally Sensitive Liposome

MRI Magnetic Resonance Imaging

TM Transition Temperature

TSL Thermo-sensitive Liposomes

ROS Reactive Oxygen Species

PSs Photosensitizers

PDT Photodynamic Therapy

TNBC Triple Negative Breast Cancer

HIFU High-intensity Focused Ultrasound

MNPs Magnetic Nanoparticles

RISC RNA-induced Silencing Protein-complex

PLK-1 Polo-like Kinase 1

DOPS DSB Double-stranded Break 1,2-dioleoyl-sn-glycero-3-phospho-L-serine

DOPG 1,2- Dioleoyl-sn-glycero-3-phospho-(10 -rac-glycerol)

ASOs Antisense Oligonucleotides

CRISPR Clustered Regularly Interspaced Short Palindromic Repeats

siRNA Small Interfering RNA

mRNA Messenger RNAs

sgRNA Single-guide RNA

Chapter 1

Introduction

An uncontrolled growth of cells following either the deactivation of oncogenes or the activation of tumor suppression genes or the both leads to carcinogenesis which is the initiation of cancer formation(Sarkar et al., 2013). Cancer is the uncontrollable division of abnormal cells and their invasion to nearby tissues occurs. Also, these cells have the ability of metastasis which is to spread over the various different parts of the body by lymph and blood systems (*National Cancer Institute*, n.d.). Cancers originating from breast tissue, mostly from the lobules supplying milk to the ducts or inner lining of milk ducts are breast cancers. In women it is by far mostly frequent reason of cancer and second most frequent reason of death for cancer (Sharma et al., n.d.).

1.1 Rationale

Despite of some major advances in the cancer therapy still it is one of the leading reasons of mortality. Additionally, traditional therapy sometimes may cause toxicity which is a major challenge to tolerability and adherence. So, researchers are in search for anticancer therapies with better efficacy and tolerance (Mun et al., 2018). The efficacy and tolerance of conventional cancer therapy can be improved and combating cancer can be easier by nanocarriers.7 Liposomes are potentially useful to bring nanocarriers in the improvement of cancer therapy by two major methods, active and passive targeting. The rationale of the study is to provide information about recent advancement to injectable liposomes-based drug delivery system with both active and passive targeting therapy in breast cancer patients (Alavi & Hamidi, 2019).

1.2 Objective

Liposomal drug delivery system has been proven successful among many nanoparticle-based drug delivery systems by clinical applications. As they are more efficacious and well stablished, they are used vastly in cancer therapy, especially in breast cancer. So, the point of the investigation is overviewing the liposome-based drug delivery systems' current position in the treatment of breast cancer including both gene therapy and chemotherapy as well as to identify some of the challenges in the development of this platform (Yang et al., 2021).

Chapter 2

Methodology

Databases with bibliography such as PubMed, Springer link, Google Scholar, Scopus and Science Direct were strenuously investigated and information like cancer, breast cancer, liposomal delivery system, pharmacokinetics of liposomal drugs, biodistribution of liposomal drugs and nanomedicines etc. were assembled. To cite and reference the information sources Mendeley desktop version 1.19.8 was used.

Chapter 3

An Overview of Breast Cancer

Breast cancer occurs if the cells of the breast begin to grow uncontrollably which can start in one breast or both. Most occurrence of breast cancer happens in women although occurrence in men also happens.

3.1 Breast cancer epidemiology:

Breast cancer is one among three most frequently occurring cancer of the world. It is the most common malignancy in women. According to a statistic, worldwide 1.7 billion cases of breast cancer were diagnosed and death from this cancer was almost half million in the year 2012. Breast

cancer in women is so common that among eight to ten women one will get affected by this. In developed countries like North America and European Union (EU) mortality rate from breast cancer has been decreased (decrease rate in EU was 8% in 2016) because of their advanced treatment facilities as well early detection. On the contrary, in less developed countries of South America, Africa, and Asia breast cancer is the most prominent reason of mortality for cancer due to lack of advancement of treatment and state of diagnosis (Harbeck & Gnant, 2017).

3.2 Types of Breast Cancer

3.2.1 Common types of breast cancers

Non-Invasive Breast Cancer: Cancerous cells are enclosed into the ducts, invading into surrounding fatty and connective tissue does not occur. The most occurring form (90%) of non-invasive breast cancer is ductal carcinoma in situ (DCIS). Lobular carcinoma in situ (LCIS) is less occurring which gives an indication of the risk for increased breast cancer.

Invasive Breast Cancer: Cancerous cells are not enclosed into the ducts rather they break through the duct and lobular wall to invade breast's fatty and connective tissue. It may not be metastatic to lymph nodes or other organs. This type of breast cancer's occurrence is very frequent.

Lobular carcinoma in situ (LCIS, lobular neoplasia): Here by "in situ' it means the type of cancer which do not spread on other area except its initial development. A notable increase in the cell number of milk glands (lobules) of breast occurs.

Ductal carcinoma in situ (DCIS): among non-invasive breast cancers DCIS is the most common which is limited to the ducts area of the breast. Ductal comedocarcinoma is an example.

Infiltrating lobular carcinoma (ILC): also called invasive lobular carcinoma which begins from the milk glands (lobules) of the breasts and metastasizes to other organs or lymph nodes of the body. 10-15% of breast cancer is this type.

Infiltrating ductal carcinoma (IDC): Also called invasive ductal carcinoma. Begin from milk ducts of the breasts, later invades the ducts wall and fatty tissue of the breast. Also, metastasizes to other organs. Its occurrence is 80% of all breast cancers which makes it as the most common type of breast cancer.

3.2.2 Breast cancer of a less prevalent kind

Medullary carcinoma: Invasive breast cancer which make a noticeable boundary among normal tissue and tumor tissue. Causes almost 5% of total breast cancer occurrence.

Mutinous carcinoma: Other name is colloid carcinoma. This rare type of breast cancer is formed by the mucus-producing cancer cells. Women have better prognosis who have mutinous carcinoma in compared to women who have more common form of invasive carcinoma.

Tubular carcinoma: A form of invasive breast cancer. Women have better prognosis who have mutinous carcinoma in compared to women who have more common form of invasive carcinoma Causes 2% of all breast cancers occurrence.

Inflammatory breast cancer: In inflammatory breast cancers inflammation (red and warm) on breasts in addition with dimples and thick ridges is seen as a result of cancer cell blocking lymph vessels on breast skin surface area. It is very first growing although its occurrence is very rare (only 1%).

Paget's disease of the nipple: Starts from the milk ducts, then spreads to the nipple skin and areola. Only causes 15 of total breast cancer occurrence.

Phylloides tumor: Can be non-cancerous or cancerous which forms in the connective tissues of the breasts. Surgical removal can treat this type of breast cancer.

3.3 Breast cancer's root causes

Prior breast cancer history: If a woman had cancer in one breast previously she has the risk of having the cancer on another breast.

Significant family history: History of cancer of several members in the family causes increased risk of of affecting with breast cancer.

Genetic causes: As mentioned family history is one of the risk factor for breast cancer. It can be either maternal or paternal relatives. If the affected relative had breast cancer in a young age, affected in both breasts in that case the risk is highest. Mother, sister or daughter are considered to be the first degree relatives who play the vital role in estimation of the risk. Some second degree relatives like grandmother or aunt having breast cancers also enhance the risk. Male breast cancer enhances the risk of all of his close female relatives. Two abnormal genes that are BRCA1 and BRCA2, if inherited it significantly enhances the lifetime risk of affecting by breast cancers to 40-80%. Women carrying these two genes have a tendency of affecting by breast cancer at a younger age.

Hormonal reasons: Changes in the level of hormone plays role in breast cancer. It can cause due to changes in menstrual cycle (starting or stopping), early pregnancy, , any oral pills usages, hormone replacement therapy etc.

Dietary and life style cause: Unhealthy life style, obesity specially during post menopause causes breast cancer. Alcohol intake in an increased amount like two to five alcoholic beverages in a daily basis have almost double chance of affecting by breast cancer in compared to nonalcoholic women.

Environmental cause: Women working for a long time in an area having radiation enhances of their risk of being affected in breast cancer.

3.4 Sign and symptoms of breast cancer

Typical symptom of breast cancer is breast area or armpit lump. Doing breast self-exam (BSE) in a monthly basis is an excellent method to know the breasts' texture, size, skin and cyclical changes. The alerting conditions of breasts that are known to be the primary symptoms of cancer are: mammary nodule, any kind of fluid coming from the nipple, lymph node swelling under the armpit, nipple discomfort or pain; inverted or pitted nipples; dented skin of the nipple; persistent breast tenderness, pain or discomfort in the breasts. The following symptoms indicate a metastatic or advanced stage of breast cancer: in cases of bone metastasis, underarm lymph nodes are present along with symptoms like headaches, bone discomfort, neurological discomfort or weakness; shortness of breath in the case of lung metastasis; loss of appetite or rapid weight loss in the case of liver metastasis (G. N. Sharma et al., 2010).

Chapter 4

Liposomal Drug

Lipid bilayer structures which can incorporate drug substances for modifying the drug's pharmacokinetic profile for improving drug delivery are called liposomes. Intravenous route is the most common route for administering liposomal products (84%) and cancer is the most common indication (63%) (Kapoor et al., 2017).

For treating a range of diseases liposomal drug delivery is a very adaptive therapeutic approach. Surfactants and natural/synthetic lipids are usually applied in the manufacturing of liposomal drug delivery vehicles. The varied molecular structure of liposomes allows for unique physiological processes like prolonged blood circulation, pH responsiveness and reduced systemic toxicity.

4.1 Liposomal drug delivery system molecular building blocks:

Polysaccharides, sterols, surfactants, and natural or synthetic lipids can all be used to make liposomes. These formulations have several distinctive qualities, including improved drug encapsulation, tissue-targeting, stimulus responsiveness, reduced toxicity in non-target tissues, longer blood circulation and diagnostic potential. When compared to free medication delivery, all of these factors increase therapeutic effectiveness.

4.1.1 Natural lipids: With a hydrophobic head group and a hydrocarbon tail group, lipids have a diverse structural makeup. The head group may be zwitterionic, which has both a negative and a positive charge, creating a neutral state, or it may be positively or negatively charged. To conjugate with different molecules, the head group has also undergone chemical change. Because charged liposomes electrostatically resist one another, the head group charge contributes to stability. The length of the acyl chain, which might be symmetric, asymmetric, saturated, or unsaturated, vary for hydrophobic tails. Glycerol, phosphate, or sphingosine groups are frequently used as the building blocks of lipids. These distinguishable chemical characteristics support liposome activities such as lipid packing, pH responsiveness, stability, drug encapsulation and release, and bilayer construction.

