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MULTIFUNCTIONAL POLYMERIC MICELLES FOR DELIVERY OF DRUGS

KONSTANTINA SOFRONIADOU

Committee members:

Asterios Pispas (supervisor)

Costas Demetzos

Maria Gazouli

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III. ABSTRACT

One of the primary goals of nanomedicine research is to develop nanoparticles as drug delivery systems with optimal properties so as to tackle diseases that are leading cause of death worldwide. Recent advances have proven that nanoparticles combined with therapeutic agents overcome problems associated with conventional therapy. The introduction of polymeric micelles loaded with several drugs as multidrug nanocarriers have gained immense popularity and can be envisaged as key to improving the efficacy of current treatments. Such multifunctional micelles have a great spectrum of advantages and more specifically, their nanoscopic dimension, stealth properties, ability to solubilize hydrophobic drugs as well as imaging agents, various ligands and stimuli-sensitive groups provide a useful bioengineering platform for tumor targeting, stimulated drug release and imaging capabilities. The purpose of the current thesis is to focus on recent advancements in the multifunctional design of micellar nanomedicine for delivery of drugs and bioimaging.

IV. INTRODUCTION

Most drugs entering the human body usually need to cross important natural barriers that are structurally and chemically composed of a wide variety of agents. Moreover, in the vast majority of cases their biological effects are not limited to targeted tissues but also at non-target sites, which often results in undesired side effects and inhibits/reduces their therapeutic potential. The particular chemical and mechanical complexity that the drug needs to surpass, while delivering its active compounds selectively to the pathological areas, makes drug delivery a very challenging process [7]. In other words, the specific delivery to an organ, a tissue or a type of cells remains even today a great need and challenge for the successful treatment of various diseases. During the latest decades, nanotechnology has offered a great many contributing steps in the international drug delivery research. The application of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems is now referred as nanomedicine and is expected to provide significant improvements, while provides innovative approaches for the management of diseases with minimal side effects. Therefore, many scientists all around the world have contributed their knowledge and expertise in the construction of various nanoscale systems (including polymeric and metallic nanoparticles, liposomes, niosomes, polymeric micelles, nanogels, nanocapsules, dendrimers, quantum dots, microcapsules, carbon nanotubes, nanocrystals, solid lipid nanoparticles and many different nanoassemblies) with the aim of using them in the delivery of pharmacological agents that target specific tissues of the body [1, 2, 7].

IV(a). Drug delivery nanosystems: Potentials and Limitations

Several nanoparticle-based drug delivery and drug targeting systems are currently developed or under development. Compared to conventional small molecule-based therapy, their use imposes specific requirements aiming to minimize drug degradation and inactivation upon administration, prevent undesirable side effects, and increase drug bioavailability and the fraction of drug delivered in the pathological area. In general, pharmaceutical drug carriers are expected to be biodegradable, easy, and

reasonably cheap to prepare, to have a small particle size, to possess a high loading capacity, to demonstrate prolonged circulation –for as long as they need to be pharmacologically effective, and, ideally, to specifically or nonspecifically accumulate in required sites in the body and interact only and exclusively with the targeted cells so as to reduce toxicity to healthy tissues [2, 6, 22].

The advantages that those nanoparticles can offer are obvious: First of all, because of their biochemical structure, are not limited from poor solubility in the body's fluids. Secondly, their activity can be pretty selective and accurate, due to their ability to penetrate through the barriers in the body and reach the desired tissues with minimal loss of their volume in the blood circulation. Third, both their distribution and their pharmacokinetics can be the best possible for each one of the patients -thus succeeding the best possible pharmacological effect for the maximum of the time indicated by the drug properties. And finally, side effects like dose-limiting toxicity and multi-drug resistance can easily be sidestepped because the selection of the targeted tissues makes these problems be almost a past [2, 3, 6, 18].

On the other hand, current nanotechnologies are characterized by a number of potential limitations. The most important of which are: Poor drug loading, which is a loading usually under 5% of the transported drug compared to the drug carrier total composition. This means, in simple words that less quantity of the drug reaches the sites of the body that need to be reached, and as a result that quantity is rarely enough to reach a pharmacologically effective concentration in the body. However, excessive drug loading is also an undesired outcome, but is much rarer than the previous one. In that case, the amount of the drug that is loaded on the nanocarrier is too high, thus leading to possible adverse effects (toxicity symptoms). Another serious limitation is rapid (or "burst") release. More specifically, the drug that is encapsulated in the nanocarrier is being released at the targeting tissues before reaching the pharmacological target, thus leading to low activity and/or toxicity issues. The difficulty of designing an efficient nanodelivery system using conventional physicochemical methods that will combine the properties of low toxicity, high immunogenicity, biodegradability and accumulation only on desired cells/tissues with maximum clinical benefit is crucial, since such nanosized materials behave differently

in various environments. During this process it is important to consider ethical issues, the long time it takes to gain regulatory approval and pass the required clinical trials. As profit/risk ratios decreases, the failure rate increases and it is a deterrent factor for investing companies. So, for the requirements to be fulfilled and for the limitations to be surpassed, nanocarriers should be developed under strict requirements and regulations [3, 22].

IV(b). Polymeric micelles: Theoretical Background

The development of drug nanocarriers for poorly soluble pharmaceuticals is a particularly important task, since a considerable number of potent drugs that have been developed are very hydrophobic. While their degree of hydrophobicity is favorable for drug permeation through cell membranes, their low water-solubility results in poor absorption and low bioavailability of the drug. Drug aggregation and formation of capillary embolisms upon intravenous administration of those drugs could also lead to local toxicity [2, 5, 9]. To overcome this problem, the use of polymeric micelles has been the subject of growing scientific attention. They are formed from the self-assembly of amphiphilic block copolymers and they have emerged as a potential carrier for poorly water soluble drugs, because their inner hydrophobic core can act as a reservoir and accommodate hydrophobic drugs, while simultaneously, their hydrophilic shell protects the micelle from rapid clearance by the reticuloendothelial system (RES), hindering the interaction with plasma proteins and cells, thus enabling stabilization in an aqueous environment (**Figure 1**) [9, 18].

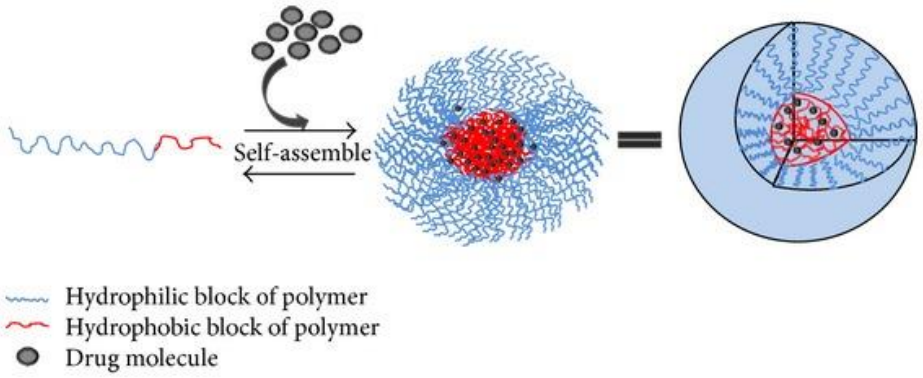


Figure 1: Schematic representation of drug loading of polymeric micelles by the self-assembly of amphiphilic block copolymers in aqueous solution (*Source: Wei Xu et al., Polymeric Micelles, a Promising Drug Delivery System to Enhance Bioavailability of Poorly Water-Soluble Drugs, J Drug Deliv. 2013; 2013: 340315*).

The formation of polymeric micelles occurs when the concentration of the block copolymer in an aqueous medium increases above a certain concentration, named as the critical micelle concentration (CMC), and leads to the aggregation and self-assembly of a core-shell micellar or vesicular structures through hydrophobic interactions. Micelle formation is mainly governed by the entropy gain derived from the reorganization of the water structure with the removal of hydrophobic segments from the aqueous phase [6, 8, 15, 17]. This concentration threshold is very low for polymeric micelles and reflects their thermodynamic stability, resulting in constructs that have a better chance of staying in a micellar form under high diluting condition and are not easily disassembled in vivo [1, 9, 13]. Generally, the more hydrophobic and the higher the molecular weight of hydrophobic blocks, the lower the CMC [6]. Furthermore, amphiphilic copolymers exhibit a much lower CMC than that of low molecular weight surfactants. Depending on whether the hydrophobic chain is randomly bound to the hydrophilic chain or grafted to one end, the micellization of amphiphilic copolymers can result in different types of micelles, by affecting their size and mobility [12]. Most drug carrier have been studied with di-block (AB)- or tri-block ABA-type copolymers, where A represents the hydrophilic block (shell) and B represents the hydrophobic block (core), and graft copolymers, which are branched polymers consisting of one hydrophilic chain and one to multiple hydrophobic chains or vice versa [5, 6]. Recent studies have been conducted for the synthesis of tetrablock and pentablock copolymers also [17]. The chemical nature of these core-forming polymers and their interaction or the degree of compatibility with drugs can strongly affect the stealth properties, stability and influence the circulation kinetics of the micellar assembly. It is also shown that the miscibility between polymers and drugs leads to increased physical drug loading. Hence, all these reasons underline the importance of selecting appropriate core-forming polymers [5, 8, 13, 14].

Polyethylene oxide (PEO), also known as polyethylene glycol (PEG), is the most commonly used hydrophilic component (shell forming block) of the block copolymers, since it is a non-toxic polymer approved by the FDA for biomedical applications as a component of various pharmaceutical formulations. As a steric protector, PEG has an extremely attractive combination of physicochemical properties (high solubility in aqueous solutions, high flexibility, very low toxicity, immunogenicity, antigenicity, biocompatibility and high hydration) that sterically stabilize surfaces in aqueous systems and provide good "stealth" properties by preventing the opsonization and adhesion of drug vehicles [1, 2, 6, 7, 8, 10]. Attachment of lipid moieties as hydrophobic blocks capping PEG chains can offer better stability than conventional amphiphilic polymer micelles and increase the miscibility of the micellar core, since the presence of two fatty acid acyls increase the hydrophobic interactions between polymeric chains in the micelle's core [12, 13]. It is also worth to mention that RNAs had been exploited as biomaterials to construct RNA-based micelles so as to improve their performance. The resulting amphiphilic RNA nanoparticles are capable of covalently attaching different chemical and functional moieties which makes them a suitable multifunctional platform for potential drug delivery [20].

The diameter of polymeric micelles resembles that of natural viruses and can be tuned from 10 to 100 nm with a substantial narrow size distribution [5, 11]. This size range is considered ideal so as to acquire stable and long-circulating carriers, while benefits the sterilization processes in pharmaceutical productions and minimizes the risks of embolism in capillaries, contrary to larger drug carriers [5, 7, 12, 17]. It could be easily controlled by varying the hydrophobic block of the amphiphilic copolymer [9] and depends on several factors including copolymer molecular weight, relative proportion of hydrophilic and hydrophobic chains, aggregation number and can also be affected by the organic solvent used to dissolve the polymer before assembling in the aqueous medium [6, 12, 17]. Polymeric micelles come to different morphologies including spherical, vesicular and cylindrical types, while spherical is the most common morphology that seems to improve cellular uptake compared to others [16]. The main factors affecting the morphology of micelles is the hydrophobic-hydrophilic balance of

the block copolymer, the length and nature of the core and corona, pH, concentration, temperature, ionic strength and the method of preparation [17].

