Chapter 5
Stealth Properties of Nanoparticles Against Cancer: Surface Modification of NPs for Passive Targeting to Human Cancer Tissue in Zebrafish Embryos

Samson A. Adeyemi, Pradeep Kumar, Yahya E. Choonara, and Viness Pillay

Abstract Cancer as a noncommunicable disease remains the major cause of death globally. A major drawback for cancer therapeutics is the lack of specific delivery to disease sites which in turn accounts for adverse effects on healthy cells. Incorporation into nanoparticle (NP) and subsequent surface functionalization remain a preferred strategy to circumvent this limitation and to achieve optimal delivery of anticancer drugs. NPs can be coated with hydrophilic and positively charged surfaces that confer on them stealth characteristics that enhance long circulation times and internalization through receptor-mediated endocytosis within the biological systems. One way of achieving this is the coating of poly ethylene glycol onto the surfaces of NPs. In this way, opsonization is reduced and engulfment through reticuloendothelial system is avoided. Central to the concept of passive targeting of NPs is the unique microvasculature of tumor. Various regulatory factors that control the blood pressure as well as maneuvering of the vasculature may shift the equilibrium towards the more captivating tumor environment for NPs. Highlighted in this chapter is how PEGylation of NPs and exploration of the EPR effect could increase the circulation times of NPs while escaping immediate elimination by the immune systems and rapid renal clearance in vivo. Similarly, the stealth properties of NPs can be explored for enhance therapeutic effects through surface modification with other nonfouling hydrophilic polymers in order to cover for the PEG dilemma. Meanwhile, the zebrafish model provides a more promising alternative to detect, view, monitor, image and characterize the interactions between NPs and neoplastic tissues in real time.

Keywords Cancer · Nanoparticles · Surface functionalization · Stealth properties PEGylation

S. A. Adeyemi · P. Kumar · Y. E. Choonara · V. Pillay (✉)
Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
e-mail: viness.pillay@wits.ac.za

© Springer Nature Switzerland AG 2019

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_5

1 Cancer: Introduction, History, Geographical Distribution, and Causes

Cancer as a noncommunicable disease remains the major cause of death globally after cardiovascular disease [1]. The global burden of cancer as reported by the International Agency for Research on Cancer (IARC) showed that cancer incidence has occurred at a rate that is twice its previous occurrence over the past 30 years. Current prediction has it that by the end of year 2030, there will be an additional 21.4 million people living with cancer. With 17 million deaths yearly due to cancer, there will be an average of 75 million cancer patients within 5 years of diagnosis. The World Health Organization report showed that 8.2 million new cases of deaths were recorded globally in 2012 due to cancer, 60% of which were from Africa, Asia, Central and South America and only 30% of cancers could be prevented [2]. In the year 2014, a total of 4500 new cases of cancer were projected to be diagnosed daily in the United States amounting to an average of 1,665,540 of which 585,720 deaths was recorded yearly [3].

The term cancer originates from the word "karkinos," which was used by a Greek doctor called Hippocrates to mean carcinoma between 460 and 370 BC. Meanwhile, prior to his discovery, records of human bone cancer discovered in mummies in ancient Egypt were documented as far back as 1600 BC. Egyptian scientists in 1500 BC were the first group of researchers that discovered and recorded the first breast cancer case without any treatment but palliative option. Through surgical procedures, tumors on the body surface were excised in the same way it is carried out today [4]. Generically, the word cancer is used to describe a large group of diseases. This disease group has the potential to affect any part of the body and is defined by abnormal cell growth both in the organ or part of the body it resides in and beyond their usual boundaries. Many types of cancer have their origin in cells and natural mutations of the DNA. However external factors are also reported to facilitate the development of cancer, including environmental toxins, radiation, exposure to certain chemicals, and more.

2 Employing the Enhanced Permeability and Retention Effect in Cancer Nanomedicines

Passive and active targeting are the two principal alternatives by which nanoparticles (NPs) can be delivered to the tumor sites. Tumor microenvironments possess unique characteristic features within the tumor cells and vasculatures that are different from that of healthy cells. Passive targeting employs this unique feature inherent within cancer cells to facilitate the deposition of NPs into the tumor milieu [5]. The characteristic features of the tumor vasculature as well as the stealth property of the NP including its shape, size and surface charge are the principal determining parameters for the delivery of NPs to cancer [6]. Meanwhile, in active targeting, various

mechanisms have been employed to facilitate the selective delivery and uptake of NPs into the tumor cells. Tumor cells overexpress some biomolecules on their surfaces as opposed to healthy cells that could serve as molecular signatures. Thus, molecular biomarkers such as ligands and short homing peptides are attached to the surface of nanovectors to target these overexpressed biomolecules on the neoplastic cells [7].

3 Passive Targeting of NPs in Cancer Nanomedicines

A unique phenomenon in passive targeting is the preferential accumulation of biomolecules including NPs into tumor tissues due to the enhanced permeability and retention (EPR) effect as first reported by Meada and Matsumura [8]. Two intrinsic properties of the tumor tissues, that is, the leaky vascular and impaired lymphatic drainage and the nanometer size distribution of the NPs are principal factors that influence the EPR phenomenon [6].