Phospholipids Natural lipids: Phospholipids Natural lipids: The two most prevalent forms of phospholipids found in mammalian cell membranes are phosphatidylcholine (PC) and phosphatidylethanolamine (PE). The majority of phospholipids in eukaryotic cells are glycerophospholipids, which are lipids with a glycerol backbone. Egg phosphatidylglycerol (EPG) and egg phosphatidylcholine (EPC) are derived from lipid component combinations found in live tissues. Therefore, the quantity of the components may vary across batches. For instance, L-aphosphatidylcholine found in EPC obtained from chicken eggs has the length of acyl chain

ranging 14 - 22 and 0 - 4 degrees of saturation. Other naturally occurring lipids that are obtained from living things, processed, and combined with other lipids to create a nearly pure lipid composition. Additionally, acyl chain's degree of saturation/unsaturation might affect the composition of a lipid. Short, unsaturated acyl chains are less stable in vivo than saturated, longer acyl chains (high Tm). Endogenous lipid-based vesicles mimic the original cell membranes, making them naturally biocompatible and ideal for medicinal uses.

Sphingolipids: Mostly found in the membranes of mammals. These molecules, having 18-carbon amino-alcohol sphingosine backbone, are crucial for the formation of cell membranes and serve as regulatory signaling molecules. Sphingolipids, such as sphingomyelin (SM), are added to liposomal formulations to extend blood circulation, boost in vivo stability, and improve therapeutic efficacy.

Sterols: The majority of living things possess them. There are three different kinds of sterols: zoosterols (found in animals), phytosterols (found in plants), and mycosterols (in microorganisms). An essential part of mammalian cell membranes is the example of an endogenous amphiphilic zoosterol that is cholesterol. Cholesterol is largely compacted with lipid rafts in the cell membrane and is essential for maintaining membrane integrity and lipid raft activity. Liposomal formulations containing cholesterol have higher in vivo stability and reduced lipid bilayer leakiness, which allows for a more gradual and controlled release of cargo. Liposomes containing cholesterol are stable in the blood stream for a long time—more than 6 hours—while those containing no cholesterol are only stable for a few minutes.

Polysaccharides: Monosaccharides that are long-chain polymeric carbohydrates which are connected together by glyosidic linkages. Since they are found in cell membranes, they are crucial for cellular activities, tissue identification, and several transport systems. Liposomes may be

directed to cell receptors or circulated longer if the lipid membrane is coated with oligo- or polysaccharides. With their biocompatibility and antiviral, antibacterial, and anti-tumor capabilities, polysaccharides support drug delivery methods. The body's mucosal surfaces are comparatively better targets for the detection of polysaccharide. Therefore, polysaccharide-coated liposomes are chosen to target the gastrointestinal, respiratory, peroral, and nasal epithelial pathways.

4.1.2 Synthetic lipids: They are commercially produced, not naturally occurring or derived from the biological sources, and are commonly employed as parts of medicinal liposomes. Although they are not the endogenous type, yet they share many of the same biological and structural characteristics as natural lipids, including excellent biocompatibility and purity. Phosphatidylcholines, phosphatidylethanolamines, and phosphatidylglycerols are a few examples. They are a better choice than natural ones because of their commercial availability, purity, cost efficiency and chemical functioning.

4.1.3 Surfactants: These molecules decrease the liquid's surface tension where they are incorporated. As edge activators they are important additives in liposomal formulations. Generally, they are single acyl-chain surfactants which destabilizes the lipid bilayer of liposomal nanoparticles, as a result enhanced vessel deformability acquired. They also enhance the skin penetrative capacity of liposomes in anti-fungal, anti-cancer, and transdermal medications. Moreover, the edge activators are too used for increasing therapeutic efficacy.

4.2 Significance of composition on the characteristics and operation of liposomes

4.2.1 Encapsulation of drug: Conventional therapeutic compositions gives effectiveness when applied in vitro but their in vivo pharmacokinetic properties are low, also they show rapid clearance, deficient biodistribution or little plasma solubility. By encapsulating the medication of interest inside a vehicle that assures the appropriate in vivo pharmacokinetics, stability and biodistribution, the liposomal drug delivery system gets beyond these restrictions. High rates of encapsulation efficiency are thought to be a crucial factor in the effectiveness of liposomal medication delivery. There are so many methods used to enhance the efficiency of encapsulation but it is tough to efficiently load any drug inside tiny liposomes which are 50–150 nm in size because of their little entrapment volumes. This is a challenge which is mentioned while using reverse phase evaporation as well as freeze-thaw cycling (Ohsawa et al., 1985; Xu et al., 2012).

Compounds that are hydrophobic and hydrophilic are effortlessly loaded inside liposomes with high encapsulation efficiency by the help of lipid bilayer or aqueous core. Active loading is one kind of encapsulation technique that changes the drug permeability (membrane). For this trans-membrane gradient can be incorporated for driving the drug substance's molecules inside empty vesicles by a pH gradient. pH gradients can be made by ammonium sulfate (Haran et al., 1993) or citrate buffer (Mayer et al., 1986) for improving the efficiency of encapsulation of the drug which are amphiphilic by an easy, cost effective and safe technique (Tazina et al., 2011). The mentioned techniques are used industrially for drug loading of various FDA approved liposomal systems like Doxil, MyocetTM, DuanoXome etc. Liposome's physicochemical properties can have effect on the efficiency of encapsulation at the time of preparation, which includes: size, surface charge, composition, and surface modifications (He et al., 2019).

Inclusion of substances can positively or negatively effects efficiency of encapsulation like adding long acyl chain lipids have positive effect on encapsulation efficiency of the drugs which are hydrophobic inside the bilayer and improves the retention of drug (Ali et al., 2013). On the other hand, adding cholesterol have negative effect on the efficiency of encapsulation if the drugs are incorporated inside the lipid membrane. So, the liposomal system can be used for controlling the rate of release of the drug, which improves the effectiveness of the drug therapeutically. Optimization in liposomal formulation may be done in zeta potential, adding cholesterol and the distinctive features of the lipid molecules (head groups, length of acyl chain, degree of saturation and temperature of phase transition) (Maritim et al., 2021). Features of the lipids in the liposome preparation have effect on the packing of the bilayer of lipid. Also, it can be optimized for achieving prolonged drug release. Again, enhancing acyl chain's unsaturation degree has positive effect on drug release rate because bilayer of lipid's leakiness (Charrois & Allen, 2004).

4.2.2 Stability: It can be chemical, physical, or biological. Chemical and physical stability maintain its properties steadily. For example, phospholipids have a tendency of various reactions of chemical degradation like ester bonds hydrolysis and unsaturated acyl chains peroxidation; these things have effect on the durable stability of a liposomal preparation. Stability has effect on liposome preparation to be regenerated from lyophilization. Liposomal features like osmolality can be modified by several excipients. The integrity of liposome in serum proteins presence is known as biological stability.

When liposome enters into the bloodstream the bind to serum proteins which is called opsonization. This enhance the clearance rate (Yadav A. et al., 2011). Avoiding this liposomal opsonization and incrassation of blood circulation time is achieved by adding PEG. Moreover, cholesterol addition in liposomal preparations (alone or conjugated with PEG) enhances the

circulation time of blood (Gabizon et al., 1989; Semple et al., 1996). All in all, stability means the maintenance of the liposomal properties primarily during various type of stresses.

4.2.3 Surface charge: Phospholipids has a net charge because of the headgroup they have and pH of the solution. The compositions of the final formulation have effect on the liposome's gross zeta potential, the therapeutic function and efficacy of the vesicles' delivery system is also affected by this. Anionic liposomes (negative surface charge containing liposomes) are made of lipids which has headgroups of negative charge. In DNA transfection anionic liposomes are used (Patil et al., 2004). The addition of negative charged lipids in liposomal preparations also amplify the cardiotoxic drugs delivery, for example doxorubicin, lessen the systemic toxicity in comparison with free drug administration (Forssen & Tokes, 1981). Furthermore, anionic liposomes help to enhance the vascular extravasation and decrease the accumulation inside the vascular endothelium, relative to liposomes which are cationic (Krasnici et al., 2003). Because of the anionic liposomes electrostatic repulsion with cell membranes negative charge surface, nonspecific uptake of cells is decreased in contrast to the cationic liposomes. This decreased nonspecific uptake permits anionic liposomes for prolonged circulation in the bloodstream compared to that of cationic ones (Kuang et al., 2012). Cationic liposomes can be synthesized by adding positively charged lipids, playing important role in transfection of nonviral gene and delivery of siRNA. Usage of cationic liposome is seen in liposomal vaccine adjuvants formulation (Christensen et al., 2007) because they have inherent immunostimulatory function(Inoh et al., 2020; Vangasseri et al., 2006). Also they are used in immunogenic response and enhancing the efficiency of vaccines (Zhuang et al., 2012). Incrassation of peritoneal retention more than that of neutral or anionic liposomes demonstrate them as the ideal drug carriers for gynecological malignancies and gastrointestinal treatment (Dadashzadeh et al., 2010)

4.2.4 Pharmacokinetics of liposomal formulations: Liposomal drug delivery system has effect on pharmacokinetic parameters (e.g. half-life and clearance rate, liposomal biodistribution) in vivo.

Half-life and clearance rate: Rate of liposomal half-life and blood clearance are very much influenced by the composition of liposome. The mononuclear phagocyte system (MPS) is consisted of tissue resident macrophages as well as blood monocytes (van Furth, 1980) which is the primary reason for the liposomal clearance from the bloodstream.

Neutrophil circulation also has a vital impact on drug delivery (Betker et al., 2018). Various research has been done to modulate composition of liposome to for decreasing MPS uptake. Opsonization of liposome after systemic administration causes liposomal interaction with receptors on the liver and spleen surface macrophages of MPS, gradually it induces internalization and degradation. It may also have impact on directing particle toward the liver by a process called hepatocytes. By PEG inclusion half-life and blood circulation time can be increased and this process is called stealth. Again, liposomes having phospholipids of neutral nature with a little charged (negative charge) glycolipid called monosialoganglioside (GM1) half-life and blood circulation time can also be increased in vivo. Here, GM1 decreases opsonization by steric hindrances by sialic acid which has negative charge and physical hindrance causes by chains of carbohydrate (Allen, 1994).

Biodistribution (Liposomal biodistribution): Distribution of liposomes all over the can be changed by the addition of various molecular components. Liposomes plays a crucial role in the area of drug delivery for its the ability of delivering toxic drugs to cancer cells for the treatment of cancer. Inside a tumor liposome can be accumulated as the process facilitates by the enhanced permeability and retention (EPR) effect which results in prolonged blood circulation. Added with

that, another factors which facilitates tumor accumulation are: liposome size , elasticity and charge of surface(Campbell et al., 2002; Krasnici et al., 2003). Change in Liposomal elasticity can be done by inclusion of cholesterol, surfactants and lipids as they change the length of acyl chain and the degree of saturation. Liposomes having low to moderate Young's Moduli (~0.045–28 MPa) can accumulate more inside the tumors in compared to high Young's modulus liposomes (Campbell et al., 2002; Wu et al., 2019).Another technique for increasing tumor accumulation of liposome is the surface modifying system of liposomes. Liposomes accumulation mainly occurs in lymphatic and renal organs. Minimal accumulation also occurs on some other organs, like the respiratory, nervous, cardiovascular system etc. By the help of this fact anti-fungal drug can be directly sent to kidney (amphoteric B).