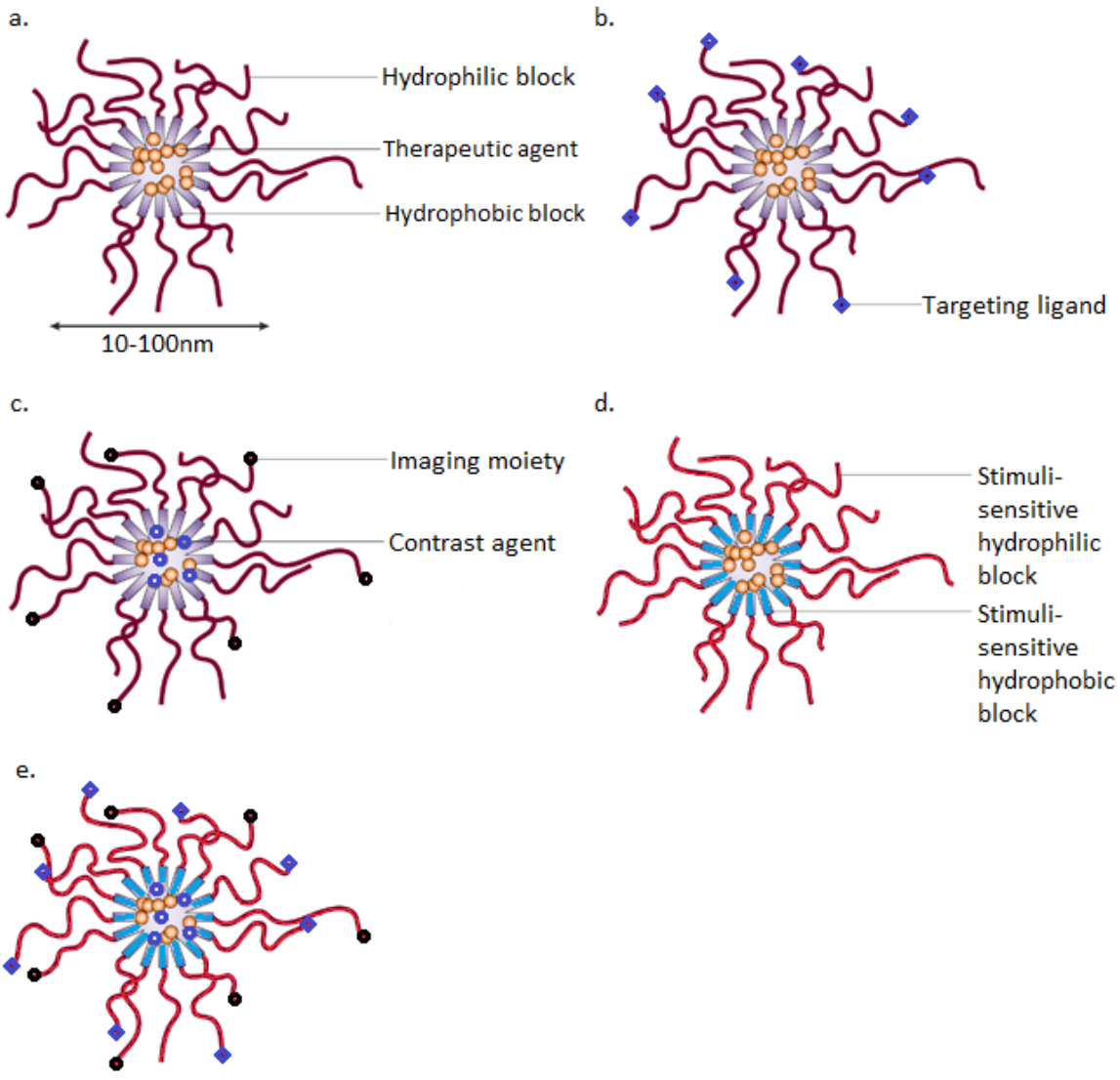
Micellar size polydispersity permits for evasion of renal filtration while allowing for increased accumulation in tumor tissue via the passive enhanced permeability and retention (EPR) effect. What is more, some other important advantages of their use in the clinical field include their tunable payload release, which is important for the timely accuracy of the pharmacological treatment plan, their ability to specifically target their payload to diseased tissues and cells by the modification of their surface chemistries by binding pilot molecules capable of recognition of cell-specific surface receptors such as antibodies and/or certain sugar moieties and -last but not least- their ability to respond to various internal and external stimuli for triggered release so as to achieve temporal and spatial control over the release of various therapeutic payloads [1, 2, 5, 6, 9, 11, 15, 17].

It should not be omitted that apart from drugs, those nano-agents can also be used to deliver other therapeutic active macromolecules like proteins, polypeptides, aptamers, DNA and small interfering RNA (siRNA) [1, 2, 11, 12, 13]. Such hydrophilic charged macromolecules have strong therapeutic potentials for many intractable diseases based on their ability to change the genetic material of specific sites of the body at a local level. However, their susceptibility to enzymatic degradation and inefficient cellular uptake have been great challenges to overcome [15]. As well as reporter molecules and contrast agents, which can greatly enhance the specificity by highlighting the area of interest for imaging modalities like nuclear imaging, magnetic resonance imaging (MRI), X-ray computed tomography (CT), positron emission tomography (PET) and single photon emission computed tomography (SPECT) [6, 9].

The strategy incorporating imaging probes and therapeutic molecules together within the same platform, in this case into polymeric micelles, allows simultaneous diagnosis, monitoring and therapy, the so called theranostics approach, and may prove essential to address the challenges of tumor heterogeneity and adaptive resistance to achieve efficacious treatment of cancer [11]. In consequence, owing to the exciting features offered by polymeric micelles, they could be used in many applications in the

pharmaceutical field, especially when they are made from suitable biodegradable polymers [17].

The next step is an attempt to engineer efficient nanocarriers that could demonstrate a combination of various features and offer a multifunctional profile (**Figure 2**). To prepare such smart nanocarriers, certain chemical moieties have to be simultaneously assembled on the surface of the same nanoparticle. Furthermore, these individual moieties have to coordinate in such a way so as to provide specific properties [19]. Designing of smart or stimulus-responsive drug-release vehicles and the possibility of simultaneous detection and imaging are additional major challenges in the drug delivery domain [3].



1. **Figure 2**: Schematic drawing of polymeric micelles: **a.** Block copolymer micelle **b.** Micelle conjugated with a targeting ligand **c.** Micelle loaded with contrast agents and/or imaging moieties **d.** Modified micelle with thermo/pH/light/ultrasound-sensitive block polymers for triggered drug release **e.** Multifunctional polymeric micelle with two or more different functions (*Source adapted with modification from: Oerlemans Chris et al., Polymeric Micelles in Anticancer Therapy: Targeting, Imaging and Triggered Release, Pharm Res. 2010 Dec; 27(12): 2569-2589)*.

Despite several potential advantages of polymeric micelles, it is worth mentioning that various challenges exist and represent an active field of research. First of all, high levels of polymer chemistry are needed, which is a great challenge in the synthesis of these structures, -especially in a large industrial scale in a highly reproducible manner. Thus, the chemical synthesis of nanoparticles and storage can be more complicated and relatively expensive. Apart from that, the path from laboratory to the market in nanoscience has a really high level of risk, due to the fragility, instability and reactivity of nanoscale materials. In addition, another important disadvantage is the small micellar size limits the amount of drug that can be incorporated within the core, with higher drug loading coming at the cost of increased micelle size and aggregation. Some other hurdles include poor blood stability upon administration and difficulty in transport through the cell membrane. In particular, the extravasation of polymeric carrier systems is much slower compared to that of low-molecular-weight drugs, which may lead to leakage and burst release of loaded drugs. Therefore, a long circulation character is needed for the sufficient delivery of drugs to the targeted sites of the body. What is more, several questions have been raised regarding the long-term stability and toxicity of polymer micelles. Drugs conjugated in the polymeric carrier systems are metabolized in liver in a slower manner than free drugs, thus, toxic side effects may be exhibited for a longer period of time. Last but not least, questions regarding the antitumor efficacy of micelles in the clinical setting have been raised [5, 9, 13, 17].

Several micellar formulations for anticancer therapy are currently at different stages of preclinical and clinical trials, and a few of them have been FDA approved for use in

patients [6]. However, as far as those nanocarriers are concerned, one should take into consideration that the rapid paces of technological progress have not let much time for toxicological studies of long duration. In other words, time is an important ally for the design of such evolved nano-particle agents and more research is needed for sure. And secondly, the amount of money needed for the research, in combination with the little time that has gone by since their first launching on markets, makes those nano-agents suggested for high budgets only -that is, they can still not be available for widespread public use.

In the following chapters of the current thesis, we will focus our attention on recent advances in the area of polymeric micelles drug targeting that are most commonly used in everyday medical practice, that is, passive and targeting, stimuli-responsive programmed, designed for specific targeting and multifunctional polymeric micelles will be highlighted. We will mention the basic guidelines of network-targeted combination therapy, and -finally- we will analyze how molecular imaging, as well as theranostics, can be used nowadays so as to serve the drug delivery field.

V. PASSIVE TARGETING OF POLYMERIC MICELLES

Passive targeting occurs due to unique characteristics of solid tumors, which include newly formed vessels (hypervascularization) with poorly aligned endothelial cells and wide fenestrations. According to the tumor model, this discontinuous endothelium has pores varying from 10 to 1000 nm [14]. Unlike vasculature of normal healthy tissues, pathological vasculature allows the preferential accumulation of large molecules and even of small particles from blood vessels in the interstitial tumor space. This vascular permeability is enhanced by the actions of various factors, like nitric oxide, bradykinin, endothelial growth factor and matrix metalloproteinases [1, 5]. In addition, the growing tumor cells compress the lymph vessels, especially in the central portion of the tumor, causing them to collapse [1]. Therefore, the lymphatic system does not operate adequately and is responsible for the drainage of macromolecules from normal tissues in tumor sites [2]. As a result, both these phenomena lead to the fact that colloidal particles with sizes up to several hundreds of nanometers, like polymeric micelles, can extravasate in the tumor interstitium and other inflamed tissues and subsequently retained there for a prolonged time and gradually reach high local concentrations [7]. This mechanism is known as the enhanced permeability and retention effect (EPR). Because of the fact that it essentially only relies on the pathophysiological properties of solid tumors, it is generally referred to as 'passive drug targeting' [4]. In the EPR effect, carriers do not have to possess specific targeting moieties and must not have any chemical structures that would be biologically recognizable by the reticuloendothelial system (RES) [5]. Nevertheless, their size and geometry have a determining role ensuring their remaining in circulation for longer intervals, as well as their uptake by tumor cells and macrophages [1]. It has been shown that grafting of hydrophilic polymers, such as PEG, on the surface of particles is effective to attenuate the uptake of particles by the RES cells [7]. Considering those requirements, polymeric micelles are preferable candidates due to their submicron size, their phase-separated spherical structure and their neutrally or weakly negatively charged block copolymers (**Figure 3**) [5]. However, the exact biodistribution and accompanying pharmacokinetics of polymeric micelles is depending on many factors, such as the particle size, the surface layer characteristics and the particle rigidity [7]. In the international bibliography, a large number of substances have been

reported to be used in passive targeting. Some examples of drugs loaded to polymeric micelles exploiting the EPR effect are:

Doxorubicin (DOX): the most effective antineoplastic drug against different types of cancer and other malignancies. DOX-loaded micelles have greater in vitro anticancer activity as compared to free DOX at the same dose, indicating their potential in pharmaceutical applications. This is attributed to the fact that lacking a protective vehicle, results in rapid recognition and clearance of free DOX by the RES. DOX-loaded polymeric micelles exhibit significantly reduced cytotoxicity toward normal tissues and organs, good biocompatibility and increased apoptosis of tumor cells [21].

Paclitaxel (PTX): very hydrophobic anticancer agent for the treatment of various cancers. Paclitaxel-loaded micelles show higher anticancer effect than that of free paclitaxel, by improving the intracellular uptake of paclitaxel in cancer cells [22].

Docetaxel (DTX): artificial, semi-synthetic, anticancer drug for the treatment of various solid tumors. Docetaxel-loaded polymeric micelles demonstrate better antitumor efficacy than common docetaxel injection, by providing improved stability, increasing the exposure time of the drug compounds in blood circulation and decreasing the toxicity of docetaxel in normal tissues [23].

Resveratrol (RES): phytochemical with diverse biological activities (anti-cancer, anti-oxidant, anti-inflammatory etc.). Resveratrol loaded polymeric micelles display improved pharmacokinetic profiles, slower clearance and smaller volume distribution compared to free resveratrol. Micelle forming ability and colloidal stability offer improved plasma stability and resistance to enzymatic cleavage [24].

Docetaxel and Resveratrol (DTX and RES): synergistic anticancer effect that generates better treatment effects on cancer compared to when each drug compound is used alone. DTX and RES loaded polymeric micelles exhibit prolonged release profiles and enhanced cytotoxicity against cancer cells. The increased sensitivity and the ability to

escape from the RES of DTX/RES loaded polymeric micelles can be attributed to the presence of PEG shell which enhance the uptake mediated through endocytosis [25].