During tumor growth, the rate of diffusion of NPs across neoplastic tissues becomes limited at a volume of 2 mm^3 or above [9]. The movement of nutritional intake, the delivery of oxygen and excretion of waste are all impaired by the diffusion limitation effect. Meanwhile, through the process of angiogenesis, increased microenvironment of the tumour vasculature assists to overcome the diffusion limitation [10]. In angiogenesis, the basement membrane is abnormal and the pericytes, which underline the endothelial cells, are absent [10]. As such, compromised tumor vasculature becomes leaky with gap sizes ranging between 100 nm to 2 μm based on the tumor type [11]. Also, the interstitial pressure at the circumference of tumors is less compared to the pressure at their centres due to the absence of a finely defined lymphatic system. Thus, increased pressure within the tumor results in an outflow of convective interstitial fluid, which limits the diffusion of drug to the center of the tumor. Howbeit, drug-loaded NPs that penetrated into the tumor possessed high retention times compared to normal tissues [12]. The combinatory effects of a leaky vasculature as well as a poor lymphatic drainage are termed as the Enhanced Permeation and Retention (EPR) effect. The overall surface charge of the NPs can also influence their passive targeting to the tumour. Positively charged liposomes were reported to bind preferentially through electrostatic interactions, to the negatively charged phospholipid surfaces expressed on endothelial cells of tumor [13, 14].

4 Factors Influencing the Enhanced Permeability and Retention Effect

Aberrant Structural Tumor Vessels and Blood Pressure The normal blood vessels have a smooth muscle layer that is used to facilitate a vasogenic response to vascular mediators and maintain a steady blood supply to the organs. Conversely, smooth muscle cells are absent in the tumor tissues' microvasculature. As such,

tumor microvessels are in permanently vasodilated and do not respond to physiological stimulus regulating blood circulation [15]. These abnormalities in tumor vasculatures account for the irregular transport dynamics of fluid and solutes across its vessels, an option that can be maximized to further enhance the EPR effects [16]. Report has shown that increasing the mean arterial blood pressure by infusing angiotensin II yields a ~5.7-fold preferential increase in blood flow in neoplastic tissues as opposed to normal tissues. More so, tumor tissue selectively accumulate drugs having molecular weight of ~80 kDa while a reduction of about 60–80% drug accumulation was recorded in healthy organs including kidney and bone marrow [17]. The open endothelial gap interphase with abnormal neoplastic blood vessels allow for an increased intratumoral blood flow in response to raise the blood pressure [18]. Similarly, aberrant leaky vasculature and increased blood supply result in the enhanced accumulation of macromolecular drugs in tissues of diverse solid tumors when angiotensin II was administered to induce hypertension [19]. In contrast to the low toxicity effects of macromolecular anticancer drugs, macromolecular drugs under hypertensive state showed a greater concentration of higher than a five-fold increase in the neoplastic tissue while the hypertension was monitored for approximately 20 min [20].

Vasogenic Mediators Several internal mediating factors regulate the tumor microvasculature. Notably among these are the vascular endothelial growth factor (VEGF), Matrix metalloproteinases (MMPs), bradykinin, prostaglandins (PGs), nitric oxide (NO), and peroxynitrite. Several studies have documented the influence of these mediators for the potential application of the EPR effect for enhanced drug targeting and delivery to neoplastic tissue [21]. A brief listing of their influence will be discussed in this section.

VEGF Previously referred to as vascular permeability factor (VPF), the role of VEGF in enhancing the EPR effect has been well documented [22]. Increased VEGF concentration up to 30-fold high has been reported in tumor tissues as opposed to that recorded in healthy tissues [23]. Meanwhile, an exception was observed in the lung. Increased vascular permeability and mitogenic property of VEGF [19] are central for endothelial cells extravasation of Evans blue dye in a dose-dependent rate through intradermal administration. In this way, the role of VEGF is pivotal for enhancing the EPR effect.

MMPs The growth and spread of solid tumor depends the degradation of their extracellular matrix thereby increasing angiogenesis. Meanwhile, MMPs are known to enhance cancer metastasis in this regard [24]. In an in vivo experiment using mice, MMPs increased the permeability of solid tumors vasculature while this effect was reduced by MMP inhibitors [25]. A number of factors have been itemized limiting the application of various MMP inhibitors that have been developed over the decades. Since neoplastic cells remain viable, they easily resume growth when treatment with anticancer chemotherapeutics are stopped vis-à-vis MMP inhibitors. Also, since MMPs are proteases, they are pivotal for cellular metabolism and a

higher dosage of MMP inhibitors results in toxicity. Owing to these negative consequences, the development of several anti-MMP based drugs has been terminated and clinically not applicable [19].