.4.3 Liposomal compositions responsible for unique functionalities

By using stimuli responsive liposomal advantages usage of nanoparticles for site-specific delivery in therapy has become evolved and speed up. Some environmental stimuli, like pH, causes liposomal membrane to lose stability which enables local deposition of drug loading inside target tissue. Moreover, some external stimuli like heat and light can have impact on the site of release and the rate of release. Stimuli responsive liposomes increase the efficacy of anti-tumor and gene delivery because of their tumor microenvironment features, such as pH and enzymatic activity. By utilizing this feature drug delivery to specific tumor site become easier as well as efficient (Lee & Thompson, 2017).

4.3.1 pH responsive liposomes: They have special ability of targeting tumors and also the site if inflammation. In acidic environment these liposomes protonate the lipid bilayer components which then discompose the lipid bilayer causing them to rupture. As a result of this behavior drug payload is released if this type of liposomes has an interaction with acidic environment (Heidarli

et al., 2017; Simões et al., 2001). These pH responsive nature of liposomes can be optimized for maintaining essential features of liposomes like prolonged circulation duration by the optimization of destabilize nature of them and accumulation their payload efficaciously at desired pH. For circulation time maintenance adding lipid at a high phase temperature like DSPC, HSPC and soy PC as well as the additives like cholesterol and/or PEG can be successful method. Previously, PE was used to synthesize pH sensitive liposomes for stabilizing carriers at physiological pH. Recently, some other methods like fusion of pH sensitive and fusogenic components are being used (Simões et al., 2004).

4.3.2 Temperature responsive liposomes: They accumulate the payload if external heat is given, but here the membrane is destabilization mood is different. Traditionally they are comprised of lipids containing a transition temperature of gel-to-liquid phase or a transition temperature which is higher in compared to normal body temperature, that is ~42 C. In recent times, polymers that are temperature sensitive or lysolipids had been used for initialization of membrane destabilization, gradually after applying heat payload deposition occurred (Kono et al., 2010). If the temperature is higher than Tm fluidity and permeability of the lipid membrane are increased for allowing maximum drug release. Traditionally, thermo-sensitive liposomes are achieved by mixing the lipids with various phase transition temperatures which aids in disorder of lipid packing and enhancing cargo permeability. But applying a high thermal threshold for this process may result in damaging healthy tissue when heat is applied. For overcoming this issue, temperature sensitive polymers are used with lysolipids which decreases the phase transition temperature but also ensures rapid drug release (Porter & Ta, 2013).

4.3.3 Theranostic liposomes: These multipurpose nanomedicines combine a therapeutic substance with a diagnostic (such an imaging modality). Theranostic liposomes are multipurpose

nanomedicines for disease treatment and monitoring because they combine a therapeutic ingredient with a diagnostic (i.e. imaging) component (Seleci et al., 2017). A variety of imaging modalities, including magnetic resonance imaging (MRI), nuclear imaging, near-infrared fluorescence (NIR), etc., can be acquired using theranostic liposomes. (Large et al., 2021)

4.4 Liposome Technologies for Delivery of Therapeutics

Purpose of using different technologies is to protect distinctive properties of therapeutic substances as well as minimizing all possible drawbacks so that optimized drug delivery is achieved. These technologies have been evolved for the liposomal products that are used clinically.

4.4.1 Stealth Liposome Technology: For protecting active drug ingredients from the immune system of the recipient this technology has been evolved. This technique is aimed to stablish a drug delivery system where their detection is tough by the mononuclear phagocyte system. For this, polymer strands have been attached with active drug substances for improving safety and efficacy of the drug. In general, as polymer polyethylene glycol (PEG) is used (PEGylation). This PEGylation can be achieved if a reactive derivative of PEG along with the target moiety incubate together. Formation of covalent linkage between them protects the active drug substances from the immune system of the recipient, thus decreased immunogenicity and antigenicity are achieved. It also alters the physiochemical features of the active moiety, for example an alteration in hydrodynamic size, which results in decreased renal clearance and prolonged circulation time. Additionally, it imparts hydrophilicity to the drugs which are hydrophobic and reduces frequency of the dose. Changing these properties reduces toxicity despite of diminishing efficacy (Oh et al., 1995). Moreover, for their leaky nature of the tumor vasculature, nano particles having prolonged circulation imparts enhanced permeation and retention (EPR) and gradually accumulates inside the tumor bed. Doxil® is a successful liposomal product achieved by

this technique which is administered as an intravenous injection in the treatment of advanced ovarian cancer, multiple myeloma and HIV-related Kaposi's sarcoma. This technique also helps to acquire a customized dosage profile

4.4.2 Non-PEGylated Liposome Technology (NPL): This unique system is considered as a breakthrough in the therapy of cancer which offers the beneficial effect of PEGylated-liposome while elimination of PEG related side effects also achieved, hand-foot syndrome (HFS) is an example of this. NPL Doxorubicin (NPLD) injection offers a greater safety profile than the conventional DOX and Doxil®. NPLD decreases DOX associated cardiac toxicity, dose-limiting toxicity, like HFS. This feature is acquired by a mixture of specific composition and a unique formation process of the NPLD liposome that provides desirable physicochemical properties.

4.4.3 DepoFoam™ Liposome Technology: This is a proprietary, extended-release drug delivery technology, that is introduced by Pacira Pharmaceuticals, Inc., Parsippany, NJ, USA. DepoFoam™ is the core technology of many of the marketed products like, Depocyt®, DepoDur™, Exparel®. DepoFoam™ technology does the drug encapsulation in multivesicular liposomal platform despite doing modification any of their molecular structure. By this technique releasing of drug(s) requires 1 to 30 days (extended release). In DepoFoam™ formulation microscopic spheroids (3–30 μm) having granular structure and lipid particles of single-layer made of a honeycomb of several nonconcentric internal aqueous chambers contains the drug inside it. The particles carry various non-concentric aqueous chambers surrounded by a lipid membrane of single bilayer. Synthetic lipid bilayer membranes partitions each chamber from its adjacent chamber (Has & Sunthar, 2020). After DepoFoam™ administration release of drugs occurs over hours to weeks, consequently deterioration and/or reorganization of the lipid membranes occurs. By this unique technology particle release the drug over a period of hours to weeks following

erosion and/or reorganization of the lipid membranes. DepoFoam™ technology aids in the enhancement of the properties of both small and large molecules. All in all, this advantageous technology helps to improve patient care as it can provide expected solution for medications if frequent multiple injections are required and/or active ingredients with a short period of action or side effects.

4.4.4 Lysolipid Thermally Sensitive Liposome (LTSL) Technology: For releasing drugs at sites where temperature is higher **t**hermosensitive liposomes is used. Lipids (DPPC, MSPC etc.) with a transition temperature range from 40 to 45 °C are used in these liposomes composition. By this temperature-dependent release of encapsulated drug(s) is done. Radiofrequency ablation, a method based on the application of radiofrequency, often raises local tissue temperature to 42 °C. At high temperatures, the liposome's lipid components change from a gel to a liquid, increasing its permeability and enabling the release of the medicine. Additionally, the administration of local hyperthermia results in blood vessel leaking inside of tumors, which boosts the formation of liposomes inside the tumor. In a phase III clinical trial, Celsion Corporation's ThermoDox® encapsulates DOX using LTSL (lysolipid thermally sensitive liposome) technology to treat a variety of solid tumors. This method enables for a 25 times higher drug concentration in the treatment region for ThermoDox® than with intravenous (i.v.) DOX. In addition, DOX content in the blood considerably rises if compared to other liposomal encapsulated form of DOX.

Chapter 5
Breast Cancer Treatment Using Liposomal Drug Delivery

The principal cancer hallmarks of breast cancer are similar with many other cancers (Gutschner & Diederichs, 2012). But it has some unique features which make it different from other malignant neoplasms. Firstly, it starts to initiate from the cells of mammary gland, those are epithelial cells of embryonic origin and ducts and lobules forming morphology. Breast cancer tumors arising from mammary gland ducts develop ductal carcinoma, while transformed lobular cells form lobular carcinomas.

Breast cancer grows inside mammary gland inside its border of the main site and then it starts to spread. By this we come to know very early and small stage of breast cancer in situ and later, invasive carcinomas. Secondary colony forms after spreading of cancer to different organs or lymph nodes (metastasis). For breast cancer, metastasis most commonly occurs on lungs, liver and brain. The stage of breast cancer is detected by investigating the size of the tumor(original one), invasion extent and metastasis occurrence which includes all organs and lymph nodes, thus tumor (T), metastasis(M), node(N) is defining the stage of this cancer. At the same time, for checking the degree of malignant transformation of the tissue of mammary glands (for rate of mitosis, differentiation loss and nuclear polymorphism). Lastly this cancer's classification is done by degree of expression of some nuclear markers, namely, HER2-receptor, progesterone receptor(PR) and estrogen receptor(ER). Also, by this it is understood that for breast cancer there are two luminal subtype(ER+, PR+, HER2−), triple negative(basal-like) breast cancer(ER−, PR−, HER2−), HER2-enriched type(ER−, PR−, HER2+) and normal-like breast cancer(ER+, PR+, HER2−, with decreased mitotic rates). For breast cancer, this classification plays crucial role to select the

treatment strategy. For example, some of the types are hormone sensitive for which hormone-targeting treatment option is chosen (Higgins & Baselga, 2011). Again, classification links up between the phenotype od cancer cell with origin. For HER2-enriched breast cancer targeted therapy is chosen where ligands are targeted to HER2-receptor (Jamdade et al., 2015). For triple negative breast cancer treatment options are limited as there is no established molecular target yet which tells about the biological behavior. As a result, invasiveness of triple negative breast cancer is highest and occurrence of hepatic metastasis is higher than other subtypes. So prognosis and survival of various breast cancer are depending on the availability of treatment options and biological features.

Nanoparticle based drug delivery system depends on certain characteristics of the tumors. With age mammary glands (place where breast cancer initially develops) are replaced with adipose and fibrous connective tissues. Even though this results in an added vulnerability of the cells of mammary gland but, by this tumor microenvironment effect on its growth and treatment becomes understandable. For instance, two signaling pathways- pro-fibrotic and pro-angiogenic, like TGF B1 are overexpressed in advanced breast cancer (de Kruijf et al., 2013). Which is turn resulted in over accumulation og collagen connective tissue and formation of scar on that spot. Increased angiogenesis causes blood vessels overgrowth and unusual structure which results in interstitial pressure and enhanced permeability and retention effect (EPR) at the tumor become higher (Maeda et al., 2000). For nanoparticle based drug delivery system these factors cause changes in biodistribution. Moreover, nanoparticle and cancer cells interaction is dependent on particle's design and modification. Furthermore, liposome's morphology plays a crucial role in cell-

mediated endocytosis (Abumanhal-Masarweh et al., 2019). So, in order to optimize therapeutic outcomes, while designing liposomal nanoparticles all these factors should be considered.