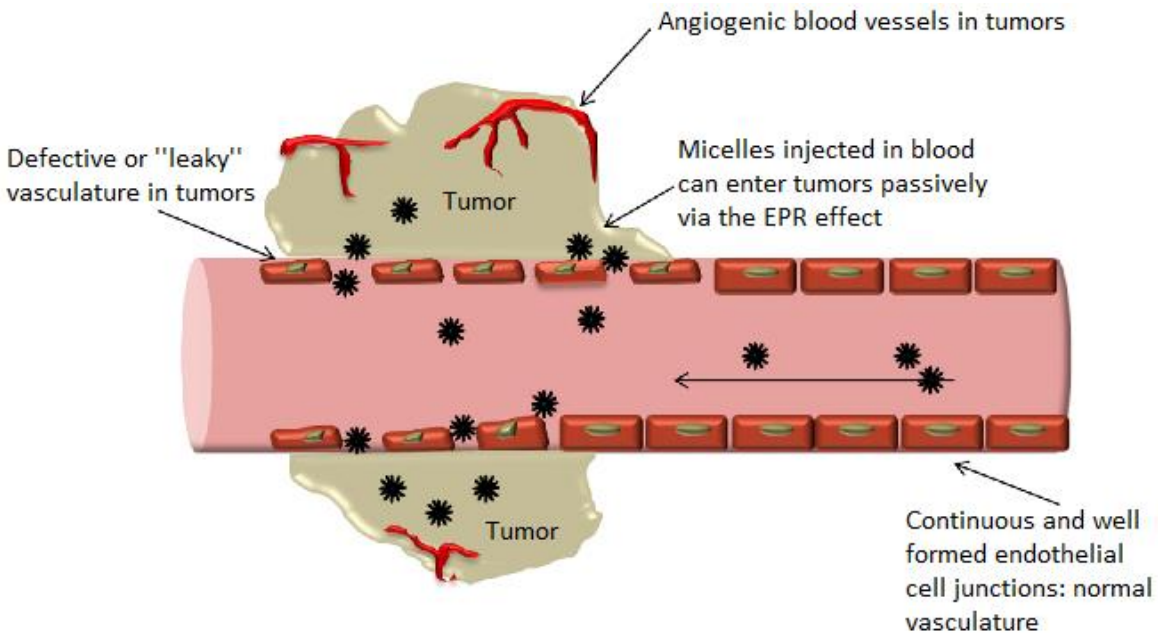


Figure 3: Graphical illustration of the enhanced permeability and retention (EPR) effect in solid tumors exemplified by micellar nanoparticles *(Source: Aditi M. Jhaveri and Vladimir P. Torchilin, Multifunctional polymeric micelles for delivery of drugs and siRNA, Front Pharmacol. 2014; 5: 77)*.

However, our current understanding of EPR effectiveness is limited by the scarcity of data, false impressions of the therapeutic outcome of nanoparticles in therapies by the heterogeneity of the EPR effect and limited patient-based experimental results. EPR phenomenon should be further investigated in human tumors and the improvement of preclinical models is therefore necessary for the design of sophisticatedly engineered nanocarriers with better delivery efficiency and maximum therapeutic benefit [26].

VI. ACTIVE TARGETING OF POLYMERIC MICELLES

Despite the fact that passive targeting increases the accumulation of nanosized anticancer drugs locally in tumor sites, it has several drawbacks. First of all, not all tumors exhibit the EPR effect. There are many factors hindering the penetration of particles inside the tumor, which are physiological (like high interstitial pressure) and physicochemical features (like composition, charge and structure) of the tumor, as well as the particle physicochemical features (like hydrophobicity, charge, geometry and size). In this case, drugs will accumulate in the tumor vicinity, but they will not be able to enter cancer cells and reach their therapeutic goals, resulting in an ineffective high local concentration [4]. The most promising strategy that has been developed to overcome this problem is to attach specific moieties or ligands, like monoclonal antibodies, polypeptides, small organic molecules, as well as high molecular weight molecules such as DNA, RNA, proteins, biomacromolecules and other receptor ligands, on the surface of the nanocarriers, -on the water-exposed free termini of hydrophilic blocks-, so they can actively recognize specific receptors or antigens uniquely overexpressed on the cell membrane of cancer cells (**Figure 4**).

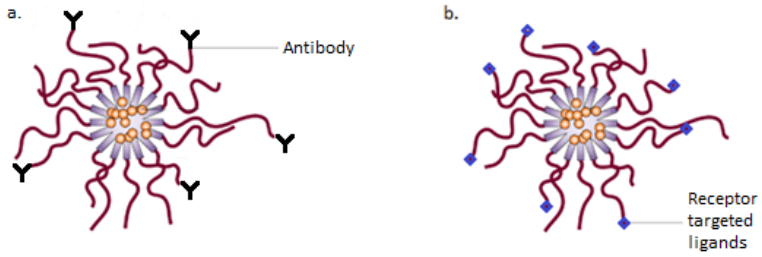


Figure 4: Schematic drawing of polymeric micelles with various targeting functions, **a.** antibody-targeted micelles, **b.** ligand-targeted micelles (*Source adapted with modification from: Aditi M. Jhaveri and Vladimir P. Torchilin, Multifunctional polymeric micelles for delivery of drugs and siRNA, Front Pharmacol. 2014; 5: 77*).

With the surface modification, the uptake of the drug is improved in the target organ or tissue and intracellular delivery of otherwise poorly internalized drugs can be achieved. Therefore, the efficacy of the drug is increased while systemic toxicity and adverse side effects are reduced compared to non-targeting nanocarriers [1, 2, 4, 6]. As mentioned

above, polymeric micelles can be modified by engineering their hydrophilic block copolymers with a broad range of bioactive ligands capable of recognition of cell-specific surface receptors, so as to improve drug loading, release rate, pharmacokinetics and tumor targeting efficiency. When the ligands bind to their specific receptors the micelles are internalized by endocytosis [6, 11]. The stability of the micelles should further contribute to better drug solubility and toxicity reduction in biological environments, because of less interaction with non-targeted organs offered by the PEG shielding [2].

An example of a small organic molecule for cancer targeting applications is biotin, a water-soluble vitamin, whose main transporter -sodium-dependent multivitamin transporter (SMVT)- is overexpressed in many cancer cells such as lung cancer cells. Wang et al. designed biotinylated polymeric micelles based on HPMAm for paclitaxel delivery. P(HPMAm) is a promising alternative for PEG, besides its biocompatible and hydrophilic profile, it could be modified with hydrophobic moieties to serve as a micellar core or it could be conjugated with multiple drugs and bioactive ligands for targeted delivery (active targeting). The synthesis of paclitaxel (PTX)-loaded micelles with biotin increased its solubility and loading capacity, while enhanced the internalization in cancer cells [27].

Another example of small organic molecule is folic acid (FA), whose receptor -folate-receptor alpha- is overexpressed in the majority of cancer tissues, such as ovarian, brain, epithelial and cervical cancers, with limited expression in healthy non-target tissues and organs. Luong et al. utilized folic acid conjugated polymeric micelles with a synthetic flavonoid anticancer compound, curcumin-difluorinated (CDF), so as to overcome CDF's extremely high hydrophobicity and susceptibility to photolysis. The use of folic acid as a targeting ligand and styrene-maleic acid (SMA) as block copolymer, does not only enhanced the aqueous solubility and the delivery of CDF specifically to cancer cells but also protected them from photolysis. *In vitro* studies showed that FA-SMA-CDF formulations have significantly enhanced stability, higher therapeutic potential and increased half-life [28].

Peptides are also actively explored as ligands for tumor-targeted drug delivery. Cai et al. investigated the use of the peptide motif Arg-Gly-Asp (RGD), which targets the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins overexpressed on the surface of tumor cells. The authors used PEGylated

stearic acid-grafted chitosan (PEG-CS-SA), as an amphiphilic block copolymer to form spherical micelles and doxorubicin, as a model anticancer drug to investigate its antitumor properties. By coupling linear RGD to these micelles, they were able to show decreased release rate of DOX from carriers before they arrive at the target cells and enhanced cellular uptake and cytotoxicity in the integrins overexpressed cell lines [29].

Carbohydrate molecules, such as galactose, have also been used to functionalize micelles. A galactosylated pluronic F68 copolymer (GF68-Gal) was used by Patil and coworkers to produce polymeric micelles encapsulating galangin, an anticancer and hepatoprotective polyphenol, so as to suppress the proliferation of hepatocellular carcinoma cells. However, despite its potent pharmacological properties, galangin has poor bioavailability due to its low water solubility, rapid hepatic metabolism and rapid systemic elimination. In order to overcome these drawbacks, galactose has been used to increase the amount of galangin in the liver through active targeting by galactose moieties having high affinity for the asialoglycoprotein receptors (ASGPRs) overexpressed on the surface of hepatocellular carcinoma cells. The cytochrome enzyme inhibition activity of pluronic F68 copolymer reduced the rate of metabolism and in turn elimination. Galangin loaded GF68-Gal micelles exhibited controlled release of galangin and increased uptake in ASGPR cells [30].

In conclusion, conjugation of targeting ligands in nanoparticles is a more complex strategy than passive targeting. However, their ability to successfully target disseminated regions throughout the body is a great advantage for the treatment of various tumors [26]. Polymeric micelles offer high versatility for incorporating a broad range of bioactive molecules by engineering the core forming segments of the block copolymers. Besides the enhanced binding and internalization by targeted cells, ligands can also be used for overcoming numerous biological barriers, including extravasation and tissue penetration in, otherwise, impermeable tissues. However, it has to be kept in mind that the efficiency of these ligands depends on various aspects, such as the density of ligands on the surface of the polymeric micelles, along with the characteristics of the selected ligand-receptor system, including the binding affinities, the receptor internalization, the biodistribution and availability of the receptors and the volatile expression of the receptors according to tumor stage [11].

VII. STIMULI-RESPONSIVE POLYMERIC MICELLES

An additional obstacle that limits the therapeutic outcome of drug delivery systems is the premature and non-specific release of the encapsulated cargos. An ideal polymeric micellar system should retain the entrapped drug in blood circulation after intravenous or subcutaneous injection and release the therapeutic agent, preferably in a controlled fashion in order to reach cytotoxic levels, only after reaching the targeted area. As mentioned above, drug release from polymeric micelles is governed by many factors (e.g., molecular weight, physicochemical characteristics, length of the hydrophobic polymer etc.). By functionalizing their surface with synthetic polymers and ligands, the release rate can be adequately controlled, and the carriers can be targeted to specific cells and organs, avoiding either precipitation upon dilution or sequestration within the micellar phase. The development of stimuli-sensitive nanocarriers can further increase the selectivity and efficiency of drug delivery. To achieve this, several methods for triggered release have been explored and are expected to expand in nanocarrier science, including pH-sensitive, thermoresponsive, ultrasound-sensitive and light-sensitive micelles. These targeting approaches rely on the fact that many pathological processes present either a certain increase in temperature or decrease in pH, while others are activated in response to exogenous stimuli, like ultrasounds and light respectively (**Figure 5**). Designing stimuli-sensitive systems has great clinical potential in the reversion of multidrug resistance, since they are designed in such a way that they can be activated only on exposure to locoregional triggers and release the conjugated or entrapped chemotherapeutic drug. Thus, these carriers can maximize drug release and uptake at the desirable pathological site, while decreasing potential damage to healthy tissues. Nonetheless, this process can be simple or highly complex, depending on the selection of the stimulus and the sensitivity of the chosen materials [2, 4, 6, 7, 9, 14, 26].

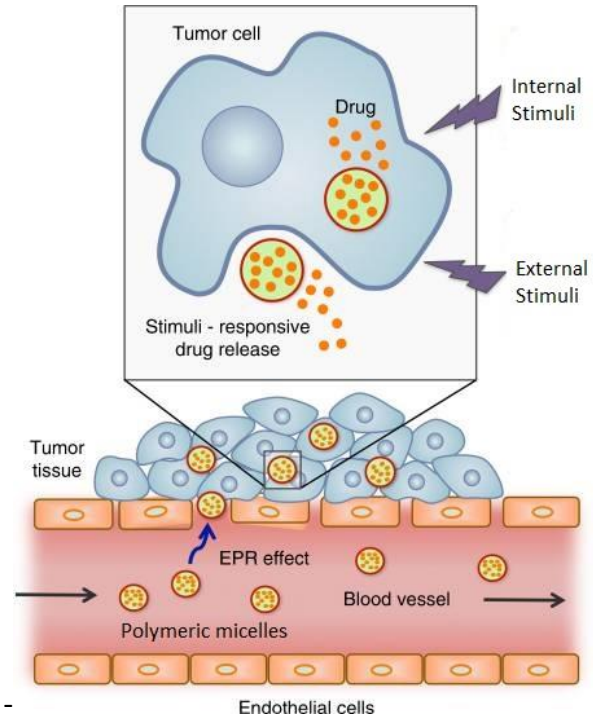


Figure 5: Schematical representation of stimuli-responsive polymeric micelles triggered by internal or external stimuli (*Source: Daniel Rosenblum, Nitin Joshi, et al., Progress and challenges towards targeted delivery of cancer therapeutics, Nat Commun. 2018; 9: 1410*).

VII(a). pH-Sensitive Polymeric Micelles

It is really important to understand that the stability of PEGylated nanocarriers may not always be favorable for drug delivery systems. Especially when they are unable to release their cargo inside the tumor cells so as to eliminate them. In the same way, the presence of the PEG coat can be an obstacle when carriers have to be taken up via an endocytic pathway [2]. To solve these problems, a drug delivery system that responds to physiological differences between the body and the tumor vicinity, such as changes in pH values, as an internal stimulus appear promising to provide a potential passage through in order to achieve stimulated release of systemically administered drugs from pH sensitive polymeric micelles [4, 9]. As is known, blood and healthy tissues have a pH of 7.4, while the intracellular pH is around 7.2 [6]. On the other hand, extracellular pH values of most solid tumors and inflammatory tissues tend to have a special biochemical and physicochemical microenvironment with lower pH values (pH ~ 6.8), due to the higher

rate of aerobic and anaerobic glycolysis compared to normal cells, as well as in the endosomal and lysosomal compartments of cells where the pH is even lower, around 5.0-6.0 in endosomes and 4.0-5.0 in lysosomes [7, 14]. Therefore, the existing pH of tumor tissues or in various subcellular organelles can be exploited as an ideal internal stimulus for triggered drug release (**Figure 6**) [31].