Bradykinin The kallikrein–kinin system is made up of a couple of proteases such as the Hageman factor XII of the coagulation cascade. Prior to the conversion of prekallikrein to kallikrein is the activation of the Hageman factor XII. Bradykinin is produced directly from kininogen through the activity of kallikrein [23]. Several research reports have identified bradykinin receptors in different types of animal and human tumors [26]. The bradykinin-producing cascade has been shown to be activated in cancer tissues [27]. Bradykinin is upregulated is both the pleural and peritoneal fluids of animals and human neoplastic tissues. Studies have also shown that bradykinin inhibits kallikrein in the extravasation of plasma components into either the pleural or peritoneal cavity [28]. As such, bradykinin plays a pivotal role as a mediator in regulating the EPR effect in tumor tissues [27]. Meanwhile, bradykinin also activates endothelial NO synthase (eNOS), which in turn triggers the production of NO [25]. The angiogenic characteristics of VEGF in human endothelial cells is enhanced by NO production [29]. The inhibition of angiotensin-converting enzyme (ACE), among other peptidases that degrade bradykinin [30], results in higher concentration of bradykinin thereby increases the permeability of neoplastic vasculature. Inhibitors of ACE including temocapril and enalapril, have been reported to enhance the EPR effect [31, 32]. Interestingly, ACE inhibitors enhance the delivery of macromolecular drugs to cancers at normal blood pressure [33]. Thus, ACE inhibitors may act preferentially at tumor sites in individuals with normal blood pressure as ACE inhibitors are only active in patients with high blood pressure, a condition that enhances the EPR effect [34].

NO The reaction between L-arginine and oxygen by three isoforms of NOS results in the production of NO the most active form of NOS is produced in macrophages and neutrophils which are readily present in neoplastic tissues [34]. NO is an established mediator of vasodilation, angiogenesis and extravasation [23]. It has been shown that NO mediates increased vascular permeability in solid tumours, a phenomenon that is hindered by NO scavengers and NO synthase inhibitors [28]. Due to its role in mediating the permeability of tumor vasculature, NO contributes immensely in facilitating the EPR effect in neoplastic tissues [30]. In addition to its direct influence on the EPR, the interaction between NO and the superoxide anion, mainly produced by leukocytes, results in the production of peroxynitrite. Peroxynitrite converts MMP precursors (proMMPs) into MMPs [35] which also play a vital role in the EPR effect [35]. Simultaneous systemic infusion of both isosorbide dinitrate—an NO-releasing agent and angiotensin II into the artery of a localized tumor resulted in an enhanced site-specific delivery of poly(styrene-co-maleic acid/anhydride) neocarzinostatin (SMANCS)-Lipiodol, corroborating the postulation that NO influences the EPR effect [36].

Prostaglandins (PGs) Among the PGs, PGE2 is a unique mediator of vascular permeability. It is produced through the activation of cyclooxygenase (COX) isozymes, like COX-2, which is highly expressed in tumors. Reports have shown that the vascular permeability in sarcoma 180 and other solid tumors are suppressed by COX inhibitors including indomethacin and salicylic acid [37]. This in turn further validates PGs as potent enhancers of vascular permeability and their subsequent role in the EPR effect. In another related report by Tanaka and colleagues [38], the systemic circulation half-life of a PG12 derivative, beraprost sodium, is significantly longer (>1 h) compared to PG12 which only lasts for a few seconds. Their finding showed that the EPR effect was increased up to about two- to three-fold by PG12 analogs which may provide an efficient delivery alternative for macromolecules.

5 Types of Nanoparticulate Systems Employed in Cancer Therapy

Various types of nanoparticulate systems are presently under investigation for the delivery of cancer drugs [12] including polymeric NPs [39], protein NPs [40], ceramic NPs [41], viral NPs [42], metallic NPs [43], carbon nanotubes [44], micelles [45], dendrimers [46] and liposomes [47]. The design and fabrication of each NP system is tailored towards its intrinsic material characteristics in order to facilitate efficient delivery to the tumor sites. NPs can be coated with hydrophilic and positively charged surfaces that confer on them stealth characteristics that enhance long circulation times and internalization through receptor-mediated endocytosis within the biological systems [48]. Quite often, serum proteins adhered to the surfaces of NPs through a process called opsonization [49]. This is turn exposes the NPs to resistance through the reticuloendothelial systems (RES) and thereby limits their circulation times within the biological systems [50]. A major way to circumvent this anomaly is to functionalize the surfaces of NPs in order to create a stealth surface from opsonization. As such, their circulation times are increased and the resistance through RES is avoided [51]. One way of achieving this is the incorporation of poly (ethylene glycol) (PEG) onto the surfaces of NPs. In this way, opsonization is reduced and engulfment through RES is avoided.

Advances in nanotechnology have given a major boost to research in the design and development of various nanoparticulate systems for cancer therapeutics [52]. Meanwhile, only few NP drug delivery systems have been approved till date by the US Federal Drug Administration and the European Agency for the treatment of cancer. Notable among those approved includes liposome–PEG doxorubicin (Doxil®, Ortho Biotech, and Caelyx®, Schering Plough), methoxy-PEG–poly(D,L-lactide) taxol (Genexol-PM®, Samyang), albumin-bound paclitaxel NPs (Abraxane®, Abraxis BioScience) [12]. Zhang and colleagues have reported a comprehensive review of the NP systems in preclinical and clinical developmental stages [53].

6 Stealth Properties of NPs and Their Possible Application in Cancer Nanomedicine

The stealth property of NP refers to the ability of a NP, through diverse modification processes, to by-pass detection and destruction by the immune systems thereby having a prolonged circulation time within the biological system and enhanced targeting potential to its site of action. This phenomenon is termed "stealth coating."