5.1 Treatment by liposome-formulated drugs

There are many side effects and other drawbacks of conventional cancer treatment which can be overcome by liposomal drug delivery system. As liposome is versatile drug delivery system for many drug encapsulation, so by this system these problems can be solved (e.g. toxicity causes by chemotherapy).

5.1.1 Breast cancer active targeting by liposomes: Active targeting liposomes means liposomes that functions with targeting reagents who have elevated affinity towards overexpressed molecules by cells of interest so that selective delivery of therapeutic agents toward the primary metastatic tumors can be done. By this delivery system possibility and severity of toxic side effects can be reduced (Olusanya et al., 2018). Moreover, this system also able to overcome the resistance occurs by conventional system of drug delivery. Receptor mediated endocytosis occurs in the functionalized liposomes by targeting ligands so that rapid cellular internalization is achieved (Muley et al., 2020). A large variety of targeted ligands, such as antibodies, peptides, small compounds, aptamers, etc., have now been developed and evaluated in breast cancer treatment (in vitro and in vivo). Despite the fact that their production costs are significant and their process of conjugation is complicated (Chapman, 2002). Similar to this, aptamers (nucleic acid strands) have a very strong affinity and specificity towards the target while being prone to nucleic degradation with time and having the ability to produce immunogenicity (Mendes et al., 2015; Vandghanooni et al., 2018). Small molecules can be produced at lower costs and with less immunogenicity and cytotoxicity via simple conjugation with nanocarriers (Yoo et al., 2019). Certain receptors are overexpressed in cancer cells, such as the folate receptor, which uses small molecules as a targeted

ligand (Sneider et al., 2017). Again, peptides provide excellent binding specificity and affinity affordable manufacturing costs, and less immunogenicity since of their smaller molecular size and lighter weight (Mendes et al., 2015).

5.1.2 Drug delivery utilizing triggerable liposomes for the treatment of breast cancer:

Recently, advancement has been done in the release of encapsulated drugs from liposomes. Many type of triggering method have been explored in order to stimulate liposomes for immediate release of drugs. Internal triggers include pH variation, enzyme effects etc. and external triggers include light, magnetic field, heat, ultra sound etc. They are applied in preclinical treatment of breast cancer.

pH-sensitive liposomes: extracellular pH of tumor cells is comparatively higher than the normal ones as a result of their production of lactate and enhanced hydrolysis of ATP. For this factor, pH sensitive liposomes maintaining stability at normal physiological state and disassemble drugs in low pH(acidic) microenvironment have been engineered (Karanth & Murthy, 2010). Releasing therapeutic payload, they are able to respond to pH variation among normal and cancer tissues. Trozan horse liposome, a pH sensitive liposome which encapsulates PTX for treatment of breast cancer (Jiang et al., 2016) to form DLD/PTX Lips, a pH-responsive dimethylmaleic amide (DMA) bond into 1,2-distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE) was introduced by the liposome via a lysine linker. Cleavage of DMA amide in a weak acidic pH microenvironment causes the change in liposome's zeta-potential to a positive state from the negative state, facilitating intercellular uptake as well as endosomal escape. Resulting in higher PTX release by the liposome and accumulation of drug occurs in tumor cells was increased. In vitro, DLD/PTX-Lips showed a very high cytotoxic effect to 4T1 murine cells of breast cancer than independent PTX at concentrations of 0.01-5 g/mL range. In vivo tumor inhibition efficiency was evaluated by

a rodent model with 4T1 cells. The rate of inhibition of the cancer by DLD/PTX-Lips was 57.4%, which was substantially more than that of independent PTX that is 25.1% and conventional liposome that is 30.4%. Furthermore, when bound with pH-sensitive liposomes for targeted delivery, certain ligands may promote receptor-mediated endocytosis (de Oliveira Silva et al., 2019). Silva et al. created folate-coated, DOX-loaded pH-sensitive liposomes (SpHL DOX-Fol), in which the liposome surface conjugation occured with folate ligand (de Oliveira Silva et al., 2019). Releasing of DOX was enhanced from 21.5% ±3.9% to 53.6% ±5.7% while lowering the pH to 5.0 from 7.4. In 4T1 cell viability this demonstrated that low concentration (0.15 μM) can have greater cytotoxicity than free drug, with no satisfactory difference. Independent DOX contrary to liposome-formulated DOX, the temperature sensitive liposomes' in vivo antitumor activities were found to be more effective (68% tumour growth decreased) in BALB/c mice harboring 4T1 cells.

Thermo-sensitive liposomes (TSL): In the gelatinous state, the lipid membrane structure of TSL is firmly packed at normal physiological temperature, preventing the encapsulated medication from diffusing by the membrane. However, when these liposomes are heated to transition temperature (Tm), the lipids experience a gel-to-liquid phase change. For instance, the Tm of dipalmitoylphosphatidylcholine (DPPC) is 41.5 °C. This caused structural destabilization and drug release. For the treatment of breast cancer, many TSL-encapsulating anticancer medicines have been created. To enhance the drug's antitumor effects, a new thermo-sensitive liposome encapsulating DTX (DTX-TL). The release of drug at 37 °C is much lower than that at 42 °C, according to in vitro tests, demonstrating the influence of temperature on drug release. For in vivo drug release, a handmade hyperthermia device coupled to a thermostatic circulator was used to heat the tumor of a rodent model carrying MCF-7 cells at the temperature of 42 °C for half an

hour. The research showed that, when compared to mouse groups treated with various conditions, mice treated with TSL had the greatest tumor size decrease. TSL may be designed to administer two medications through a single platform, increasing therapeutic effectiveness. Jose et al. developed TSL to administer tamoxifen and imatinib synergistically for the treatment of breast cancer (Jose et al., 2019). After the temperature is raised over the transition point of 39.4 °C, more than 80% of the medicines were released from the TSL in half an hour. At a similar concentration, liposomes containing the singlet drug (for tamoxifen and 43.0±3.3% for imatinib) in comparison with the growth inhibition of MCF-7 cells treated with this liposome formulation co-encapsulating 5 μM tamoxifen and 3.75 M imatinib increased to 86.31.5% at 40 °C. It was also noted that the identical liposomes had improved in vitro therapeutic effectiveness in MDA-MB-231 cells, with a growth inhibition of 66.5±3.9%. TSL demonstrated the advantages of temperature-triggered medication release and the hyperthermia effect when combined with chemotherapy and thermotherapy. In addition to releasing chemotherapy drugs when heated, hyperthermia directly kills cancer cells in the exposed region, increasing therapeutic effectiveness (Porter & Ta, 2013). In order to create moderate hyperthermia and release DOX from TSL upon irradiation by a near-infrared laser with a wavelength of 808 nm, Ou et al. used gold nanoantennas. In order to activate hyperthermia and drug release from TLS concurrently, the energy transfer from light to heat was made possible by the special geometry of multibranched gold plated on the liposomes' surface (Ou et al., 2016). Even at a low drug concentration of 0.5 g/mL, in vitro tests revealed that such TSL was more hazardous to MDA-MB-231 cells than free DOX (33% vs. 17%). The in vivo therapeutic efficacy of the combination therapy via the TSL was not, however, confirmed in this investigation. In Phase I/II clinical studies for cancer treatment, ThermoDOX® was the only thermosensitive liposome formulation researchers are aware of (Celsion, 2009). Lysolipids were added to the

formulation of ThermoDOX® to slow the liposome phase transition at room temperature, significantly accelerated drug release when heated. Needham et al. developed this lysolipid-based liposome formulation using DOX, and Celsion Corp. has invested in it (Needham et al., 2000). It was used to treat patients with breast cancer with chest wall recurrence by combining heat and chemotherapy.

Light-sensitive liposomes: Due to its adjustable spectrum features, illumination intensities, and timing, external light source is a useful stimulation used to activate the liposomes' on-demand release. Furthermore, the ability to precisely regulate the release of cargo is made possible by the spatial and temporal control of light sources. The mechanism of light-sensitive liposomes can be divided into two categories: photochemical activation effect, which includes photosensitization-induced oxidation, photocleavage, photoisomerization; and photophysical effect, which includes inorganic nanomaterials, plasmonic nanoparticles and molecular absorbers (Chen et al., 2020). Reactive oxygen species (ROS) are produced by photosensitizers (PSs) when light exposure activation occurs at certain wavelengths in photosensitization-induced oxidation techniques (Albert W. Girotti, 1990; Karanth & Murthy, 2010; Pooler, 1989; Robertson et al., 2009). One kind of ROS produced by photosensitizers is singlet oxygen, which possesses unpaired electrons and unstable bonds. Singlet oxygen may oxidize the unsaturated carbon-carbon link in lipid chains to create hydroperoxides, which then cause the lipid bilayers to break down (Tejero et al., 2004). By include PS in the formulation of the liposomes, it is possible to create light-sensitive liposomes based on the triggering mechanism stated above. The lipid components of the liposome were oxidized, leading to the destabilization of the liposome structure, when PS was activated under light irradiation to produce singlet oxygen or other ROS. Verteporfn is a well-known PS used to treat tumors such as dermatological, small cell lung, head and neck, gastroenterological, brain,

ophthalmic and gynecological cancers. It is also clinically licensed for the photodynamic therapy (PDT) of macular degeneration (Z., 2005). To treat triple negative breast cancer (TNBC), Sneider et al. created liposomes containing verteporfn (Sneider et al., 2016). To provide liposomes the ability to absorb more nutrients from cells and capture cancer-related tar, DSPE PEG2000-folic acid was added to the liposomes. A 33% cell viability was seen in MDA-MB-231 cells treated with the light-sensitive liposomes at 690 nm light, according to in vitro tests. Although this work used light-sensitive liposomes to apply PDT effect to cancer cells, medication release might potentially be accomplished with the use of this triggering mechanism. The limitations of the light source utilized in this study prevent visible light (380–740 nm) from being used for in vivo treatments. First, the visible light's limited tissue penetration depth prevents it from properly treating deep tissues. Secondly, several endogenous fuorophores, such as the pigments in the epidermis, hemoglobin, and chlorophyll, can absorb light energy in the 200-650 nm range (Son et al., 2019).