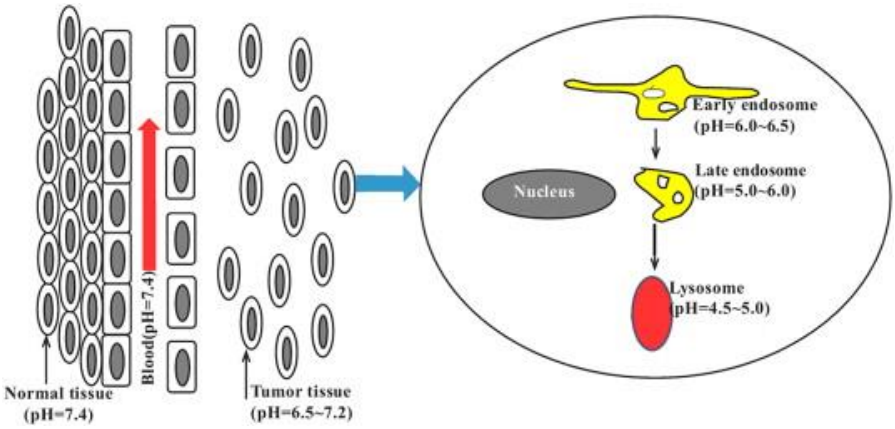


Figure 6: Schematic illustration of various pH gradients of normal tissue, blood, tumor tissue and endosome-lysosome process (*Source: Yanhua Liu et al., pH-sensitive polymeric micelles triggered drug release for extracellular and intracellular drug targeting delivery, Asian Journal of Pharmaceutical Sciences, Volume 8, Issue 3, Pages 159-167*).

Additionally, there could be a drug delivery system stable at low pH values, as within the stomach (pH 1.0-2.5), but degradable at neutral or slightly alkaline pH values, as at the intestines (pH 5.1-7.8), for controlled release upon oral administration [7, 8]. To achieve this, pH-tunable moieties, like polyacids or polybases, may be used as building blocks that impart pH sensitivity to drug release. Basic core monomeric units (e.g., amines) are uncharged and thus hydrophobic at high pH, while hydrophilic upon protonation at low pH. On the other hand, acidic core monomeric units (e.g., carboxylic acids) are hydrophobic when protonated at low pH and hydrophilic at high pH. The protonation and deprotonation of the polymers alter their hydrophilicity and increase electrostatic repulsions, causing the destabilization of the protonatable groups and yet the polymeric micelles, thus affecting the local release of the drugs [4, 6, 8]. This ability can come in handy when cancer cells show high resistance in various drugs [4]. Therefore, a variety of pH-sensitive polymeric micellar systems have been explored that are stable at

physiological pH but deform to free their incorporated anticancer drug molecules precisely at tumors and/or enter into their cytoplasm only when subjected to mild acidic conditions.

By taking advantage of these pH variations, researchers have developed various polymeric micelles suited to releasing drugs in a controlled manner in tumor tissues, endosomes, and lysosomes. An example of a novel pH-sensitive polymeric micelle was designed by Zhou and his coworkers by developing a urushiol-loaded tumor-targeted micelle delivery system based on amphiphilic block copolymer poly(ethylene glycol)-b-poly-(β-amino ester) (mPEG-PBAE) (**Figure 7**). Urushiol is a natural pro-electrophilic quinone compound that is capable of promoting tumor cell apoptotic death in a range of tumor types, thus it has been used as antitumor chemotherapeutic agent [32]. However, urushiol has limited solubility in water and poor tumor selectivity, increasing the risk of adverse side effects. The incorporation of urushiol into pH-sensitive polymeric micelles improved its stability and water solubility when exposed to a normal physiological pH, while simultaneously promoted the controlled release in response to acidic conditions at tumor sites in vivo. These findings suggest that urushiol encapsulation can highly increase intra-tumoral drug concentrations, thus improving its therapeutic efficiency and lowering its off-target toxicity [33].

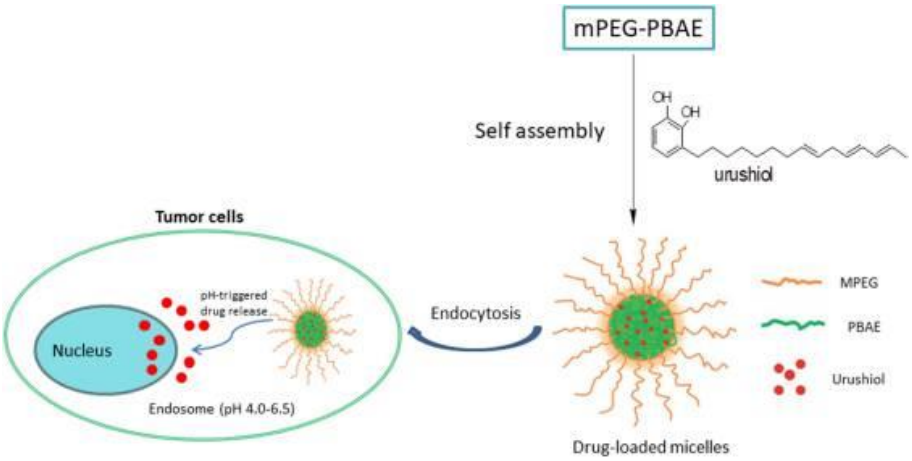


Figure 7: Schematic illustration of forming pH-responsive urushiol-loaded polymeric micelles and the pH-triggered drug-release mechanism (*Source: Zhou Hao, Novel pH-sensitive Urushiol-Loaded Polymeric Micelles for Enhanced Anticancer Activity, Int J Nanomedicine 2020; 15: 3851-3868*).

In another interesting approach of this strategy, Cavalcante et al. successfully formulated doxorubicin-loaded pH-sensitive micelles composed of DSPE-PEG_{2000}, acting as the hydrophobic block of the micelles, and oleic acid (OA) (**Figure 8**). The ion pair between OA and DOX seems to play an important role in pH-dependent release of DOX, due to the carboxyl acid of OA that is protonated at acidic pH, triggering the destabilization of the interaction between DOX and OA, thus leading to rapid release of the drug in these conditions. The resulting micelles had high encapsulation percentage, near to 90% and pH sensitive release; 60% of the drug was released after 24 hours in acidic pH, while only 30% release of the drug was achieved at the same time at physiological pH. In addition, *in vivo* studies showed more effective inhibition of tumor growth of DSPE-PEG/OA6/DOX micelles (81.3%) compared to the free drug (38%) and significantly lower systemic toxicity, indicating a promising pH-dependent drug release profile [34].

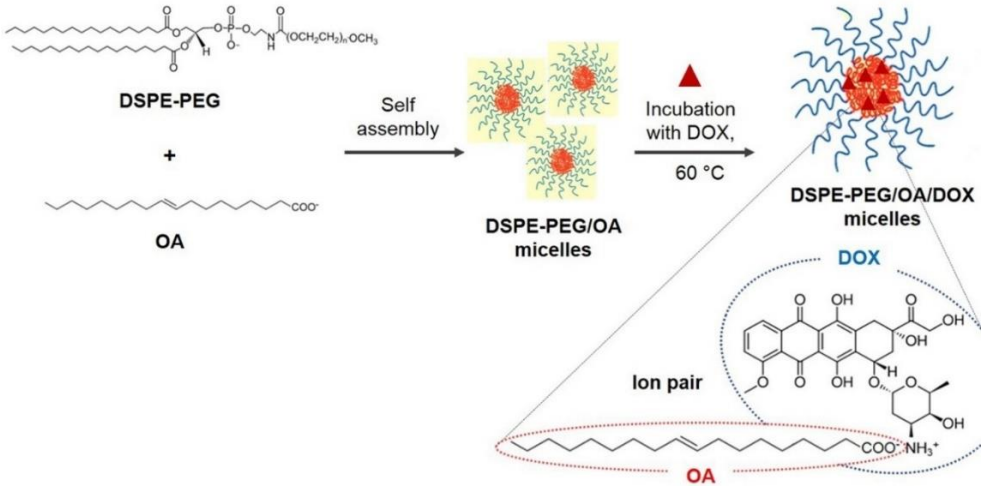


Figure 8: Schematic illustration of the self-assembly of DOX-loaded DSPE-PEG/OA polymeric micelles (*Source: Carolina Henriques Cavalcante et al., Doxorubicin-loaded pH-sensitive micelles: A promising alternative to enhance antitumor activity and reduce toxicity, Biomed Pharmacother. 2021;134:111076*).

Such micelles can be formed with different components and loaded with a variety of drugs, and they have already showed their utility *in vivo*. The stimuli sensitivity of PEG coats allows for the preparation of multifunctional drug delivery systems with multiple functions [2]. However, in most cases it is hard to obtain high sensitivity and specificity of stimuli responsiveness. The carriers either release serious amounts of drug without

actually being triggered or they appear so stable that the triggering environment does not affect the release effectively. Over the years scientist are putting their efforts to overcome these obstacles, focusing on improving the effectiveness of tumor-targeted nanomedicines.

VII(b). Thermoresponsive Polymeric Micelles

It is well known that the temperature in tumor microenvironment is around 3°C to 5°C higher than that in normal tissues. Thermoresponsive polymeric micelles are a new opportunity for development of nanocarriers with biomedical and pharmaceutical use [35]. Typically, an aqueous solution of a thermoresponsive polymer is based on the lower critical solution temperature (LCST) or cloud point (CP), which depends on the hydrophilic and hydrophobic balance of the block copolymers (hydrophobic monomers decrease the LCST, while it is increased by hydrophilic monomers), along with the chemical characteristics and molecular weight of the blocks [36]. Below the LCST, water is bound to the polymer, creating a water-soluble compound that prevents intra- and interpolymer interaction. Once the solution is heated and exceeds this temperature the polymer becomes insoluble as a result of disruption of hydrogen bonds between water molecules and the polymer chains, leading to collapse or precipitation of the polymer [6, 7]. This has been exploited for the development of polymeric micelles that can be formed or destabilized depending on the solution temperature. The most frequently investigated polymer for such micelles is poly(N-isopropyl acrylamide) (pNIPAAm), which has a tunable phase transition temperature. It switches from a water-soluble hydrophilic polymer to a hydrophobic insoluble polymer above 32^{o}C. It can be used either as the hydrophobic core of the micelles conjugated to a permanently hydrophilic block (e.g., PEG) or the hydrophilic micellar corona when combined with a permanently hydrophobic block. Hence for both situations, the polymeric micelles can disintegrate after the induction of mild hyperthermia, which for instance can be induced by ultrasounds, or hypothermia. As pNIPAAm has LCST of 32^{o}C, it means that it is hydrophobic at body temperature, therefore after arrival of the micelles at the target site the encapsulated drug can be released by local hypothermia [36A-36C].

A prominent example of such nanomedicine came from Su et al. by designing a shikonin-loaded thermoresponsive micelle with a LCST of ~40°C. Shikonin has been characterized as a great antitumor agent used for breast cancer therapy, improving the safety and efficacy of the treatment. In order to improve its low aqueous solubility and tumor accumulation, a micelle with thermoresponsive shell and pH degradable core was formed. NIPAAm and DMAAm were used as hydrophilic monomers and the LCST increased to ~39°C, as well as polylactic acid (PLA) as hydrophobic core-forming blocks (**Figure 9**). The shikonin release at physiological temperature (T=37^{o}C) was very slow, compared to the enhanced drug release rate (~90%) within 10 hours at 40^{o}C. In addition, the tumor-cell killing efficacy was found higher at 40°C resulting in a 74% decrease of the tumor volume after 27 days compared to 37^{o}C. The stability of the micelles during blood circulation and high tumor accumulation resulted in low tissue distribution, thus low systemic cytotoxicity. All these data confirmed that the thermoresponsive micelles are promising nanocarriers of shikonin for breast cancer therapy [35].

Figure 9: The assembly process of the shikonin-loaded thermoresponsive nanomicelle (STN) with PNIPAM-co-DMAAm (PID) as the hydrophilic corona and polylactic acid (PLA) as the hydrophobic reservoir (*Source: Yonghua Su et al., Successful in vivo hyperthermal therapy toward breast cancer by Chinese medicine shikonin-loaded thermosensitive micelle, Int J Nanomedicine 2017; 12:4019-4035*).