Study has shown that opsonization of NPs remained a major factor the influenced the clearance of NPs by RES within few minutes upon their intravenous administration into the blood [49]. The intrinsic chemical composition of the NP matrix [54], its shape/modulus [55], surface architecture [56], surface charge [57], and size [58] determine its circulation half-life within the biological system (Fig. 5.1). Research has shown that both hydrophobic and charged NPs possess shorter circulation times as a result of their ability to evade the opsonization process [60]. As such, coating the surfaces of NPs intended for systemic application with neutrally charged hydrophilic surface layer is a preferred alternative. Thereby, the circulation half-life of surface modified NPs is prolonged to more than 40 h by the stealth process [61].

The intrinsic material composition of NPs is directly linked to their ability to interact with their environment [62]. The rate at which NPs deliver their payloads at the site of interest can be engineered based on material degradation, or the diffusion through the NP matrix or pores [63]. To facilitate the level of control,

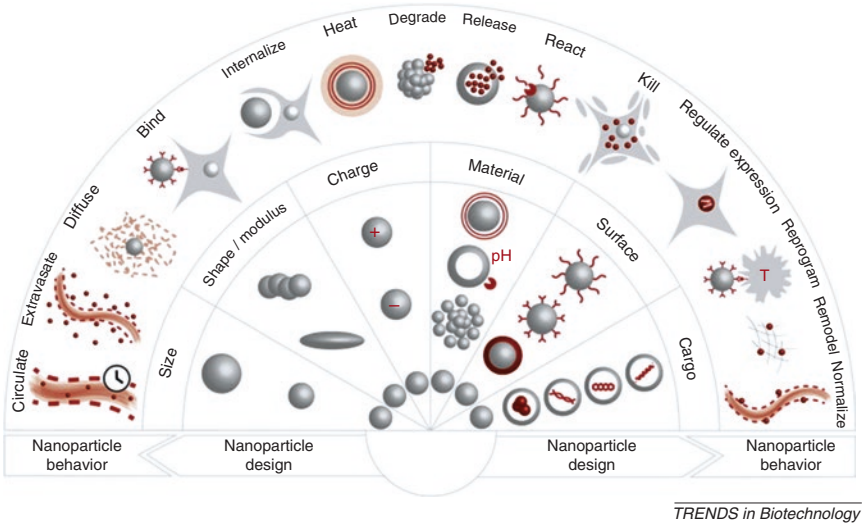

Fig. 5.1 Stealth properties of NPs. NPs' behavior are influenced by their size, shape, modulus, charge, material, and surface architecture. Reprinted under the permission of [59] (Copyright Elsevier Publishers, 2018)

materials can either be designed to release their payload at the action site or adopt new physical characteristics in response to internal stimuli in tumor microenvironments including changes in pH condition or enzymatic activities [64, 65]. External stimuli such as magnetic, sound or light waves can also be employed to activate materials which are responsive to energy for theranostics applications [66, 67].

The size effect on the circulation time, extravasation, internalization and diffusion into the cellular compartment has been widely reported [58]. NPs with smaller size below 5 nm possessed lower circulation half-life and gain rapid entrance into neoplastic cells and tissues [68]. Meanwhile, their disappearance from the tumor cells is rapid due to enhanced filtration by the kidney and rapid renal clearance from the urine. Conversely, NPs with larger size ranging between 5 nm and 500 nm have a higher circulatory retention time and accumulate in neoplastic tissues by exploiting the enhanced permeability and retention (EPR) effect [69]. Also, the size of NP influences its cellular internalization since different NP sizes are transported through diverse endocytic channels [70].

The shape of a NP also has a direct effect on its cellular uptake. Spherically shaped NPs showed increased cellular uptake into the tumor microenvironments. NPs that possessed high aspect ratios and have rigid shape were reported to accumulate more slowly in macrophages than those that have small and flexible morphology. This in turn enhances their retention time within the system and ultimately minimize their clearance time from the circulation [71].

The surface charge of NP influences its circulation time. Charged NPs are highly opsonized and are rapidly engulfed by the immune [72]. Meanwhile, positively charged NPs bind and are preferentially internalized by tumor cells than their uncharged pairs once they are in a tumor environment [73]. Hence, studies to shield NPs once they enter the tumor environment, and release their charged interiors and encapsulated payloads, as a result of the changes in pH or enzymatic activity within the tumor microenvironment, are the paradigm in cancer nanomedicines [74]. Therefore, passive coating, by using polyethylene glycol to confer neutral surface architecture, has been well documented to shield the charged surfaces of NPs, enhance their circulation time, and accumulation in tumor tissues [75]. Similarly, the threat faced by the phagocytes that engulf NPs are being eliminated by shielding NPs with “self-peptides” derived from the human CD47 receptor as later amplified in this review [52, 76].

In all, the potential of NPs to detect, navigate, and deliver their payloads in the body is influenced by their fabrication and interactions with their macro/microenvironments. Maneuvering the properties and subsequent behavior of NPs has become increasingly possible vis-à-vis the deepening knowledge expansion and understanding of molecular biology and NP transport. More important is the impressive nanotechnological techniques and toolbox available to bioengineers to modify NPs and biological systems.