Ultrasound- and magnetic-sensitive liposomes: In addition to the TSL outlined above, magnetic fields and ultrasound waves were extensively investigated as an external triggering mechanism (Alawak et al., 2021; Amrahli et al., 2021). Due to the physical characteristics of magnetic fields and acoustic waves, local heat may be produced from these two external sources with high intensities, making it more effective and non-invasive for treating specific tumor sites. They both demonstrated remarkable tissue penetration abilities as well (Van Ballegooie et al., 2019). High-intensity focused ultrasound (HIFU) or magnetic fields can destroy cancer cells through the process of hyperthermia in addition to causing medication release from TSL. On stage IV HER2-negative breast cancer patients, magnetic resonance guided HIFU in combination with ThermoDox® was being studied in a phase I clinical trial (Maar et al., n.d.). Another method of

activating liposomes that are sensitive to ultrasound relies on mechanical cavitation by incorporation of the liposomes with the microbubbles. Stable cavitation or internal cavitation will happen as a result of the ultrasonic triggering, depending on the strength and frequency of the ultrasound waves as well as the size and characteristics of the microbubbles. Lower intensities cause the microbubbles to oscillate (stable cavitation), which causes fluid convection and localized spinning. The drug release can be caused by liposomes rupturing and deforming as a result of the associated shear forces in the surrounding fluid (Marmottant & Hilgenfeldt, 2003). Low-intensity ultrasound has a negligible impact on the chemical composition and anti-tumor effects of medicines that are encapsulated (Schroeder et al., 2007). Internal cavitation, in contrast to stable cavitation, occurs when high intensity ultrasound causes microbubble collapses and shockwave generation, both of which can enhance membrane permeability (Pecha & Gompf, 2000). This impact not only triggers the release of the medication from liposomes but also makes it easier for cells to absorb liposomes. Additionally, the utilization of magnetic-sensitive liposomes for MRI-guided cancer treatment is possible (Song et al., 2019). In order to accomplish MRI and medication release concurrently, magnetic nanoparticles (MNPs), such as iron oxides, are often enclosed in liposomes. The liposomes may be ruptured mechanically by the motions of MNPs that are aligned with external magnetic fields. Furthermore, external magnetic field direction can also promote liposome accumulation at the location of the tumor (Y. Wang & Kohane, 2017).

5.1.4 Breast cancer gene therapy using liposomes: Each breast cancer subtype was linked to gene mutations that led to the malignant transformation of certain breast cells. In particular, for triple negative breast cancers, which cannot be effectively treated by effective targeted treatments due to the absence of receptors, gene therapy is a potential technique for treating breast cancer subtypes harboring different genetic abnormalities (Bottai et al., 2017).

Potential gene delivery devices, cationic liposomes naturally interact with negatively charged DNA (Simões et al., 2005). Complex nucleic acids can be shielded by liposome bilayers against cellular lysis and antibody neutralization (Judge et al., 2006). Additionally, the positive charge of cationic liposomes may make it easier for them to engage with the negatively charged cell membrane through endocytosis, leading to more effective cellular absorption and content release into the cytoplasm. Plasmids and oligonucleotides are encapsulated in cationic liposomes in the method for cancer gene therapy. We'll talk separately about the CRISPR/Cas9 system, which is the most promising gene-editing tool for cancer gene therapy.

5.1.5 Liposome-formulated oligonucleotide therapeutic: Short synthesized nucleic acids known as oligonucleotides have the potential to cure or manage a variety of ailments (Roberts et al., 2020) . By attaching to certain regions in a genome or RNA, these gene agents can modify the levels of expression of protein-coding genes (Wan et al., 2013). Small interfering ribonucleic acids (siRNAs) and antisense oligonucleotides (ASOs) were the oligonucleotide-based treatments that were most thoroughly investigated and applied in both laboratory and clinical settings for breast cancer treatment. ASOs are distinct from messenger RNAs (mRNA), which are accountable for protein coding and are made up of a single strand of RNA. Through hybridisation to a particular mRNA region, which prevents the creation of the corresponding proteins. A non-coding RNA (ncRNA) segment is carried by ASOs as well. SiRNA are double-stranded RNA molecules that have been created synthetically. They are frequently employed for temporary gene silencing, which entails the creation of a sequence unique to the target mRNA (J. Wang et al., 2010). Through a mechanism involving the RNA-induced silencing protein-complex (RISC), siRNA cuts the mRNA. More clinical studies on cancer therapy will be made possible by the effectiveness of ASO and siRNA-based medicines. These drugs' limited cellular uptake, quick disintegration and quick

renal clearance after systemic injection presented some difficulties for their use, albeit (Ahmadzada et al., 2018; Eloy et al., 2018). Many attempts have been undertaken to produce nanocarriers carrying siRNA and ASO, such as liposomes, which are capable of being an effective vehicle having safety profiles and enhanced effectiveness, in order to get around these restrictions and improve therapeutic results. An oncogenic miRNA overexpressed on breast tumors and associated with the course of malignant transformation for suppressing miRNA-191. A cationic liposomal delivery system loaded with ASO was designed as well (S. Sharma et al., 2017). liposomal delivery platform's in vitro inhibitory effectiveness was examined in ZR-75-1 and MCF-7 cell lines of breast cancer after encapsulating the appropriate antisense oligonucleotide against miRNA-191. The scientists discovered that anti-miR-191 transfection efficiency in breast cancer cells was improved by liposome-mediated anti-miR-191 delivery. Another intriguing finding from this research suggested that breast cancer cell proliferation might be inhibited by the modified liposomes alone. Therefore, the synergistic impact of stearylamine-liposome combined with anti-miR-191 revealed increased levels of cell apoptosis and migratory suppression, as well as increased chemosensitivity of breast cancer cells to anti-cancer medications (S. Sharma et al., 2017). Through the use of cationic liposome delivery methods, another recent study revealed synergistic anti-tumor effect of PTX and siRNA that targets Polo-like kinase 1 (PLK-1) in breast cancer (Yu et al., 2019). These liposomes were designed to co-load PTX and siPLK-1, and their surface was modified with a targeting aptamer (AS1411) to improve their ability to target tumors. After being treated with liposomes, PLK1 mRNA expression in breast cancer cells (MCF-7) was noticeably downregulated, with a 79% knockdown. Additionally, after being treated with such liposomes, tumor-bearing mice's survival rates increased and tumor development was markedly suppressed. Chemotherapeutic medicines and siRNA delivered simultaneously via this liposome

technology may have a synergistic anti-breast cancer impact. Although cationic liposomes were the most popular and extensively researched nanocarriers for the transport of siRNA and ASO, they may alter the behavior of cells by causing cell shrinkage, fewer mitoses, and cytoplasmic vacuolization (Kedmi et al., 2010). As a result, non-cationic liposomes' potential as gene delivery devices was looked at. An siRNA-protamine (siRNA/prot) complex-loaded non-cationic liposomal delivery method was created by Alshaer et al. In order to aggressively target TNBC cells that express CD44, its surface was further modified with the anti-CD44 aptamer (Apt1) (Alshaer et al., 2018). The cell surface glycoprotein CD44, which is superficially overexpressed on tumors, is a suitable targeting receptor for targeted therapies. Both in vitro (MDA-MB-231-Luc2- eGFP cells) and in vivo environments were used to investigate the targeted liposomal system's ability to silence the luciferase (luc2) gene (TNBC mouse model). Compared to non-targeted liposomes (47.2±10.6%), Apt1 functionalized liposomes showed the greatest in vitro gene silencing activity in the cells they were applied to (25.7±15.1% gene expression level). When compared to the PBS control group, the in vivo research further showed that Apt1 functionalized liposomes significantly reduced the level of Luc2 mRNA expression. Additionally, the detected bioluminescence signal from the tumors illustrates how the siRNA-loaded liposome systems might prevent the growth of tumors. MicroRNA is a substitute gene treatment for breast cancer (miRNA). A miRNA is a small non-coding RNA molecule of 20 nucleotides or less. It was discovered in plants, animals, and certain viral cells, and it has the ability to control post-transcriptional gene expression (Cannell et al., 2008). By base-pairing with complementary sequences found in mRNA molecules, miRNA serve as a guide to inhibit the production of mRNA. Through the use of designed extrinsic miRNA, this characteristic is utilized to silence a particular oncogene. For cancer gene therapy, a variety of miRNA formulations have been investigated (Mathiyalagan & Sahoo, 2017; Rupaimoole & Slack,

2017). Yan et al. created a useful miRNA liposome to treat TNBC by shutting down the Sluggene. Functional liposomes modified with the DSPE-PEG2000-tLyp-1 peptide were used to encapsulate the miRNA's 25-nucleotide sense strand. According to in vitro findings, TNBC cells' ability to invade and develop was decreased by silencing the Slug gene and inhibiting the TGF-1/Smad pathway. Functional miRNA liposomes and functional vinorelbine liposomes were combined to provide virtually total tumor growth suppression and a higher anticancer effect than functional vinorelbine liposomes in TNBC cancer-bearing mice (Yan et al., 2019).

5.1.6 CRISPR therapies using liposome formulation: In the past years, new genetic editing techniques like transcription activator-like effector nucleases (TALENs), clustered regularly inter spaced short palindromic repeats (CRISPR) and zinc-finger nucleases (ZFNs) have emerged as a prominent therapeutic option for many cancers, including breast cancer. Due to the ease of preparation, high gene editing effectiveness, and simultaneous editing of numerous loci, CRISPR has emerged as a viable substitute for TALENs and ZFNs among these genome editing methods. Comparatively speaking, it became highly appropriate for clinical or preclinical applications. In this method, the guide RNA (gRNA) repair template directs the introduction of a double-stranded break (DSB) DNA molecule’s target region by a nuclease protein called Cas9. This allows for the inclusion of a new sequence into the genome. CRISPR has so far proven successful in treating primary immune system abnormalities, hemophilia, hemoglobinopathies, muscular dystrophy and metabolic diseases using CAR-T immunotherapy for cancer (Xia et al., 2019). Through the creation of therapies that may selectively alter the expression of disease-relevant genes, significant progress has recently been achieved in the clinical uses of CRISPR. However, this technology is still in its infancy and has not yet undergone a clinical trial for the treatment of breast cancer. This is a result of ineffective delivery mechanisms, poor transfection

effectiveness, rapid biodegradability, and possible off-target effects (Tao et al., 2019). The majority of CRISPR transfections have been carried out using viral-based delivery vehicles. The primary obstacle, however, was brought on by viral vectors' induction of CRISPR/Cas9-specific immunogenicity (Mintz et al., 2018). Different non-viral delivery tactics, such as liposome delivery systems, have been investigated and developed as a possible delivery alternative (Jain et al., 2020). Although there aren't many studies about liposome-based CRISPR treatments for breast cancer right now, they seem to hold promise for the future of cancer gene therapy (Eoh & Gu, 2019). The insufficient transfection efficiency of CRISPR was solved by Zhang et al. using a cationic liposomal method. A Cas9/single-guide RNA (sgRNA) plasmid was enclosed in a polyethylene-glycol-phospholipid-modified (PLNP) liposome system that was created by the scientists (DNA). The scientists chose to use these nanocarriers to knock down the polo-like kinase 1 (PLK-1) gene, a key regulator of cancer cell proliferation, in order to show the effectiveness of such modified liposomes for transfection. Breast cancer cells (MCF-7) treated with PLNP including CRISPR/sgRNA plasmid showed a better transfection efficiency of 37.8% in in vitro transfection experiments, as compared to Lipofectamine2000 (a commercial liposome transfection agent), which showed just 3.15%. The in vivo therapeutic effects of this liposome-formulated CRISPR technology on breast cancer were not demonstrated in this study. However, the in vivo efficacy of these liposomes in a mouse model carrying melanoma cells was asserted by the scientists (A375). Guo et al. used a noncationic, tumor-targeting liposome-hydrogel hybrid technology to eliminate the breast cancer-promoting gene Lipocalin 2 (Lcn2) through CRISPR technique. This method contained three CRISPR plasmids that encoded the Cas9 nuclease and a guide RNA sequence to recognize and disrupt the Lcn2 gene in the targeted human TNBC cells' genomes. Lcn2 mRNA expression levels in TNBC cells were significantly decreased, as evidenced