VII(c). Ultrasound Sensitive Polymeric Micelles

The application of ultrasound, with frequencies from 20 to 100 kHz, has been extensively studied so as to improve disease diagnosis and therapeutics in clinical practice. As an external stimulus, ultrasound possesses several favorable characteristics. First of all, it can

penetrate through a number of tissues in the body in a safe and controlled manner, creating a hydrophilic porous network in the skin and cell membranes, through which drug molecules can reach systemic circulation and accumulate intracellular in tumor tissues [37]. In addition, it is a versatile tool able to trigger both thermal phenomena, by absorption of energy causing large temperature increase at the focal region, and mechanical effects, associated with oscillating or cavitating microbubbles that allow direct passage of the therapeutics into the cytosol [6]. Furthermore, it is an effective and low cost tool for visualizing the target during the therapeutic action. Thus, ultrasound stimulation raises great interest in the biomedical community [38].

However, there are various factors that should be considered in order to apply ultrasound, such as time of application, continuous or pulsed mode of application, frequency and power density. All these factors depend on the location of the tumor in the body. For small superficial tumors it is preferable to use high frequencies (1 to 3 MHz), due to their higher power densities and the fact that they can be more narrowly focused. On the other hand, for deeply located tumors low frequencies are more appropriate because they can penetrate deeper into the body but can also damage healthy tissues due to a strong cavitation effect [6].

In a pioneering study by combining polymeric micelles and ultrasound, a new ultrasound-sensitive polymeric micellar drug delivery system has been developed and has recently received great attention. Salgarella and her co-workers investigate the effect of ultrasound on the release of an anti-inflammatory hydrophobic drug, dexamethasone, from poly(2-oxazoline)-based micelles. Poly(2-oxazoline)s are synthetic polymers that exhibit stealth behavior and have been found to be non-toxic both *in vitro* and *in vivo*. The results showed that ultrasound of 40 kHz increased the amount of the released drug by 6% to 105%, depending on the type of the copolymer used and stimulation time point, compared to the spontaneous release (**Figure 10**) [38].

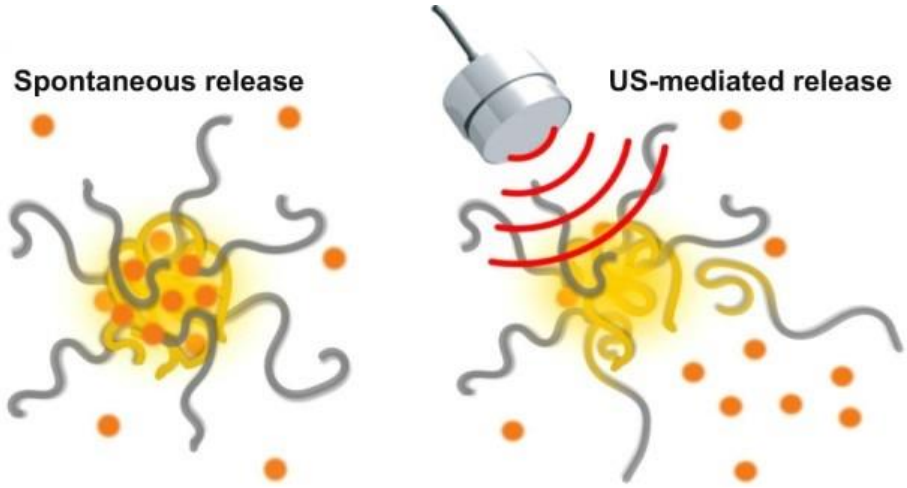


Figure 10: Representation of spontaneous release (left) and ultrasonic-mediated release (right) from poly(2-oxazoline)-based micelles *(Source: Alice Rita Salgarella et al., Investigation of drug release modulation from poly(2-oxazoline) micelles through ultrasound, Sci Rep. 2018; 8:9893)*.

Another interesting example of such technology came from Yugo Martins and his coworkers by combining ultrasound with sonosensitizer drugs, known as sonodynamic therapy. They developed a drug delivery system based on polymeric micelles to transport zinc phthalocyanine (ZnPc) to the skin by the application of low frequency ultrasound (20 kHz) with the dual purpose of generating reactive oxygen species (ROS). The survey showed that the application of ultrasound resulted in 40.5 times more penetration and better distribution of ZnPc into deeper layers of the skin than passive treatment. In addition, ultrasound irradiation of ZnPc-loaded micelles significantly increased singlet oxygen generation and the viability of tumor cells was reduced to approximately 60%. Consequently, the above results demonstrated that the application of low frequency ultrasound on the skin has the double potential of improving the topical sonodynamic therapy for the treatment of skin tumors [37].

VII(d). Light-sensitive Polymeric Micelles

Compared with other external stimuli, light as a trigger for drug release has received a lot of interest, since it is a clean and remote stimulus that allow site-specific release of payload at desired disease sites and can be spatially and temporally controlled by tuning the intensity, wavelength and site of irradiation [39]. Either ultraviolet (UV), visible (VIS) or near infrared (NIR) light can be applied as the trigger, while the use of NIR can be used for deeper tissue penetration, up to 10cm, and less damage to healthy tissues, because hemoglobin, water and lipids have low absorption in the NIR region (650-900 nm) [7]. However, UV light can be used to trigger drug release for topical treatment of the skin and mucosa, since it has low penetration in biological tissues.

Polymeric micelles can be light-sensitive and thus deliver therapeutic agents in response to all kind of light by incorporation or/and conjugation of a chromophore structure to the hydrophobic polymer. When light-sensitive polymeric micelles are illuminated, there is a change in polarity and hydrophobic-hydrophilic balance changes resulting to dissociated micelles and drug release. This change can be either reversible, thus when the photoreaction ends the initial polarity is returned and the micelles are re-established, or a second photoreaction might occur converting the hydrophobic block into a hydrophilic polymer leading to an irreversible micelle destabilization [6].

For example, Kim et al. prepared micelles conjugated with the hydrophobic chromophore diazonaphthoquinone (DNQ) that absorbs strongly at 365 nm and showed a change in polarity of the micellar core from hydrophobic to hydrophilic under UV light irradiation, releasing doxetaxel (DTX), a well-known anti-cancer drug. The results revealed that the polymeric micelles dissociated under UV light, which was attributed to the rearrangement of DNQ moieties. In addition, cancer cell viability was decreased from 55% to approximately 36%, since more DTX was released into them. However, without light irradiation, the cytotoxicity to healthy endothelial cells were lower compared to free drug, making them suitable for biomedical applications within a living system [39].

A novel UV-sensitive polymeric micelle system based on coumarin was designed by Zhang and his coworkers, indicating that the micelle itself can generate CO_2 bubbles under UV irradiation for direct tumor ablation, so as to avoid harming normal cells and tissues with

conventional anticancer drugs (**Figure 11**). A coumarin ester (DEACM) was used as the hydrophobic block of the micelles conjugated with PEG to form the amphiphile DEACM-PEG derivatives, that leads to the cleavage of coumarins and production of CO_2 under UV light exposure (365 nm). As a proof of concept, the results showed that UV exposure of appropriate energy was able to cleave the amphiphile and thereby a large number of bubbles containing CO_2 exploded and induce the necrosis of ~86% of the cancer cells. As a result, this project indicates the capability of killing tumor cells under UV exposure through CO_2 generation and should be further investigated [40].

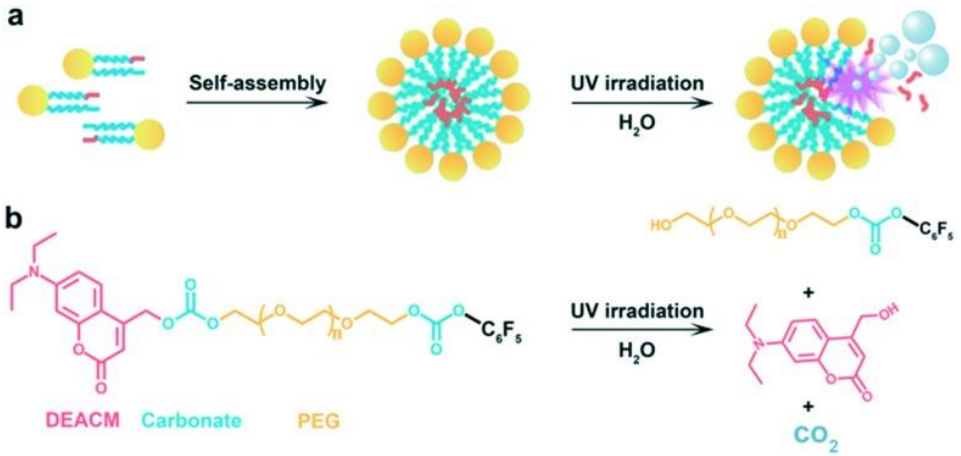


Figure 11: **a)** Schematic illustration of the self-assembly and UV-induced gas generation process of the polymeric micelles, **b)** changes of the copolymer structure before and after UV irradiation (*Source: Yifan Zhang et al., Light-responsive CO_2 bubble-generating polymeric micelles for tumor cell ablation, Medchemcomm. 2017 Feb 1; 8(2):405-407*).

VIII. MOLECULAR IMAGING IN THE DRUG DELIVERY FIELD

The research on polymeric micelles as drug delivery systems has shown superior results compared to conventional cancer therapies. To fully exploit the clinical possibilities of this new generation of nanomedicines, polymeric micelles has also been used for bioimaging applications. Since imaging provides a more detailed and comprehensive understanding of cancer metabolism, it is important to include recent studies in this field.

Medical imaging modalities such as magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), positron emission tomography (PET) and X-ray computed tomography (CT) play an important role in the diagnosis of cancer and therapy response evaluation. Unfortunately, nonenhanced imaging techniques are useful only when relatively large tissue areas are involved in the pathological process. To solve this problem, the use of contrast agents specific for each imaging modality that are able to absorb certain types of signals much stronger than surrounding tissues, greatly increases contrast specificity between tumor and healthy tissues by highlighting the area of interest compared to conventional image diagnosis of tumors [2, 9]. Furthermore, they can lower the size limit of small tumor detection, which is a valuable feature considering the struggle of detecting tumors smaller than 1 cm in diameter with present cancer medicine [5]. To further increase local spatial concentration of contrast agents, a combination of certain nanoparticulate carriers that are able to carry multiple moieties to areas of interest and enhance the signal from these areas is suggested [2]. Thus, polymeric micellar carrier systems have received great attention because of their ability to incorporate various kinds of chemical species, contrast agents and anticancer drugs. Therefore, they can combine diagnostic imaging applications and therapy systems [5]. This section reports on the most recent developments in this area by giving examples of polymeric micelles that have been used for imaging techniques.

VIII(a). Magnetic Resonance Imaging (MRI)

In MRI a sufficient magnetic field is applied to detect changes in magnetization of hydrogen atoms in tissues within the body [6]. After relaxation to their ground state, a radiofrequency pulse is generated that is identified and converted into a picture. However, this technique has low sensitivity or poor contrast, thus the use of MRI contrast

agents is necessary so as to alter the relaxation times of protons in various organs through their involvement with the magnetic field [41]. These agents are usually paramagnetic elements and their encapsulation by polymeric micelles limits their contact with other intracellular structures, preventing the formation of free radicals and thereby lowering systemic cytotoxicity [42]. For example, super paramagnetic iron oxide nanoparticles (SPIONs) can significantly shorten the signal intensity by shortening the hydrogen transverse relaxation time (T2) and cause darkening of the surrounding regions. A normal tissue should take a substantial amount of SPIONs and therefore, reduce the T2 signal intensity of MRI. On the other hand, tumor cells have less SPIONs uptake, which leads to relatively high signal intensity. Based on this fact, an interesting effort combining SPIONs and Nile red into polymeric micelles assembled from the telodendrimer mPEG-*b*-dendritic oligo-cholic acid (mPEG-Lys3-CA4) was made by Li and his colleagues so as to investigate the feasibility of MRI in vitro (**Figure 12**).