7 Surface Modification and Optimization of NPs for Passive Targeting to Human Cancer Tissues

7.1 PEGylation Chemistry: Stealth Coating of NPs for Biological Application in Cancer Nanomedicines

The covalent attachment of the polyethylene glycol (PEG) chain unto specific given molecule for an enhanced systemic delivery is termed PEGylation. The stealth characteristics conferred on PEGylated molecules allow for their long circulation within the biological system as well as provide a "shield effect" from the RES systems and other blood phagocytes [77]. The first attempt by Abuchowski and colleagues using PEG coating on bovine serum albumin and bovine liver catalase significantly altered both their immunological properties and stability by covalently linking them to methoxy PEG (mPEG) using cyanuric chloride activation [78, 79]. Several other biomolecules including peptides, enzymes, liposomes, carbohydrates, nucleotides, antibody fragments, as well as small organic molecules and various nanoparticulate systems have all been modified using PEGylation chemistry [80–82]. While mPEG is mainly employed for the modification of polypeptides, several PEG variants having different molecular weights and structures including linear, branched, PEG dendrimers and of recent, multiarm PEGs are now being used in PEGylation chemistry [83]. The first approach in the PEGylation process is the activation of native PEG molecule through conjugation of its functional derivative at either one or both terminals of the PEG chain. The PEGylation conjugation process can either be by the first generation randomized technique or the second generation site-specific procedure [84]. Meanwhile, the focused has been given to second generation site-specific PEGylation because it produces well-defined conjugated products having improved product profiles more than those obtained using the randomized technique [77]. Similarly, both reversible and irreversible mechanisms are employed in PEGylation conjugation. However, irreversible PEG conjugation technique showed some negative effects on the biological activity of some therapeutics. As such, the reversible PGylation strategy has been adopted in order to reduce the loss of biological activity of potent therapeutics. In reversible PEGylation technique, drugs are attached to PEG variants through cleavable linkages. Thereafter, the drug is release through various cleaving agents including enzyme and hydrolytic cleavage, or degradation within the biological system at a predetermined release rate over a period of time (Fig. 5.2) [85].

A critical aim of most PEGylation conjugation process is to improve the circulation half-life of therapeutic biomolecules without affecting their activity. The unique advancement in PEG conjugation chemistry and the difference in both structural and molecular architectures of PEGs employed for the conjugation contribute immensely to the increasing demand for PEgylated products in the pharmaceutical industry. Surface modification of drugs and biomolecules enhance their therapeutic efficacy with several advantages over non-PEGylated products. Noteworthy among these systemic modifications are presented in Fig. 5.3. Increased circulation half-life of the PEGylated conjugated product in the blood is the major way to enhance

its therapeutic effect. PEGylation increases the circulation time of the PEG-modified therapeutics by reducing its renal clearance with increasing hydrophilicity [86]. PEGylation confers on the PEG-modified product protection from reticuloendothelial cells, degradation by proteolytic enzymes, reduced formation of neutralizing antibodies against the protein by hiding antigenic sites through the formation of a protective hydrophilic shield [87]. This in essence improves the pharmacokinetic profile of the PEGylated conjugates. Previous reports showed that the absorption half-life of therapeutics administered subcutaneously increased when PEGylated and showed reduced distribution volume [86].

As a nonbiodegradable polymer, the use of PEG is limited in its application. Previous reports showed that PEGs having molecular weight of about 20 kDa are easily cleared by the renal system while those with higher molecular weight were eliminated by fecal excretion [88]. Though PEGylation confers stealth property on conjugated therapeutics and biomolecules with prolong serum half-life, some hurdles were recorded on liposomes particular for the delivery of genes and nucleic acids in anticancer nanomedicines. The surface modification of lipoconjugates by PEG due to its hydrophilic shield decrease their cellular uptake with increasing stability of the lipid envelop, thereby results in lysosomal degradation of the conjugated vector due to poor endosomal escape through membrane fusion [89]. As such, PEG application