by the in vitro genome editing effectiveness, with 80% of Lcn2 deletion found in both MDA MB-231 and MDA-MB-436 cell lines. This liposome system's therapeutic efficacy was evaluated in vivo using a mouse model carrying MDA-MB-231 cells. When compared to other treatment scenarios, the mouse group treated with nanocarriers showed a considerable suppression of tumor development by 77% in volume. According to the findings of these two research, liposomes are a potential delivery formulation for improving CRISPR transfection, which will thereafter have a therapeutic effect on breast cancer. It is noteworthy that the authors of these two research employed CRISPR plasmid DNA to accomplish the desired gene knockdown result. Due to the relatively long time that plasmids remain within cells, the main problem with plasmid DNA was high rates of unintentional gene editing, which would hinder the clinical application of CRISPR technology (Peng et al., 2016).

Chapter 6: Conclusions

Rapid advancements in the experimental advancement of liposomal delivery systems are being made in response to the desire for fresh approaches to the treatment of breast cancer. On the creation of the novel liposome formulation for breast cancer, there is, however, no comprehensive knowledge or road plan. Most publications base their choice of the targeting and triggering modalities in significant part on the molecular subtypes of the tumor and the currently being used conventional therapies. There are several obstacles to the clinical implementation of these novel liposome formulations, despite the fact that standard development of chemotherapeutic liposomal medicines has been utilized extensively the clinical setting of treating breast cancer. When creating these liposome formulations, more research into the triggering mechanisms for the triggerable liposomes is required.

Reference

Abumanhal-Masarweh, H., da Silva, D., Poley, M., Zinger, A., Goldman, E., Krinsky, N., Kleiner, R., Shenbach, G., Schroeder, J. E., Shklover, J., Shainsky-Roitman, J., & Schroeder, A. (2019). Tailoring the lipid composition of nanoparticles modulates their cellular uptake and affects the viability of triple negative breast cancer cells. *Journal of Controlled Release*, *307*, 331–341. https://doi.org/10.1016/j.jconrel.2019.06.025

Ahmadzada, T., Reid, G., & McKenzie, D. R. (2018). Fundamentals of siRNA and miRNA therapeutics and a review of targeted nanoparticle delivery systems in breast cancer. *Biophysical Reviews*, *10*(1), 69–86. https://doi.org/10.1007/s12551-017-0392-1

Alawak, M., Abu Dayyih, A., Mahmoud, G., Tariq, I., Duse, L., Goergen, N., Engelhardt, K., Reddy Pinnapireddy, S., Jedelská, J., Awak, M., König, A. M., Brüßler, J., Bartsch, J. W., & Bakowsky, U. (2021). ADAM 8 as a novel target for doxorubicin delivery to TNBC cells using magnetic thermosensitive liposomes. *European Journal of Pharmaceutics and Biopharmaceutics*, *158*, 390–400. https://doi.org/10.1016/j.ejpb.2020.12.012

Albert W. Girotti. (1990). Photodynamic Lipid Peroxidation in Biological Systems*. *Photochemistry and Photobiology*, *51*(4), 497–509. http://dx.doi.org/10.1111/j.1751-1097.1990.tb01744.x

Ali, M. H., Moghaddam, B., Kirby, D. J., Mohammed, A. R., & Perrie, Y. (2013). The role of lipid geometry in designing liposomes for the solubilisation of poorly water soluble drugs. *International Journal of Pharmaceutics*, *453*(1), 225–232. https://doi.org/10.1016/j.ijpharm.2012.06.056

Allen, T. M. (1994). The use of glycolipids and hydrophilic polymers in avoiding rapid uptake of

liposomes by the mononuclear phagocyte system. *Advanced Drug Delivery Reviews*, *13*(3), 285–309. https://doi.org/10.1016/0169-409X(94)90016-7

Alshaer, W., Hillaireau, H., Vergnaud, J., Mura, S., Deloménie, C., Sauvage, F., Ismail, S., & Fattal, E. (2018). Aptamer-guided siRNA-loaded nanomedicines for systemic gene silencing in CD-44 expressing murine triple-negative breast cancer model. *Journal of Controlled Release*, *271*, 98–106. https://doi.org/10.1016/j.jconrel.2017.12.022

Amrahli, M., Centelles, M., Cressey, P., Prusevicius, M., Gedroyc, W., Xu, X. Y., So, P. W., Wright, M., & Thanou, M. (2021). Mr-labelled liposomes and focused ultrasound for spatiotemporally controlled drug release in triple negative breast cancers in mice. *Nanotheranostics*, *5*(2), 125–142. https://doi.org/10.7150/ntno.52168

Betker, J. L., Jones, D., Childs, C. R., Helm, K. M., Terrell, K., Nagel, M. A., & Anchordoquy, T. J. (2018). Nanoparticle uptake by circulating leukocytes: A major barrier to tumor delivery. *Journal of Controlled Release*, *286*, 85–93. https://doi.org/10.1016/j.jconrel.2018.07.031

Bottai, G., Truffi, M., Corsi, F., & Santarpia, L. (2017). Progress in nonviral gene therapy for breast cancer and what comes next? *Expert Opinion on Biological Therapy*, *17*(5), 595–611. https://doi.org/10.1080/14712598.2017.1305351

Campbell, R. B., Fukumura, D., Brown, E. B., Mazzola, L. M., Izumi, Y., Jain, R. K., Torchilin, V. P., & Munn, L. L. (2002). Cationic charge determines the distribution of liposomes between the vascular and extravascular compartments of tumors. *Cancer Research*, *62*(23), 6831–6836.

Cannell, I. G., Kong, Y. W., & Bushell, M. (2008). How do microRNAs regulate gene expression? *Biochemical Society Transactions*, *36*(6), 1224–1231. https://doi.org/10.1042/BST0361224

Celsion. (2009). Phase 1/2 Study of ThermoDox With Approved Hyperthermia in Treatment of

Breast Cancer Recurrence at the Chest Wall (DIGNITY). *Https://Www.Clinicaltrials.Gov/Ct2/Show/NCT00826085?Term=thermodox+rcw&rank=1*, NCT00826085. https://www.clinicaltrials.gov/ct2/show/NCT00826085?term=thermodox+rcw&rank=1

Chapman, A. P. (2002). PEGylated antibodies and antibody fragments for improved therapy: A review. *Advanced Drug Delivery Reviews*, *54*(4), 531–545. https://doi.org/10.1016/S0169-409X(02)00026-1

Charrois, G. J. R., & Allen, T. M. (2004). Drug release rate influences the pharmacokinetics, biodistribution, therapeutic activity, and toxicity of pegylated liposomal doxorubicin formulations in murine breast cancer. *Biochimica et Biophysica Acta - Biomembranes*, *1663*(1–2), 167–177. https://doi.org/10.1016/j.bbamem.2004.03.006

Chen, W., Goldys, E. M., & Deng, W. (2020). Light-induced liposomes for cancer therapeutics. *Progress in Lipid Research*, *79*. https://doi.org/10.1016/j.plipres.2020.101052

Christensen, D., Korsholm, K. S., Rosenkrands, I., Lindenstrøm, T., Andersen, P., & Agger, E. M. (2007). Cationic liposomes as vaccine adjuvants. *Expert Review of Vaccines*, *6*(5), 785–796. https://doi.org/10.1586/14760584.6.5.785

Dadashzadeh, S., Mirahmadi, N., Babaei, M. H., & Vali, A. M. (2010). Peritoneal retention of liposomes: Effects of lipid composition, PEG coating and liposome charge. *Journal of Controlled Release*, *148*(2), 177–186. https://doi.org/10.1016/j.jconrel.2010.08.026

de Kruijf, E. M., Dekker, T. J. A., Hawinkels, L. J. A. C., Putter, H., Smit, V. T. H. B. M., Kroep, J. R., Kuppen, P. J. K., Van de Velde, C. J. H., Ten Dijke, P., Tollenaar, R. A. E. M., & Mesker, W. E. (2013). The prognostic role of TGF-β signaling pathway in breast cancer patients. *Annals of Oncology*, *24*(2), 384–390. https://doi.org/10.1093/annonc/mds333

de Oliveira Silva, J., Fernandes, R. S., Ramos Oda, C. M., Ferreira, T. H., Machado Botelho, A. F., Martins Melo, M., de Miranda, M. C., Assis Gomes, D., Dantas Cassali, G., Townsend, D. M., Rubello, D., Oliveira, M. C., & de Barros, A. L. B. (2019). Folate-coated, long-circulating and pH-sensitive liposomes enhance doxorubicin antitumor effect in a breast cancer animal model. *Biomedicine and Pharmacotherapy*, *118*. https://doi.org/10.1016/j.biopha.2019.109323

Eloy, J. O., Petrilli, R., Raspantini, G. L., & Lee, R. J. (2018). Targeted Liposomes for siRNA Delivery to Cancer. *Current Pharmaceutical Design*, *24*(23), 2664–2672. https://doi.org/10.2174/1381612824666180807121935

Eoh, J., & Gu, L. (2019). Biomaterials as vectors for the delivery of CRISPR-Cas9. *Biomaterials Science*, *7*(4), 1240–1261. https://doi.org/10.1039/c8bm01310a

Forssen, E. A., & Tokes, Z. A. (1981). Use of anionic liposomes for the reduction of chronic doxorubicin-induced cardiotoxicity. *Proceedings of the National Academy of Sciences of the United States of America*, *78*(3 I), 1873–1877. https://doi.org/10.1073/pnas.78.3.1873

Gabizon, A., Shiota, R., & Papahadjopoulos, D. (1989). Pharmacokinetics and tissue distribution of doxorubicin encapsulated in stable liposomes with long circulation times. *Journal of the National Cancer Institute*, *81*(19), 1484–1488. https://doi.org/10.1093/jnci/81.19.1484

Gutschner, T., & Diederichs, S. (2012). The hallmarks of cancer. *RNA Biology*, *9*(6), 703–719. https://doi.org/10.4161/rna.20481

Haran, G., Cohen, R., Bar, L. K., & Barenholz, Y. (1993). Transmembrane ammonium sulfate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases. *BBA - Biomembranes*, *1151*(2), 201–215. https://doi.org/10.1016/0005-2736(93)90105-9

Has, C., & Sunthar, P. (2020). A comprehensive review on recent preparation techniques of

liposomes. *Journal of Liposome Research*, *30*(4), 336–365. https://doi.org/10.1080/08982104.2019.1668010

He, H., Yuan, D., Wu, Y., & Cao, Y. (2019). Pharmacokinetics and pharmacodynamics modeling and simulation systems to support the development and regulation of liposomal drugs. *Pharmaceutics*, *11*(3). https://doi.org/10.3390/pharmaceutics11030110

Heidarli, E., Dadashzadeh, S., & Haeri, A. (2017). State of the art of stimuli-responsive liposomes for cancer therapy. *Iranian Journal of Pharmaceutical Research*, *16*(4), 1273–1304.