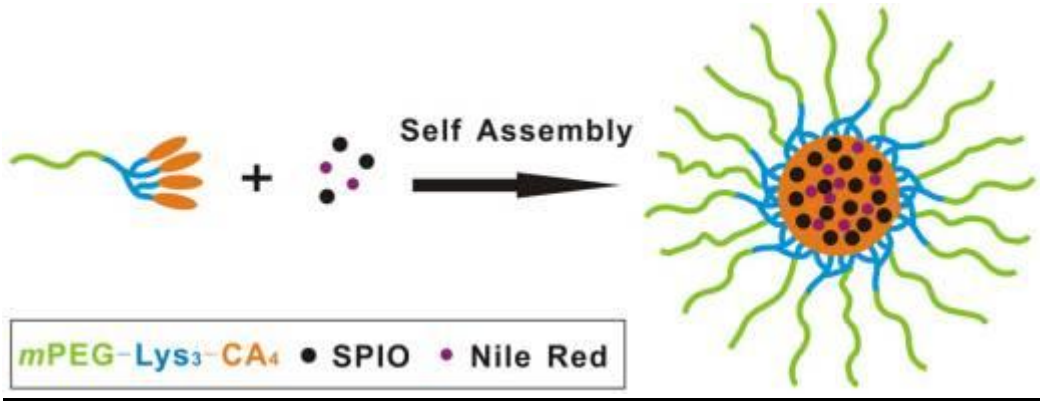


Figure 12: Formation of mPEG-Lys3-CA4-NR/SPION polymeric micelles for MR imaging (*Source: Wen-Juan Li et al., Preliminary Study of MR and Fluorescence Dual-mode Imaging Combined Macrophage-Targeted and Superparamagnetic Polymeric Micelles, Int J Med Sci. 2018 Jan 1;15(2)129-141*).

The cytotoxicity of the micelles in Raw264.7 cell line was slightly increased in a dose-dependent manner, while 40 μg/mL seems sufficient dosage for signal reduction in MRI (77.5%) and incubation time of 6 hours was most favorable for cell labeling. The results revealed mPEG-Lys3-CA4-Ne/SPIONs with high MRI sensitivity and super small particle size that are expected to provide a new strategy for targeted therapy and clinical potentials [43].

With a different strategy, Zhang et al. synthesized an amphiphilic block copolymer via the ring-opening polymerization of ε-caprolactone (CL) initiated by PEG, in which the tumor-targeting ligand cyclic pentapeptide Arg-Gly-Asp (cRGD) was conjugated with the terminal block. Superparamagnetic γ-Fe_2O_3 nanoparticles was loaded into the core of the polymeric micelles so as to explore the targeting effect in cancer cells and to evaluate the magnetic sensitivity as an effective MRI contrast agent. The results indicated that γ-Fe_2O_3-loaded cRGD-PEG-PCL micelles can undoubtedly reduce the systemic cytotoxicity of γ-Fe_2O_3 nanoparticles and showed better active uptake by human hepatic carcinoma vascular endothelial cells. In addition, there was a gradual decrease in signal intensity according to the increase in iron concentration, due to the shorter T2 relaxation time. In vitro MRI confirmed that γ-Fe_2O_3-loaded cRGD-PEG-PCL micelles can be monitored using clinical 1.5 T MRI scanner, thereby it appears to be a promising drug-delivery system for imaging contrast compounds and anti-angiogenics [44].

VIII(b). Computed tomography (CT)

Computed tomography utilizes ionizing X-rays for the production of high-resolution 3D images by spinning an X-ray tube and a detector over a patient's sides. The differences in absorption of X-rays discriminate different tissues within the body. The most frequently employed contrast agents for CT are iodine-based small molecules. However, such molecules have limited blood circulation, suffering from rapid clearance by the mononuclear phagocyte system [40]. In addition, the need for high concentration of contrast agents makes CT less suitable for molecular imaging [6]. Over the past years, there has been a great increase in the development of nanoparticles as contrast agents, which can overcome these problems. Polymeric micelles can be multifunctional and so can offer contrast for multiple imaging modalities or provide therapeutic effects as well [45].

Zaki et al. took advantage of this technology by creating gold-loaded polymeric micelles (GPMs) for computed tomography-guided radiation therapy treatment. Gold nanoparticles (AuNPs) have been used as both imaging and therapeutic agents, while they can provide greater X-ray attenuation than iodine and have also shown potentials as radiosensitizers. AuNPs in combination with radiation treatment can lead to increased

number of DNA double-strand breaks compared with radiation therapy alone. Although, because of the rapid clearance a nanoplatform, such polymeric micelles, is needed to prolong circulation time. For this reason, AuNPs were encapsulated within the hydrophobic core of micelles formed with the amphiphilic diblock copolymer PEG-*b*-poly(ε-caprolactone) (PEG-*b*-PCL) (**Figure 13**).

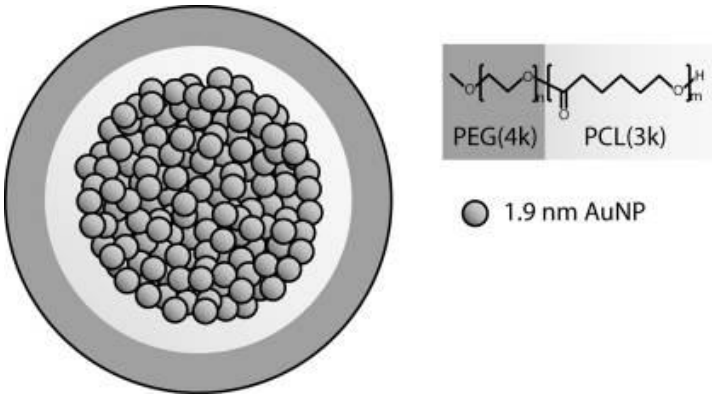


Figure 13: Schematic illustration of gold-loaded polymeric micelles with the amphiphilic diblock copolymer PEG-*b*-PCL (*Source: Ajlan Al Zaki et al., Gold-Loaded Polymeric Micelles for Computed Tomography-Guided Radiation Therapy Treatment and Radiosensitization, ACS Nano. 2014 Jan 28; 8(1): 104-112*).

Compared to human fibrosarcoma cells receiving radiation only, the cells that were irradiated in the presence of GPMs exhibited 2.2 times higher density of DNA double-strand breaks. Moreover, no evidence of leaching AuNPs from the micelles was observed, suggesting that GMPs are sufficiently stable *in vivo*. The ability of GMPs to accumulate into tumors to provide CT contrast was confirmed by displaying a statistically significant 6-fold increase in gold accumulation owing to the EPR effect. Additionally, the combination of CT-guided radiation therapy and gold-mediated radiosensitization led to a significant increase in the mean survival time of tumor-bearing mice. Therefore, GMPs can be used to guide and enhance the efficacy of radiation therapy [46].

VIII(c). Single-photon emission computed tomography (SPECT)

SPECT is a nuclear imaging technique using γ-rays that enables assessment of biochemical changes and levels of molecular targets within the body. More specifically, a gamma camera is used which rotates around the patient to capture data from various positions to

get a tomographic reconstruction. The radioisotopes preferred for SPECT imaging are either used only for diagnosis or for diagnosis and therapy as well. Polymeric micelles can be easily radiolabeled with suitable SPECT radionuclides while protecting entrapped drugs from degradation during their delivery. Gamma-emitting radioisotopes can act as drugs for radiation therapy and also as probes for monitoring the efficacy of the therapeutic approach [47].

The development of a polystyrene-b-poly(ethylene oxide) diblock copolymer micelle (PS-b-PEO) was reported by Laan et al. The radionuclide ^{111}In was entrapped within the micellar core during the formation of the polymeric micelles by using tropolone as lipophilic ligand, leaving the PEO corona unaffected. When the PS-b-PEO block copolymers examined *in vivo* with SPECT/CT imaging, a particular pattern of accumulation has been observed in the spleen and liver, possibly, due to clearance via the mononuclear phagocyte system. Although circulation is not detectable via nuclear imaging after 24 hours, *ex vivo* data revealed that the blood samples exhibit high percentage of activity, indicating that the micelles are still in circulation. Based on these findings, it is expected that PS-b-PEO polymeric micelles can have a long circulation time to ensure high tumor uptake via EPR effect [48]. The tumor uptake can be improved by conjugation with suitable targeting ligands and this approach might come in handy in the development of polymeric micelles for multifunctional imaging, tumor diagnosis, drug delivery and anticancer applications.

In a different study, Kurihara et al. reported the synthesis of ^{111}In-labeled polymeric micelles of the A_3B-type lactosome comprising (poly(sarcosine))$_3$-*b*-poly(L-lactic acid), so as to identify the meningeal dissemination in mice bearing this condition as well as bone metastasis at mandible. *In vivo* SPECT/CT imaging and biodistribution studies in mice confirmed the accumulation of ^{111}In-lactosome in the brain metastases. Compared to ^{201}TlCl, except of the accumulation gains in the brain metastases, there were also observed accumulation of radioactivity in the muscle around the neck, which makes it difficult to recognize the metastasis. So as in the case of ^{99m}Tc-HMDP, which failed to image the melanoma and was accumulated in a broad range of bones in the head and neck. Nevertheless, it was demonstrated that the accumulation amount of ^{111}In-lactosome was doubled in comparison with that of ^{201}TlCl, indicating a superiority in

imaging abilities of brain and bone metastases, and therefore it is suitable as a diagnostic agent [49].

VIII(d). Positron emission tomography (PET)

PET imaging modality is a clinically used noninvasive functional imaging technique that facilitates quantitative analysis of pharmacokinetics and biodistribution due to its high sensitivity, accurate quantification and endless penetration depth [51]. It can be used in drug delivery by assessing drug biodistribution and accumulation at regions of interest, thus measuring biological processes at the molecular and the metabolic levels *in vivo* in real time (**Figure 14**). Nonetheless, the limited spatial resolution of PET images makes it difficult to accurately characterize target regions [50]. Unnecessary radiation exposure to healthy organs should be avoided, while it is known that high radiolabeling specific activity is crucial for nanoparticle-based PET imaging to collect high-quality images [51].

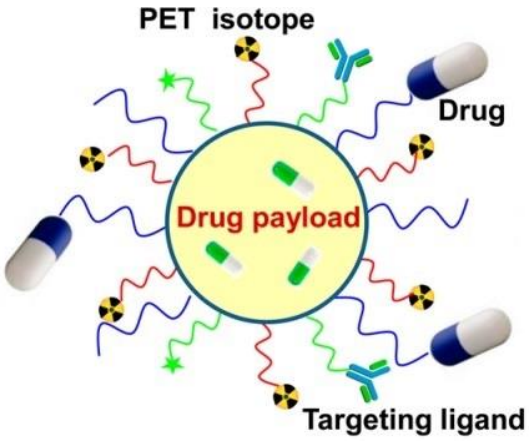


Figure 14: Schematic illustration of a radiolabeled polymeric micelle for use in PET image-guided drug delivery (*Source: Rubel Chakravarty et al., Positron Emission Tomography Image-Guided Drug Delivery: Current Status and Future Perspectives, Mol Pharm. 2014 Nov 3; 11(11): 3777-3797*).

Sun et al. in order to create a multifunctional polymeric micellar carrier offering imaging and therapeutic functions, they synthesized a farnesylthiosalicylate (FTS) based triblock copolymer POEG-*b*-PVBA-*b*-PFTS (POVF) that is able to release paclitaxel (PTX), a known anti-cancer drug. POVF polymer can inhibit tumor growth *in vitro* and *in vivo* by itself, however co-delivery of PTX and FTS by the carrier can improve further the therapeutic effect. ^{89}Zr and ^{64}Cu were successfully used as positron-emitting radionuclides. The

resulting radiolabeled PTX/POVF micelles were used for PET imaging of mice bearing 4T1.2 tumor xenografts. At early time points, there was a wide biodistribution in liver, kidney and blood, while the signals of the micelles in tumor increased over time. In addition, the data exhibited an excellent serum stability (less than 5% disassociation after 24 hours), rapid tumor uptake (at 1 hour post injection) and slow clearance (large amounts of radioactive signals after 96 hours). It was also noted a significantly high anti-tumor activity *in vivo*, by demonstrating a large area of cancer cell necrosis, probably due to the combination of synergistic effect between FTS and PTX. No apparent changes were observed in the surrounding healthy tissues and organs, indicting little systemic toxicity. The above results suggest that PTX/POVF polymeric micelles can be used as a potential PET-image guided multifunctional drug delivery system with superior anticancer efficacy and minimum adverse effects [51].

In another recent study, Huang et al. designed a pH-sensitive positron-emitting neutral copolymer micelle labelled with the radionuclide ^{64}Cu, which are then internalized and retained by cancer cells in mice models for dual PET and fluorescence functions. A pH-activatable indocyanine green-encoded nanosensor (PINS) [52] was used as a ''chemical transistor'', which improved the sharpness of pH response and allowed deeper fluorescence penetration in tissues. Fluorescence microscopy data confirmed that labelled micelles can cross the blood tumor barrier. In addition, positron signals in PET imaging further illustrated accumulation of the micelles indicating malignancies in brain, head, neck and breast. There was also a clear indication of pH-dependance, since a five-fold increase in cellular uptake was detected at pH 6.5 in 1 hour compared to that at pH 7.4. Autoradiography analysis showed sporadic capture of the micelles in tumors, while in order to reduce false positive signals from healthy tissues an evaluation in tumor-free mice exhibited a slightly higher signal over the control, which considered as statistically insignificant. All these results corroborated the feasibility of ^{64}Cu-PINS sensors to detect a broad range of occult cancer types. On the other hand, high uptake in the reticuloendothelial system may be an obstacle for the detection of cancers in spleen and liver. Overall, incorporation of both PET and fluorescence applications in one nanosensor results in a whole-body assessment of tumors followed by high resolution imaging for local interventions [53].