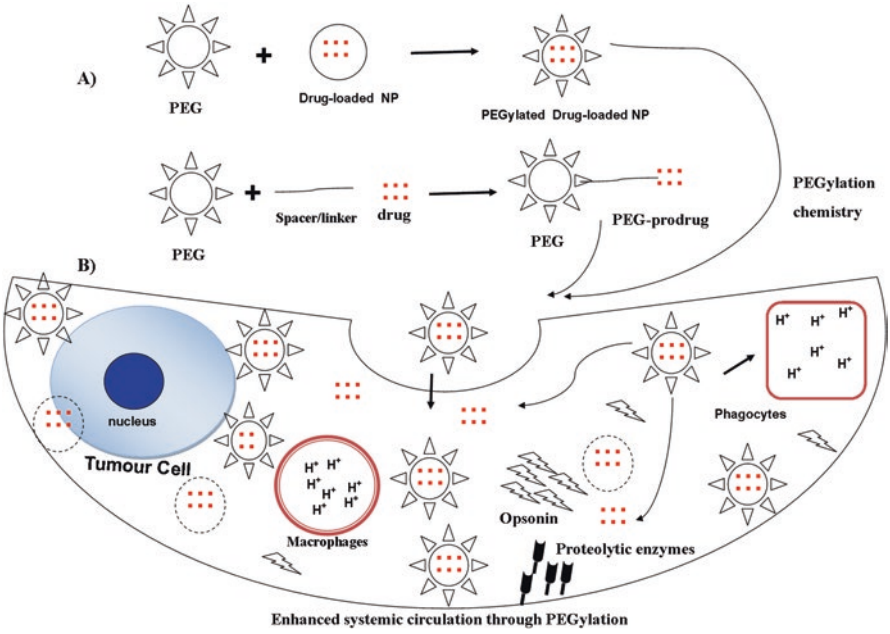

Fig. 5.2 Schematic diagram showing PEGylation and its influence on NP delivery (**a**) Surface modification using PEG on either preformed NPs loaded with drugs or attaching the PEG chain to drug through a linker (**b**) The stealth effect of PEGylation against blood barriers thereby enhancing prolonged systemic circulation of PEGylated drug-loaded NPs or PEG-prodrug as the case may be

in gene and nucleic acid delivery to tumor cells is termed “the PEG dilemma” [90]. Meanwhile, this limitation in “lipo-PEGylation” can be successively tackled by fabricating tumor-specific and pH-sensitive targeted PEG conjugated therapeutics [91, 92]. Nonsystemic delivery approach has also been shown to be efficient for the delivery of PEGylated NPs. In this way, PEGylated therapeutics is delivered locally rather than into the systemic circulation, thereby improving their efficacy while minimizing nontargeted side effects. A detail listing of various local administration strategies including vaginal delivery, pulmonary administration, gastrointestinal tract delivery, ocular and vaccine based delivery systems, of PEGylated NPs for improved delivery of their payloads has been well documented by Jung and coworkers [93].

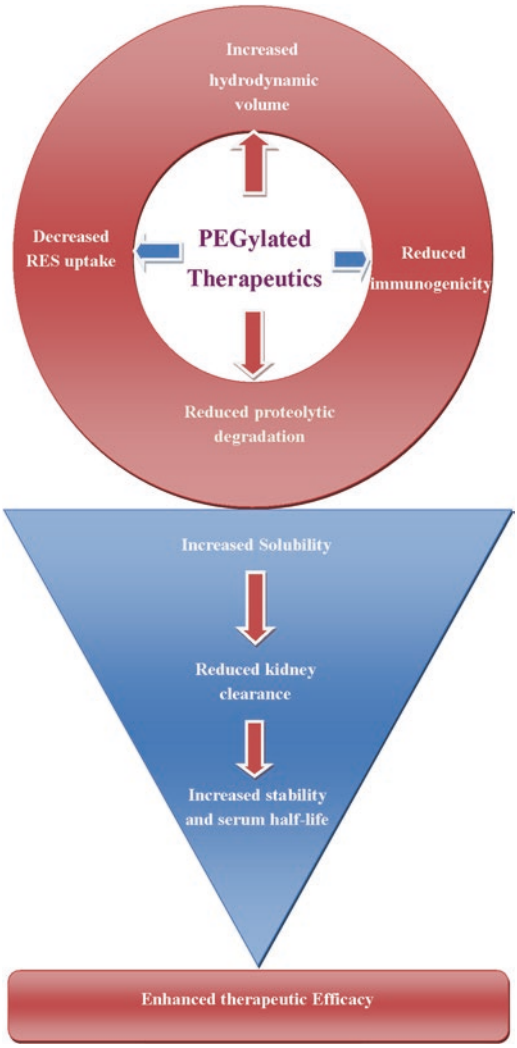

Fig. 5.3 Importance of PEGylated therapeutics

8 Surface Modification of Nanoparticles Using PEG Chains

The surface characteristics of NPs play a principal role as a determining factor that regulates the extent of their cellular internalization. Meanwhile, NPs surfaces can be engineered by the composition of the polymer to regulates either their hydrophobic or hydrophilic surface composition. The use of Polyethylene Glycol (PEG) is foremost in surface medication of these polymers in order to shield these nanosystems from opsonization and clearance by the RES as previously discussed [94]. Also, increased PEG molecular chains has been shown to enhance NPs circulation time as PEG chains provide a shielding effect particularly for negatively charged particles to protect them from immediate clearance in vivo [95].

Two major techniques that are employed in formulating PEGylated NPs including polymeric formulation, lipid-based NPs or micelle type NPs are the self-assembly of PEG-containing biomolecules, or surface modification of preformed NPs with PEG. It should be noted that the density of PEG on the surface of PEGylated NPs is a major factor that enhances their delivery in vivo [93]. In this chapter, effort will be focused on the surface modification of polymeric formulations using PEG chain.