Hemmer, E., Acosta-Mora, P., Méndez-Ramos, J., & Fischer, S. (2017). Optical nanoprobes for biomedical applications: Shining a light on upconverting and near-infrared emitting nanoparticles for imaging, thermal sensing, and photodynamic therapy. *Journal of Materials Chemistry B*, *5*(23), 4365–4392. https://doi.org/10.1039/c7tb00403f

Higgins, M. J., & Baselga, J. (2011). Targeted therapies for breast cancer. *Journal of Clinical Investigation*, *121*(10), 3797–3803. https://doi.org/10.1172/JCI57152

Inoh, Y., Hirose, T., Yokoi, A., Yokawa, S., & Furuno, T. (2020). Effects of lipid composition in cationic liposomes on suppression of mast cell activation. *Chemistry and Physics of Lipids*, *231*. https://doi.org/10.1016/j.chemphyslip.2020.104948

Jain, V., Kumar, H., Anod, H. V., Chand, P., Gupta, N. V., Dey, S., & Kesharwani, S. S. (2020). A review of nanotechnology-based approaches for breast cancer and triple-negative breast cancer. *Journal of Controlled Release*, *326*, 628–647. https://doi.org/10.1016/j.jconrel.2020.07.003

Jamdade, V. S., Sethi, N., Mundhe, N. A., Kumar, P., Lahkar, M., & Sinha, N. (2015). Therapeutic targets of triple-negative breast cancer: A review. *British Journal of Pharmacology*, *172*(17), 4228–4237. https://doi.org/10.1111/bph.13211

Jiang, L., Li, L., He, B., Pan, D., Luo, K., Yi, Q., & Gu, Z. (2016). Anti-cancer efficacy of Paclitaxel loaded in pH triggered liposomes. *Journal of Biomedical Nanotechnology*, *12*(1), 79–90. https://doi.org/10.1166/jbn.2016.2123

Jose, A., Ninave, K. M., Karnam, S., & Venuganti, V. V. K. (2019). Temperature-sensitive liposomes for co-delivery of tamoxifen and imatinib for synergistic breast cancer treatment. *Journal of Liposome Research*, *29*(2), 153–162. https://doi.org/10.1080/08982104.2018.1502315

Judge, A. D., Bola, G., Lee, A. C. H., & MacLachlan, I. (2006). Design of noninflammatory synthetic siRNA mediating potent gene silencing in vivo. *Molecular Therapy*, *13*(3), 494–505. https://doi.org/10.1016/j.ymthe.2005.11.002

Kapoor, M., Lee, S. L., & Tyner, K. M. (2017). Liposomal Drug Product Development and Quality: Current US Experience and Perspective. *AAPS Journal*, *19*(3), 632–641. https://doi.org/10.1208/s12248-017-0049-9

Karanth, H., & Murthy, R. S. R. (2010). pH-Sensitive liposomes-principle and application in cancer therapy. *Journal of Pharmacy and Pharmacology*, *59*(4), 469–483. https://doi.org/10.1211/jpp.59.4.0001

Kedmi, R., Ben-Arie, N., & Peer, D. (2010). The systemic toxicity of positively charged lipid nanoparticles and the role of Toll-like receptor 4 in immune activation. *Biomaterials*, *31*(26), 6867–6875. https://doi.org/10.1016/j.biomaterials.2010.05.027

Kono, K., Ozawa, T., Yoshida, T., Ozaki, F., Ishizaka, Y., Maruyama, K., Kojima, C., Harada, A., & Aoshima, S. (2010). Highly temperature-sensitive liposomes based on a thermosensitive block copolymer for tumor-specific chemotherapy. *Biomaterials*, *31*(27), 7096–7105. https://doi.org/10.1016/j.biomaterials.2010.05.045

Krasnici, S., Werner, A., Eichhorn, M. E., Schmitt-Sody, M., Pahernik, S. A., Sauer, B., Schulze, B., Teifel, M., Michaelis, U., Naujoks, K., & Dellian, M. (2003). Effect of the surface charge of liposomes on their uptake by angiogenic tumor vessels. *International Journal of Cancer*, *105*(4), 561–567. https://doi.org/10.1002/ijc.11108

Kuang, Y., Liu, J., Liu, Z., & Zhuo, R. (2012). Cholesterol-based anionic long-circulating cisplatin liposomes with reduced renal toxicity. *Biomaterials*, *33*(5), 1596–1606. https://doi.org/10.1016/j.biomaterials.2011.10.081

Large, D. E., Abdelmessih, R. G., Fink, E. A., & Auguste, D. T. (2021). Liposome composition in drug delivery design, synthesis, characterization, and clinical application. *Advanced Drug Delivery Reviews*, *176*, 113851. https://doi.org/10.1016/j.addr.2021.113851

Lee, Y., & Thompson, D. H. (2017). Stimuli-responsive liposomes for drug delivery. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *9*(5). https://doi.org/10.1002/wnan.1450

Maar, J. de, Suelmann, B., Braat, M., open, P. van D.-B., & 2020, undefined. (n.d.). Phase I feasibility study of Magnetic Resonance guided High Intensity Focused Ultrasound-induced hyperthermia, Lyso-Thermosensitive Liposomal. *Bmjopen.Bmj.Com*. https://bmjopen.bmj.com/content/10/11/e040162.abstract

Maeda, H., Wu, J., Sawa, T., Matsumura, Y., & Hori, K. (2000). Tumor vascular permeability and the EPR effect in macromolecular therapeutics: A review. *Journal of Controlled Release*, *65*(1–2), 271–284. https://doi.org/10.1016/S0168-3659(99)00248-5

Maritim, S., Boulas, P., & Lin, Y. (2021). Comprehensive analysis of liposome formulation parameters and their influence on encapsulation, stability and drug release in glibenclamide liposomes. *International Journal of Pharmaceutics*, *592*.

https://doi.org/10.1016/j.ijpharm.2020.120051

Marmottant, P., & Hilgenfeldt, S. (2003). Controlled vesicle deformation and lysis by single oscillating bubbles. *Nature*, *423*(6936), 153–156. https://doi.org/10.1038/nature01613

Mathiyalagan, P., & Sahoo, S. (2017). Exosomes-based gene therapy for MicroRNA delivery. *Methods in Molecular Biology*, *1521*, 139–152. https://doi.org/10.1007/978-1-4939-6588-5_9

Mayer, L. D., Bally, M. B., & Cullis, P. R. (1986). Uptake of adriamycin into large unilamellar vesicles in response to a pH gradient. *BBA - Biomembranes*, *857*(1), 123–126. https://doi.org/10.1016/0005-2736(86)90105-7

Mendes, T. F. S., Kluskens, L. D., & Rodrigues, L. R. (2015). Triple Negative Breast Cancer: Nanosolutions for a Big Challenge. *Advanced Science*, *2*(11). https://doi.org/10.1002/advs.201500053

Mintz, R. L., Gao, M. A., Lo, K., Lao, Y. H., Li, M., & Leong, K. W. (2018). CRISPR Technology for Breast Cancer: Diagnostics, Modeling, and Therapy. *Advanced Biosystems*, *2*(11). https://doi.org/10.1002/adbi.201800132

Muley, H., Fadó, R., Rodríguez-Rodríguez, R., & Casals, N. (2020). Drug uptake-based chemoresistance in breast cancer treatment. *Biochemical Pharmacology*, *177*. https://doi.org/10.1016/j.bcp.2020.113959

Needham, D., Anyarambhatla, G., Kong, G., & Dewhirst, M. W. (2000). A new temperature-sensitive liposome for use with mild hyperthermia: Characterization and testing in a human tumor xenograft model. *Cancer Research*, *60*(5), 1197–1201.

Oh, Y. K., Nix, D. E., & Straubinger, R. M. (1995). Formulation and efficacy of liposome-encapsulated antibiotics for therapy of intracellular Mycobacterium avium infection.

Antimicrobial Agents and Chemotherapy, *39*(9), 2104–2111. https://doi.org/10.1128/AAC.39.9.2104

Ohsawa, T., Miura, H., & Harada, K. (1985). Improvement of Encapsulation Efficiency of Water-Soluble Drugs in Liposomes Formed by the Freeze-Thawing Method. *Chemical and Pharmaceutical Bulletin*, *33*(9), 3945–3952. https://doi.org/10.1248/cpb.33.3945

Olusanya, T. O. B., Ahmad, R. R. H., Ibegbu, D. M., Smith, J. R., & Elkordy, A. A. (2018). Liposomal drug delivery systems and anticancer drugs. *Molecules*, *23*(4). https://doi.org/10.3390/molecules23040907

Ou, Y. C., Webb, J. A., Faley, S., Shae, D., Talbert, E. M., Lin, S., Cutright, C. C., Wilson, J. T., Bellan, L. M., & Bardhan, R. (2016). Gold Nanoantenna-Mediated Photothermal Drug Delivery from Thermosensitive Liposomes in Breast Cancer. *ACS Omega*, *1*(2), 234–243. https://doi.org/10.1021/acsomega.6b00079

Patil, S. D., Rhodes, D. G., & Burgess, D. J. (2004). Anionic liposomal delivery system for DNA transfection. *AAPS Journal*, *6*(4). https://doi.org/10.1208/aapsj060429

Pecha, R., & Gompf, B. (2000). Microimplosions: Cavitation collapse and shock wave emission on a nanosecond time scale. *Physical Review Letters*, *84*(6), 1328–1330. https://doi.org/10.1103/PhysRevLett.84.1328

Peng, R., Lin, G., & Li, J. (2016). Potential pitfalls of CRISPR/Cas9-mediated genome editing. *FEBS Journal*, *283*(7), 1218–1231. https://doi.org/10.1111/febs.13586

Pooler, J. P. (1989). Photooxidation of Cell Membranes Using Eosin Derivatives That Locate in Lipid or Protein To Study the Role of Diffusible Intermediates. *Photochemistry and Photobiology*, *50*(1), 55–68. https://doi.org/10.1111/j.1751-1097.1989.tb04129.x