IX. THERANOSTICS IN THE DRUG DELIVERY FIELD

Cancer is a complex cluster of diseases characterized by an abnormal and uncontrolled cellular expansion coupled with malignant behavior such as invasion and metastasis. The fact that it is highly unpredictable results in numerous obstacles for early diagnosis and effective treatment. Current therapies include surgery, radiotherapy, chemotherapy, hormone therapy, stem cell therapy and immunotherapy. Early detection of the tumor is the most critical factor that determines whether it can be surgically removed, and/or whether it will require a combination of approaches. Radiotherapy and chemotherapy are the principal treatment modalities focused on the eradication of deep located solid tumors. However, these approaches suffer from serious limitations, such as lack of specificity of drug molecules to reach tumor sites, the fact that tumors tend to become more and more resistant to therapies and the development of long-term off-target toxic effects that not only kill cancer cells but also harm several types of healthy cells at the same time. The concept of developing a multifunctional targeted drug delivery system that can help diagnose the location and stage of the tumor, provide the necessary concentrations of the therapeutic agent at tumor sites and minimize toxic side effects, while simultaneously monitor their therapeutic response *in vivo* by visualizing tumor regions in the body, has inspired scientists all over the world [54, 55, 56]. The integration of therapy and diagnosis is defined as theranostics. A wide range of nanostructured materials can be applied for theranostic applications. Polymeric micelles have garnered increasing research interest in this field due to their possession of multiple functional entities for the effective attachment of imaging, therapeutic and targeting agents (**Figure 15**) [41].

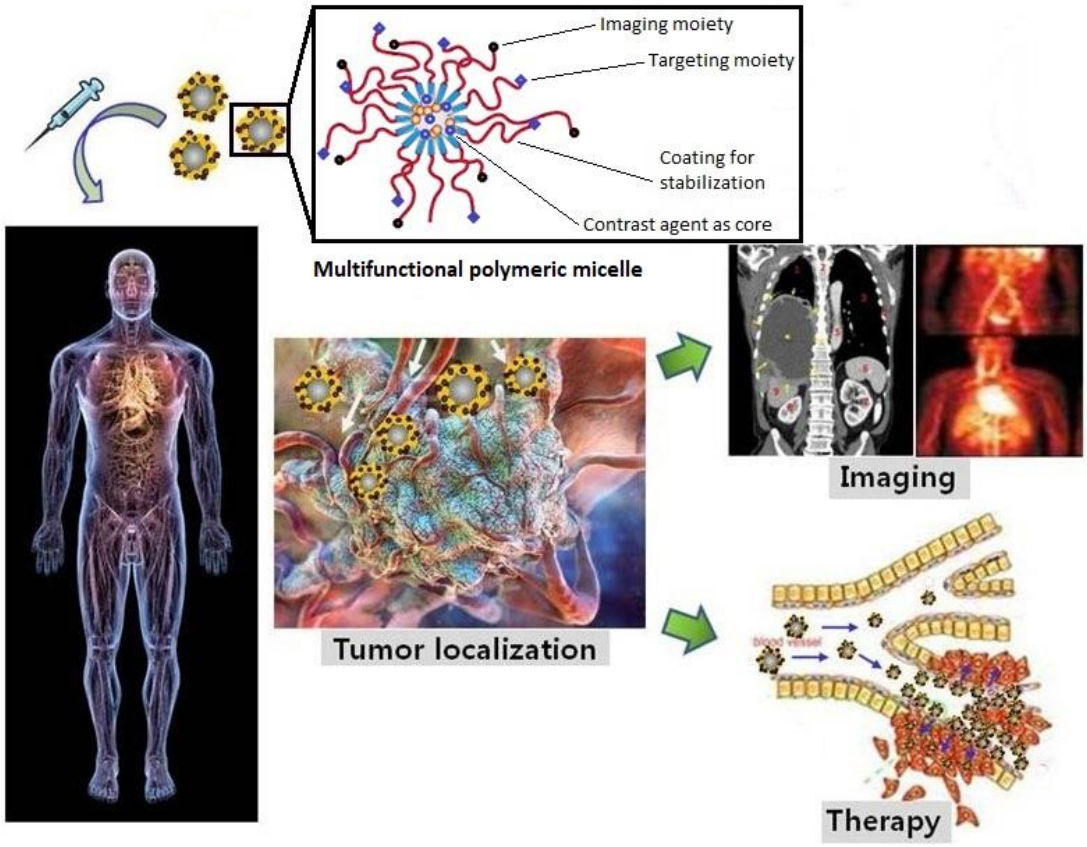


Figure 15: Multifunctional polymeric micelles carrying anti-cancer drug for theranostic applications (*Source: Ki Hyun Bae et al., Nanomaterials for Cancer Therapy and Imaging, Mol Cells. 2011 Apr 30; 31(4): 295-302*).

An example showing the importance of theranostics was made by Zhuang et al. by designing stimuli-responsive multifunctional polymeric micelles with fluorescence imaging features. More precisely, they used two-photon fluorescence imaging since it has better biological response with lower toxicity to healthy cells and longer exciting wavelengths with high resolution due to the ability to penetrate deeper in tissues. Furthermore, in order to exploit the tumor microenvironment for enhanced triggered release, they combined pH and redox responsiveness in one smart drug delivery system. The resulting polymeric micelles was based on mPEG-SS-Poly (mPEATss) amphiphilic copolymer and were equipped with a two-photon fluorescence probe, as well as with the antitumor drug DOX for both *in vivo imaging* and therapy. One more important feature is that mPEATss micelles have great sensitivity to acid and high concentration of glutathione (GSH) along with charge-conversion under acidic environment. In fact, the results showed that DOX-loaded mPEATss micelles were rapidly disassembled at acidic medium

containing 10 mM of GSH. Furthermore, they emitted strong fluorescence in water and the intensity was slightly reduced under acidic environment with high levels of GSH. They also exhibited great biocompatibility and their bioimaging ability was confirmed by red and green fluorescence. GSH triggered drug release was only observed in DOX-loaded mPEATss micelles, while it was much quicker at pH 6.0, suggesting that pH and redox dual-trigger can enhance antitumor efficacy. *In vivo* pharmacokinetic and imaging studies in 4T1 tumor bearing mice models demonstrated significantly extended circulation time and high accumulation in tumor sections respectively. As expected, DOX-loaded mPEATss micelles exhibited better tumor-controlling capacity, by suppressing tumor angiogenesis and causing apoptotic death of tumor cells, and limited side effects, which could be attributed to the controlled release of DOX (**Figure 16**). In this way, this multifunctional micellar-based carrier revealed superior therapeutic potential for cancer theranostics [57].

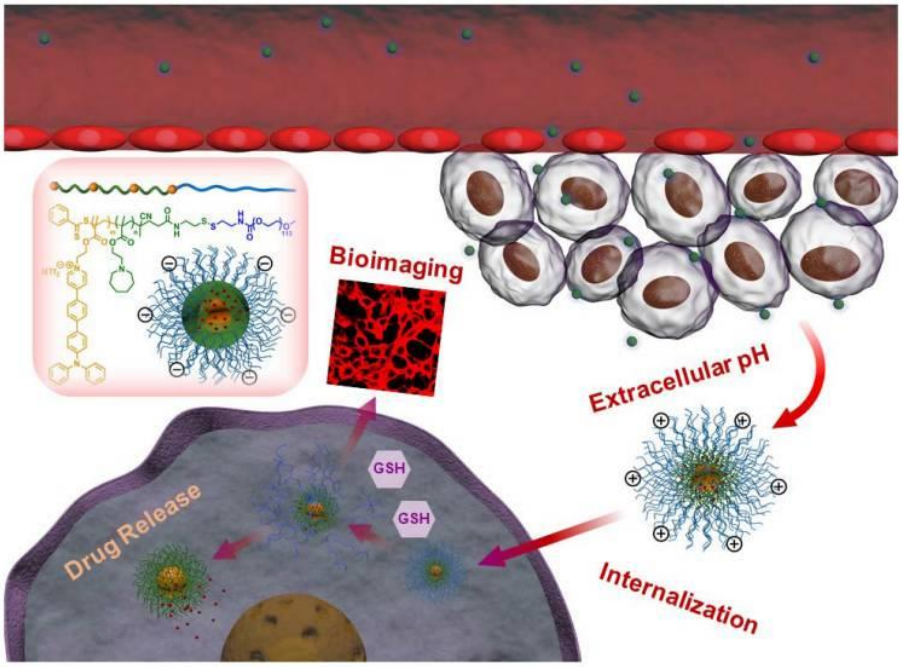


Figure 16: Schematic illustration of DOX-loaded mPEATss micelles for pH-triggered charge-conversion enhanced cellular internalization, pH and redox dual triggered drug release and two-photon cell imaging (*Source: Weihua Zhuang et al., Two-photon AIE luminogen labeled multifunctional polymeric micelles for theranostics, Theranostics. 2019; 9(22): 6618-6630*).

Similarly in another study, Xu et al. developed polymeric prodrug micelles with two-photon and aggregation-induced emission bioimaging abilities and programmed pH-sensitive charge-conversion and drug release under acidic environment. The micelles were constructed based on the two-photon fluorophore (TP) and DOX labeled prodrug TP-PEI (DA/DOX)-PEG copolymer, which consists of dimethylmaleic anhydride (DA) grafted polyethyleneimine (PEI) and PEG. The resulting micelles showed a satisfactory stability with only 34% of DOX released at physiological pH 7.4 after 48 h, while under acidic environment at pH ~6.8 the outside PEG segment gradually disassembled along with DA and DOX, leading to accelerated drug release that reached 85.1% after 48 h. Two-photon cellular imaging in 4T1 cells revealed stronger fluorescence signal at pH 6.8 in contrast to the cells at pH 7.4 in the same incubation time, illustrating that the acidic environment can trigger the charge-conversion and enhance endocytosis of the micelles to the tumor cells. So as to determine the micellar distribution after injection, *ex vivo* fluorescence signals were seen in livers and kidneys, indicating the micelle systemic metabolism and distribution in tumors, a feature attributed to the EPR effect. In addition, deep-tissue bioimaging ability of the micelles was confirmed under 800 nm two-photon excitation by observing fluorescence signals with a depth of up to 150 μm. The prodrug micelles also displayed enhanced cellular uptake and tumor inhibition from 78% to 18% after 48 h at pH 6.8, as well as no obvious weight loss was recorded compared to free DOX-treated mice with about 11% of their body weight lost. Histological studies verified the great biocompatibility and outstanding therapeutic efficacy of the prodrug micelles, since few inflammations and low toxicity were found in heart, liver, spleen and lungs of mice. Overall, TP-PEI (DA/DOX) prodrug micelles could be an efficient nanoplatform for theranostic applications in cancer treatment [58].