A more assuring way to ascertain that a vast amount of the PEG molecules coated on NPs remained on their surfaces is to modify preformed NPs with PEG. One way to achieve this is through the adsorption technique. Using this method, PEG derivatives are dissolved in an aqueous phase and binds to the NP surface through hydrophobic and electrostatic interactions or ligand-binding. In polyethylene oxide–b-polypropylene oxide–b-polyethylene oxide (PEO-PPO-PEO) triblock copolymer popularly known as pluronics, the hydrophobic PPO component is surrounded by two molecules of PEO chains which are hydrophilic (PEO is a variant form of PEG) [96]. Using their hydrophobic PPO segment, pluronic molecules can interact with and adsorb on NP hydrophobic surfaces. In a report by Yang et al., PPO chain length was observed to contribute immensely in the interaction with NP surface. At a PPO chain length of ~3 kDa molecular weight (MW) pluronic molecules, a densely coated PEG surface that could enhance the diffusion of NP in human mucus was achieved [96]. In other developments, PEGylated phospholipids [97], fatty acid-PEG-esters [98], Vitamin-E-TPGS-PEG [99] among others can be engineered to absorb on polymeric NPs as well as inorganic NPs to form a PEG corona. Through electrostatic interactions, NPs with charged outer surfaces can coated with PEG-containing molecules having opposite charge. For instance, positively charged PEG-PLL and PEG-PEI block polymers can bind to negatively charged PLGA NPs [100]. This noncovalent adsorption technique is a weak interaction that allows for easy desorption of the PEG molecules from the NP surface. Furthermore, using ligand-interactions, a more stable biotinylated PEGylated avidin-functionalized NPs are formed as biotin–avidin interactions can be employed in attaching either ligands or drugs to NP surface for targeted delivery. Meanwhile, it is harder to separate and remove the nonadsorbed PEG molecules from the adsorbed ones using this procedure. Such unintended aftermaths on both delivery and translational mechanisms should be considered prior to preparation [101].

Chemical conjugation is another technique employed to PEGylate preformed NPs. This procedure is also termed grafting. PEGylated polystyrene (PS) NPs [102], PEGYlated gold NPs [103] as well as PEGylated dendrimers [104] have all been produced using this strategy. Meanwhile, a major setback using this approach is the steric hindrance that can limit the amount of PEG that can be coated (grafted) onto the surface of NP. In a comparison research, Nance et al. found that the amount of sufficiently dense mPEG-amine coating on PS NPs required to penetrate the human mucus is less as opposed to that required to penetrate the extracellular matrix of the brain tissue [105]. A limitation to this approach is the possible leakage of the encapsulated drug from the preformed NPs and variation from one batch to another.

The lipid bilayer of preformed liposomes can be explored in PEG-lipid conjugation. PEGylated lipid conjugates can be grafted preferentially to preformed liposomes using their hydrophobic lipid tail for interaction with the lipid bilayer. Attention must be given to the temperature and concentration of the lipids in the PEG-lipid solutions. While the temperature should be closer to the melting temperature of the lipid components and added at a slower rate, formation of micelles must be avoided by keeping the concentration of the PEG-lipid below the critical micellar concentration [106]. An interesting advantage of post-insertion technique as opposed to the self-assembly process is the allowance to modify the outer surface of liposome bilayer rather than the nonspecific insertion of PEG into the interior of liposome using the self-assembly strategy. As such, the post-insertion technique provides for the use of lesser PEG-lipid conjugate to achieve the same surface PEGylation compared to preinsertion method and provides for prolonged blood circulation half-life [107]. Another application using postinsertion conjugation technique is in the preparation of PEGylated liposomes when their surfaces are to be functionalized with targeting ligands. Tendered reaction conditions that underline the reactions between amino and carboxylic groups, pyridyldithiols and thiols, maleimide and thiols are fundamental for lipid PEGylation due to the fragile nature of liposomes [108]. A novel approach termed "click chemistry" has also been employed for PEG post-conjugation including interactions between the azide-modified PEG and the alkynyl groups on the surface of liposome [109, 110].

9 Alternative Polymers Used to Coat Nanoparticles

The paradigm in surface modification strategy employs PEGYlation—modifying the NPs' surfaces with PEG to reduce abrupt clearance of NPs from the systemic circulation. Notwithstanding, several reports have shown the some undesired negative influence PEGylation may presents on the performance of NPs as a vector in drug delivery. Meanwhile, alternative surface modification techniques, using other polymers to coat and provide stealth property on NPs, may offer possible solutions to the PEG dilemma [61]. Some of these alternative polymers for surface modification of NPs are highlighted below:

Polyoxazolines (POXs) POXs are group of potential alternative stealth polymers that have been explored in amphiphilic block copolymer as the hydrophilic components. POXs are generated through living cationic ring-opening polymerization (LCROP) of 2-oxazolines. They are versatile with a number of available variants with end-group and side-chains that could be functionalized [111]. POX has been used to coat proteins, micelle-based and liposomal formulations and have shown good nonbiofouling characteristics comparable to PEG in stealth effects [112]. More so, POXylated therapeutics have shown increased bioavailability compared to PEGylated formulations [113, 114] with more stability under physiological conditions [115]. For instance, poly(2-methyl-2-oxazoline) is more hydrophilic than PEG without amphiphilicity [116], having high biocompatibility with no cytotoxicity recorded at concentrations up to 20 g/L in cell culture [117] and up to 2 g/kg upon its intravenous administration in rats [113]. Poly(2-methyl-2-oxazoline) was coupled to poly(L-lysine) in exchange for PEG for nonviral gene delivery [118]. Poly(2-ethyl-2-oxazoline) was grafted to poly(caprolactone) [119], poly(1,3-trimethylene carbonate) [120], and poly(aspartic acid) [121], to produce polymeric micelles.