Porter, T. M., & Ta, T. (2013). Thermosensitive liposomes for localized delivery and triggered

release of chemotherapy. *Journal of Controlled Release*, *169*(1–2), 112–125. http://linkinghub.elsevier.com/retrieve/pii/S0168365913001958%0Afile:///Files/3E/3E983B25-AB7D-419E-A519-8DF7F8C98428.pdf%0Apapers3://publication/doi/10.1016/j.jconrel.2013.03.036

Roberts, T. C., Langer, R., & Wood, M. J. A. (2020). Advances in oligonucleotide drug delivery. *Nature Reviews Drug Discovery*, *19*(10), 673–694. https://doi.org/10.1038/s41573-020-0075-7

Robertson, C. A., Evans, D. H., & Abrahamse, H. (2009). Photodynamic therapy (PDT): A short review on cellular mechanisms and cancer research applications for PDT. *Journal of Photochemistry and Photobiology B: Biology*, *96*(1), 1–8. https://doi.org/10.1016/j.jphotobiol.2009.04.001

Rupaimoole, R., & Slack, F. J. (2017). MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. *Nature Reviews Drug Discovery*, *16*(3), 203–221. https://doi.org/10.1038/nrd.2016.246

Schroeder, A., Avnir, Y., Weisman, S., Najajreh, Y., Gabizon, A., Talmon, Y., Kost, J., & Barenholz, Y. (2007). Controlling liposomal drug release with low frequency ultrasound: Mechanism and feasibility. *Langmuir*, *23*(7), 4019–4025. https://doi.org/10.1021/la0631668

Seleci, M., Seleci, D. A., Scheper, T., & Stahl, F. (2017). Theranostic liposome–nanoparticle hybrids for drug delivery and bioimaging. *International Journal of Molecular Sciences*, *18*(7). https://doi.org/10.3390/ijms18071415

Semple, S. C., Chonn, A., & Cullis, P. R. (1996). Influence of cholesterol on the association of plasma proteins with liposomes. *Biochemistry*, *35*(8), 2521–2525. https://doi.org/10.1021/bi950414i

Sharma, G. N., Dave, R., Sanadya, J., Sharma, P., & Sharma, K. K. (2010). Various types and management of breast cancer: An overview. *Journal of Advanced Pharmaceutical Technology and Research*, *1*(2), 109–126. www.japtr.org

Sharma, S., Rajendran, V., Kulshreshtha, R., & Ghosh, P. C. (2017). Enhanced efficacy of anti-miR-191 delivery through stearylamine liposome formulation for the treatment of breast cancer cells. *International Journal of Pharmaceutics*, *530*(1–2), 387–400. https://doi.org/10.1016/j.ijpharm.2017.07.079

Simões, S., Filipe, A., Faneca, H., Mano, M., Penacho, N., Düzgünes, N., & de Lima, M. P. (2005). Cationic liposomes for gene delivery. *Expert Opinion on Drug Delivery*, *2*(2), 237–254. https://doi.org/10.1517/17425247.2.2.237

Simões, S., Nuno Moreira, J., Fonseca, C., Düzgüneş, N., & Pedroso De Lima, M. C. (2004). On the formulation of pH-sensitive liposomes with long circulation times. *Advanced Drug Delivery Reviews*, *56*(7), 947–965. https://doi.org/10.1016/j.addr.2003.10.038

Simões, S., Slepushkin, V., Düzgünes, N., & Pedroso de Lima, M. C. (2001). On the mechanisms of internalization and intracellular delivery mediated by pH-sensitive liposomes. *Biochimica et Biophysica Acta - Biomembranes*, *1515*(1), 23–37. https://doi.org/10.1016/S0005-2736(01)00389-3

Sneider, A., Jadia, R., Piel, B., VanDyke, Derek Tsiros, C., & Rai, P. (2017). Engineering Remotely Triggered Liposomes to Target Triple Negative Breast Cancer. *Oncomedicine*, *2*, 1–13.

Sneider, A., Jadia, R., Piel, B., VanDyke, D., Tsiros, C., & Rai, P. (2016). Engineering Remotely Triggered Liposomes to Target Triple Negative Breast Cancer. *Oncomedicine*, *2*, 1–13. https://doi.org/10.7150/oncm.17406

Son, J., Yi, G., Yoo, J., Park, C., Koo, H., & Choi, H. S. (2019). Light-responsive nanomedicine for biophotonic imaging and targeted therapy. *Advanced Drug Delivery Reviews*, *138*, 133–147. https://doi.org/10.1016/j.addr.2018.10.002

Song, Y., Sheng, Z., Xu, Y., Dong, L., Xu, W., Li, F., Wang, J., Wu, Z., Yang, Y., Su, Y., Sun, X., Ling, D., & Lu, Y. (2019). Magnetic liposomal emodin composite with enhanced killing efficiency against breast cancer. *Biomaterials Science*, *7*(3), 867–875. https://doi.org/10.1039/c8bm01530a

Tao, Y., Hou, X., Zuo, F., Li, X., Pang, Y., & Jiang, G. (2019). Application of nanoparticle-based siRNA and CRISPR/Cas9 delivery systems in gene-targeted therapy. *Nanomedicine*, *8*(1), 511–514. https://doi.org/10.2217/nnm-2018-0522

Tazina, E. V., Kostin, K. V., & Oborotova, N. A. (2011). Specific features of drug encapsulation in liposomes (A review). *Pharmaceutical Chemistry Journal*, *45*(8), 481–490. https://doi.org/10.1007/s11094-011-0661-4

Tejero, I., González-Lafont, A., Lluch, J. M., & Eriksson, L. A. (2004). Photo-oxidation of lipids by singlet oxygen: A theoretical study. *Chemical Physics Letters*, *398*(4–6), 336–342. https://doi.org/10.1016/j.cplett.2004.09.093

Van Ballegooie, C., Man, A., Win, M., & Yapp, D. T. (2019). Spatially specific liposomal cancer therapy triggered by clinical external sources of energy. *Pharmaceutics*, *11*(3). https://doi.org/10.3390/pharmaceutics11030125

van Furth, R. (1980). The mononuclear phagocyte system. *Verhandlungen Der Deutschen Gesellschaft Fur Pathologie*, *64*, 1–11.

Vandghanooni, S., Eskandani, M., Barar, J., & Omidi, Y. (2018). Bispecific therapeutic aptamers for targeted therapy of cancer: a review on cellular perspective. *Journal of Molecular*

Medicine, *96*(9), 885–902. https://doi.org/10.1007/s00109-018-1669-y

Vangasseri, D. P., Cui, Z., Chen, W., Hokey, D. A., Falo, L. D., & Huang, L. (2006). Immunostimulation of dendritic cells by cationic liposomes. *Molecular Membrane Biology*, *23*(5), 385–395. https://doi.org/10.1080/09687860600790537

Wan, J., Bauman, J. A., Graziewicz, M. A., Sazani, P., & Kole, R. (2013). Oligonucleotide therapeutics in cancer. *Cancer Treatment and Research*, 213–233. https://doi.org/10.1007/978-3-642-31659-3_9

Wang, J., Lu, Z., Wientjes, M. G., & Au, J. L. S. (2010). Delivery of siRNA therapeutics: Barriers and carriers. *AAPS Journal*, *12*(4), 492–503. https://doi.org/10.1208/s12248-010-9210-4

Wang, Y., & Kohane, D. S. (2017). External triggering and triggered targeting strategies for drug delivery. *Nature Reviews Materials*, *2*. https://doi.org/10.1038/natrevmats.2017.20

Wu, H., Yu, M., Miao, Y., He, S., Dai, Z., Song, W., Liu, Y., Song, S., Ahmad, E., Wang, D., & Gan, Y. (2019). Cholesterol-tuned liposomal membrane rigidity directs tumor penetration and anti-tumor effect. *Acta Pharmaceutica Sinica B*, *9*(4), 858–870. https://doi.org/10.1016/j.apsb.2019.02.010

Xia, A. L., He, Q. F., Wang, J. C., Zhu, J., Sha, Y. Q., Sun, B., & Lu, X. J. (2019). Applications and advances of CRISPR-Cas9 in cancer immunotherapy. *Journal of Medical Genetics*, *56*(1), 4–9. https://doi.org/10.1136/jmedgenet-2018-105422

Xu, X., Costa, A., & Burgess, D. J. (2012). Protein encapsulation in unilamellar liposomes: High encapsulation efficiency and a novel technique to assess lipid-protein interaction. *Pharmaceutical Research*, *29*(7), 1919–1931. https://doi.org/10.1007/s11095-012-0720-x

Yadav A., Murthy M., Shete A., & Sfurti Sakhare. (2011). Stability Aspects of Liposomes. *Indian Journal of Pharmaceutical Education and Research*, *45*(2), 128–138.

Yan, Y., Li, X. Q., Duan, J. L., Bao, C. J., Cui, Y. N., Su, Z. B., Xu, J. R., Luo, Q., Chen, M., Xie, Y., & Lu, W. L. (2019). Nanosized functional miRNA liposomes and application in the treatment of TNBC by silencing slug gene. *International Journal of Nanomedicine*, *14*, 3645–3667. https://doi.org/10.2147/IJN.S207837

Yang, Y., Yang, X., Li, H., Li, C., Ding, H., Zhang, M., Guo, Y., & Sun, M. (2019). Near-infrared light triggered liposomes combining photodynamic and chemotherapy for synergistic breast tumor therapy. *Colloids and Surfaces B: Biointerfaces*, *173*, 564–570. https://doi.org/10.1016/j.colsurfb.2018.10.019

Yoo, J., Park, C., Yi, G., Lee, D., & Koo, H. (2019). Active targeting strategies using biological ligands for nanoparticle drug delivery systems. *Cancers*, *11*(5). https://doi.org/10.3390/cancers11050640

Yu, S., Bi, X., Yang, L., Wu, S., Yu, Y., Jiang, B., Zhang, A., Lan, K., & Duan, S. (2019). Co-delivery of paclitaxel and PLK1-targeted siRNA using aptamer-functionalized cationic liposome for synergistic anti-breast cancer effects in vivo. *Journal of Biomedical Nanotechnology*, *15*(6), 1135–1148. https://doi.org/10.1166/jbn.2019.2751

Z., H. (2005). A review of progress in clinical photodynamic therapy. *Technology in Cancer Research and Treatment*, *4*(3), 283–293. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed7&NEWS=N&AN=2005263033

Zhuang, Y., Ma, Y., Wang, C., Hai, L., Yan, C., Zhang, Y., Liu, F., & Cai, L. (2012). PEGylated cationic liposomes robustly augment vaccine-induced immune responses: Role of lymphatic trafficking and biodistribution. *Journal of Controlled Release*, *159*(1), 135–142. https://doi.org/10.1016/j.jconrel.2011.12.017