In another recent study, Yang and his coworkers tested the ability of DOX- and IR780-loaded polymeric micelles to effectively achieve both photothermal therapy and dual stimuli-responsive drug release. For this reason, a thermo- and pH-responsive polymer (mPEG-PAAV) was used with different upper critical solution temperature (UCST) at various pH values. IR780 can convert NIR laser energy into heat, thus triggering DOX release, as well as for photoacoustic (PA) imaging due to its strong NIR absorption peak (**Figure 17**). The resulting mPEG-PAAV micelles possessed good stability in simulated

physiological conditions, high hemocompatibility and limited cytotoxicity. Increasing the test temperature from 25°C to 65°C caused the swelling of the micelles and eventually their dissociation, establishing their thermal responsiveness. In addition, as pH value decreases so does micelle UCST, respectively. DOX- and IR780-loaded micelles were investigated under laser irradiation and the results demonstrated a significant temperature elevation induced by IR780, suggesting that varying the concentration of IR780 and the laser power density can control temperature increase, which is helpful for improved drug distribution and avoidance of drug leakage in the bloodstream. Subsequently, the samples were irradiated with NIR laser for three cycles so as to further investigate their photothermal efficiency. Compared to severe decrease of temperature in the free IR780+DOX group after the third cycle, mPEG-PAAV micelles/IR780+DOX degraded more slowly and still produced hyperthermia effect, due to the photostability protection of mPEG-PAAV micelles. In order to test the thermo- and pH-sensitive drug release ability, the micelles were heated at different pH values. As expected, the release of DOX was obviously higher at 45^{o}C in pH 7.4 than at 37^{o}C (body temperature) and 25^{o}C (room temperature). Remaining at 45^{o}C, DOX release was accelerated up to 32.5% in pH 6.5 and 55.3% in pH 5.0 within 24 h, implying that both increase of temperature and decrease of pH value are determining factors in DOX release rate. Moreover, NIR laser irradiation was able to enhance cellular uptake of the micelles in 4T1 cells due to the increased permeability of the plasma membrane caused by IR780-induced hyperthermia. The viability of 4T1 cells was measured so as to estimate *in vitro* the chemo-photothermal therapy efficacy of the micelles. There was no obvious tumor growth inhibition and cell viability was not significantly enhanced at pH 6.5 in comparison to that at pH 7.4 without NIR laser irradiation. On the contrary, when the treatment was combined with NIR laser irradiation, the hyperthermia (44.6^{o}C after 5 min of irradiation) not only induced cancer cell apoptosis (up to 22.4%) and accelerated DOX release, but also enhanced cytotoxicity of DOX to contribute to the synergistic chemo-photothermal therapy effect. According to biodistribution data through fluorescence imaging, it was found that the micelles were effectively targeted to solid breast tumor *in vivo* owing to the EPR effect, while *ex vivo* analysis in major organs showed weaker signals than that of tumors, indicating that the micelles achieved a preferable passive targeting ability, which is of great importance for superior combination therapy. As discussed in the beginning, IR780 can be used as

photoacoustic imaging agent to obtain more information about the distribution of the micelles and the microstructure of the tumor, which was confirmed as depicted in the *in vitro* and *in vivo* PA imaging of the micelles. The PA signal in the tumor was 1.5-fold higher after mice treated with 0.9% NaCl were injected with mPEG-PAAV micelles/IR780+DOX compared to free IR780+DOX. What is more, in order to further estimate the *in vivo* therapy efficacy of the micelles, tumor growth was measured for 29 days of treatment with and without NIR laser irradiation. Remarkably on day 9, mPEG-PAAV micelles/IR780+DOX plus NIR laser irradiation inhibited tumor growth and no recurrence was observed during the rest experimental period. Due to the fact that lung metastasis is a common phenomenon following breast cancer, more studies were needed to confirm the complete elimination of cancer cells. Fortunately, no mice treated with mPEG-PAAV micelles/IR780+DOX plus NIR laser irradiation developed lung metastasis of breast cancer cells, as well as no obvious adverse effects were displayed in healthy organs. Collectively, these results suggest that the hyperthermia-assisted chemotherapy using IR780- and DOX-loaded mPEG-PAAV micelles introduces a powerful combined treatment against metastatic breast tumors [59].

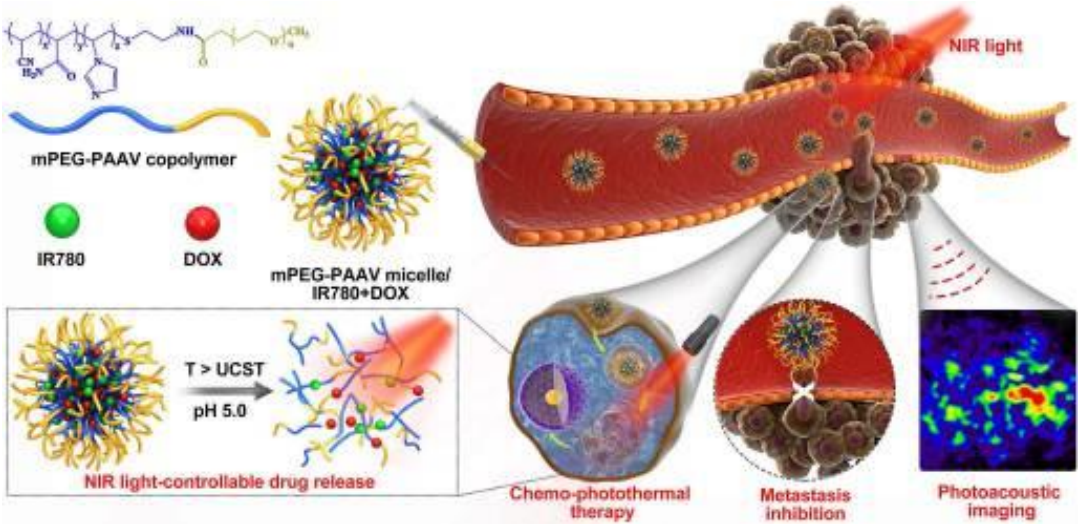


Figure 17: Schematic illustration of the chemo-photothermal therapy of DOX- and IR780-loaded mPEG-PAAV micelle with NIR laser-controlled drug release (*Source: Zhe Yang et al., Thermo- and pH-dual responsive polymeric micelles with upper critical solution temperature behavior for photoacoustic imaging-guided synergistic chemo-photothermal therapy against subcutaneous and metastatic breast tumors, Theranostics. 2018; 8(15): 4097-4115*).

X. METHODS

Knowing where to search and how to search information about a topic is considered to be a great part of how fully the topic is developed. That is, for the current thesis to be accomplished, the main search engine used was Google Search, the world's most frequently used search machine. Nevertheless, one simple search engine is never enough for a subject to be fully understood and deeply analyzed.

For that reason, we had to also add in our searching strategy some additional scientific information databases so as to offer a more detailed description of the search methodology and classification process, initial searches were conducted through web-based search engines including PubMed database, ResearchGate database, Science Direct database and MedlinePlus database. Those databases helped us find specific articles from the medical, the biochemical and the pharmacology world – written by recognized scientists on those specific fields all over the world. This ensured that the bibliographical sources of our thesis are valid, up-to-date and rich in the proper information.

Regarding the main terms searched, those included the following words (or combination of words): ''drug AND delivery AND nanosystems'', ''multifunctional AND polymeric AND micelles'', ''polymeric AND micelles AND passive AND active AND targeting'', ''stimuli AND responsive AND polymeric AND micelles'', ''polymeric AND micelles AND combination AND therapy'', ''polymeric AND micelles AND imaging'' and ''polymeric AND micelles AND theranostics''.

The more of those terms we combined in one searching trial, the better and more specified results we would get. For instance, googling "network-targeted combination therapy" is considered to be much more efficient and to the point than googling "combination therapy" on its own. No significant filtering of the applications and products themselves was performed at this point. All applications and products identified as nanotechnology related to medicine were recorded for further analysis.

Non-journal resources were not needed or used anywhere in our searching strategy-as the online bibliographical sources were found to be much richer and more updated than the respective books.

And, last but not least, the types of papers used in the current thesis were both qualitative and quantitative, but -also- some mixed ones were included, because each one of these article types offered us a different kind of scientific data that was useful in our perception of the specific research area: thus, while qualitative research papers offered a wide variety of information about the most commonly used drug delivery methods that are available up to today, quantitative research papers would show much more clearly (with the use of tables, clear diagrams and colorful graphics) the advantages and the limitations of each method -hence helping us come to important conclusions about each method's clinical efficacy. In that way, both quantitative and qualitative papers offered (each one, in a different way) precious information that was needed for the writing of the current thesis.

XII. DISCUSSION

The reason why the subject of the current thesis is important is because it gathers the majority of the information existing so far (in the online bibliographical sources) on the topic of the already existing drug delivery methods- giving a special emphasis on the use of multifunctional polymeric micelles. As it has already been cited above, multifunctional polymeric micelles are one of the most commonly used drug delivery methods nowadays, mainly because of the advantages of their biochemical composition and structure- which permits an efficient transport of a wide variety of pharmacological agents inside the human body, while at the same time permitting a good bioavailability of the carried drug and an exceptional targeting.

What is more, the drug delivery methods cover the vast majority of the protocols used in the designing of each new drug -globally. As a result, it would be a great challenge for a researcher to combine the already existing data about multifunctional polymeric micelles with the other, already used drug delivery methods, and see what is the ranking of the efficiency of multifunctional polymeric micelles amongst all kinds of drug delivery methods that have been existing up to nowadays.

On the other hand, the new technological advancements and the recent biochemical knowledge in the fields of chemistry, biology and medicine has brought new products on the pharmacological research field (new kinds of synthetic nanocarriers included), which makes it necessary for the researcher to investigate all the already existing articles, old and recent, referring to the topic and to make a summary of all the important conclusions so far.

All the above explain why the topic of multifunctional polymeric micelles needs to be examined more deeply, with the use of a detailed theses -like the current one.

XIII. CONCLUSIONS

The requirements of complex diseases, like cancer, lead scientists to seek more advanced therapeutics which eventually find their place in modern therapies. It is important to remember that during the process of administering anticancer agents, they often fail to reach target tissues due to their poor selectivity and have tremendous side effects on healthy organs. For this reason, the applications of nanomedicine in cancer diagnostics and therapy have been the most fascinating areas of research in the last decades, since they are designed to improve the pharmacological and therapeutic properties of conventional drugs. Numerous drug delivery nanoplatforms have been used for this purpose and have attracted widespread interest by virtue of their controllable size, high surface area to volume ratio, and customized chemical modifications. Their selection is primarily based on the physicochemical features of the targeted drugs being selected for the treatment and the specificity and pharmacokinetics of the delivery.

Polymeric micelles are important nanotools thanks to their composition of amphiphilic block copolymers that self-assemble in an aqueous environment so as to form structures with a hydrophobic core stabilized by a hydrophilic shell. Their unique structural, chemical and biological properties aid in preventing water-insoluble drugs from clearance by RES, while allow for the incorporation of multiple functional components within a single structure with well-defined dimensions and morphologies. Being nanosized helps these structures to stay in the bloodstream for a prolonged period of time and to facilitate higher uptake by cancer cells via the EPR effect, ensuring efficient drug delivery at the targeted location within the body after intravenous or subcutaneous injection. However, the permeability of tumor vasculature can be very different in humans compared to animal models, resulting in an unsatisfied EPR effect. Decorating the surface of polymeric micelles with targeting moieties is an interesting approach to overcome this problem, where the subsequent intracellular delivery could be mediated by certain internalizable ligands that interact with specific molecular targets overexpressed in cancer cells. However, the severe complexity of the construction of such systems and the unclear data of their uptake mechanism imply that it is urgent to improve the progression of active targeting. To that end, stimuli-sensitive systems can be engineered in such a way that they could achieve more precise drug delivery and avoid premature destabilization of the

micelles and unfavorable leakage of their payload in bloodstream. This approach relies on the spatial-temporal control of drug release at tumor sites only on exposure to internal or external triggers, such as environmental pH, temperature, ultrasound and light, thus maximizing drug uptake at the desirable pathological region and lowering potential damage to healthy cells and tissues.

Since not all cells within a tumor will respond equally to a single therapeutic approach, the incorporation of imaging or reporter molecules into the structure of polymeric micelles has gained immense popularity and is an essential step towards overcoming the biological complexity and therapeutic challenges during cancer treatment. Such systems allow for simultaneous diagnosis and precise delivery of higher concentrations of anti-cancer drugs with responsive release to target tissues along with reduced or negligible side effects. Combining several therapeutic agents into a delivery vehicle can intensify the effectiveness of cancer interventions. Recent advances in bioimaging have allowed the development of nanoplatforms that can be used in image-guided modalities, such as MRI, CT, PET and SPECT, while delivering anticancer agents. The main aim of image-guided targeted drug delivery is to optimize the therapeutic outcome through *in vivo* monitoring of nanoparticles regarding their pharmacokinetics, biodistribution, drug release and biological performance. Each modality has its own advantages and disadvantages, regarding spatial and temporal resolution, probe sensitivity and signal penetration depth, thus the selection of the appropriate imaging system depends on the type of information required each time. Merging two or more molecular imaging applications is motivated by the desire to mark multiple molecular targets simultaneously. The obtained imaging information can then be employed to identify suitable patients so as to improve the future of individualized healthcare (personalized medicine). In conclusion, by integrating imaging properties in stimuli-sensitive drug nanocarriers have higher potential to achieve superior clinical benefit. Nanotheranostics can positively impact the overall process of cancer diagnosis and therapy with the best response and highest safety margin, resulting in enhanced quality of life for cancer patients.

The present review refers to a number of examples which cover the recent advances in the development of novel polymeric micelles as drug delivery systems from polymeric micelles which are adjusted for passive targeting and rely on the EPR effect, to

progressively more complicated systems conjugated with targeting ligands, capable of active targeting and responding to various external and internal stimuli. Furthermore, micelles incorporating imaging agents are mentioned for diagnostic and monitoring purposes (image-guided drug delivery) and finally systems that combine multiple functions so as to give rise to multifunctional polymeric micelles.

Despite the overwhelming potential of polymeric micelles, it must be admitted that the field of drug delivery in nanomedicine is still facing numerous obstacles and more systematic studies to understand the mechanisms for targeting and cancer metabolism would be required in order to implement these novel discoveries into clinical anticancer therapies. However, with the growing global interest to seek for more precise medicines and diagnosis, the future for nanomedicine and nano-drug delivery technology looks bright. To that end, polymeric micelles are already well ahead compared to other nanocarriers in terms of proven clinical success, thus one can expect their use in drug delivery, bioimaging and theranostics in the near future.

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