Polyglycerols Either in their linear of branched forms, polyglycerols which are also referred to as polyglycidols, are flexible and biocompatible hydrophilic aliphatic polyether polyols [122]. Highly branched polyglycerols possess antifouling properties with reduced susceptibility to oxidation or thermal stress that is comparable to PEG [123]. Also, the presence of multiple hydroxyl groups in polyglycerols allow for easy functionalization with other moieties [123]. The enhanced circulation half-life of hyper branched polyglycerols showed their potential as stealth polymer for surface modification of NPs [124]. Surface coated nanoliposomes were reported to show prolonged plasma circulation [125] and prevent opsonization to gold surface [123]. Recently, a multifactorial strategy in which both hyper branched polyglycerol and PEG were used as a block-copolymer to coat liposome was reported [126]. In this liposomal system, the polyglycerol moieties allowed for the multivalent functionalization of the liposome.

Poly (Amino Acids) Previous studies have reported the potential stealth characteristics of poly (amino acids) such as the poly hydroxyethyls of both L-asparagine and L-glutamine. Because poly (amino acids) are susceptible to protease degradation, there are limited chances that they will be accumulated in the biosystem [127]. Prolonged plasma circulation of NPs was observed using poly (amino acids) surface coating comparable to PEGylation [127]. Particularly, poly(hydroxyethyl-L-asparagine)-coated liposomes showed enhanced ABC resistance with higher stealth property than PEGylated liposomes after repeated low dose lipid administrations [128].

Polybetaines Zwitterionic molecules including sulfobetaine and carboxybetain as betaine derivatives interact with water molecules through electrostatic bonds [129] with stronger binding force as opposed to weak interaction via hydrogen bonding

[130]. Betaine-based polymers have been shown to decrease nontargeted protein adsorption [131], formation of biofilm [132], and bacterial adhesion on diverse surfaces. Interestingly, the carboxyl derivative of betaine (polycarboxybetaine) possess multiple functional groups which are responsive to multivalent conjugations thereby allows for multiple surface functionalization that could be explored in nanomedicines [133]. Based on these unique qualities, the use of polybetaines as alternative nonfouling materials for the surface medication of NPs has gained a lot of recognition. For instance, various nanomaterials including iron oxide [134], silica [135], gold [136], PLGA [137], and hydrogels have been modified using poly(carboxybetaine). These polybetaine-modified NPs exhibited enhanced size stability in protein solutions such as serum, which showed their potent resistance to nonspecific protein adsorption [134, 136].

Polysaccharides Another class of polymers that could provide excellent stealth property employed in surface medication of NPs are the polysaccharides. Surface engineered polysaccharide-coated NPs, having hydrophilic surfaces conferred on their surfaces, by polysaccharide derivatives such as chitosan [138], hyaluronic acid [139], dextran [140], and heparin [141] are well documented. The biodegradable, low immunogenic and less toxic properties of polysaccharides make them advantageous in surface medication chemistry [142]. Also, the presence of multiple functional groups that could be implored for conjugation with drugs molecules as well as cell-penetrating ligands allows for their application for surface modification. Reports have shown that polysaccharide-based NPs increase circulation half-lives of loaded therapeutics with enhanced accumulation in neoplastic tissues. For example, at lower acidic pH, chitosan molecules become positively charged and can facilitate cellular binding of coated NPs. This unique characteristic can be explored for direct targeting and delivery of anticancer drugs to the negatively charged surfaces of tumor tissues [143]. In another development, Papisov and colleagues employed acyclic hydrophilic polyacetals, a derivative of polycarbohydrates, instead of PEG for surface modification [144]. The high hydrophilicity and multiple modifiable functional groups of polyacetals make it more advantageous compared to PEG [144]. More importantly, the conjugation of polylysine to polyacetal produced a prolonged circulation time than a polylysine grafted dextran—a polysaccharide precursor for polyacetal. This was however attributed to changes in the rigid stereochemical structure of dextran [144].

10 The Rationale for Zebrafish as a Vertebrate Model for Human Disease

The completion of the human genome project as well as the complete sequencing of the zebrafish (ZF) genome allow for their comparative analyses in scientific research. Overall, ZF possess several unique features as a model organism in human disease

including their high fecundity, fast growth rate, easy and cost-effective maintenance, relatively small morphology having embryos and larvae that are optically translucent [145, 146]. Most importantly, a comparison between the ZF genome and that of human showed that over 70% of the human genes have their orthologs in the ZF genome [146]. ZF are readily available as transgenic lines having specialized marker genes for fluorescent macrophages, endothelial and the lymphatic systems. Meanwhile, diverse genetic tools have been developed for ZF mutation including zinc finger nucleases and knockdown using morpholino antisense oligonucleotides [147, 148]. These unique characteristics have popularized the increasing use of ZF as a model in scientific research of human disease including cancer over the past two decades [149].

11 Zebrafish as a Model for the Characterization of Human Cancer

Over the years, the ZF model has gained recognition as a diverse model for human diseases including cancer. Through transgenic techniques and gene-specific mutations in ZF cell lines, different models including that for melanomas, rhabdomyosarcoma and several other solid tumors are available [150]. There exists a large similarities between the growth of human cancer cell lines in ZF to the behavior of tumor xenografts in mammalian models like mice [151, 152]. Interestingly, tumors are developed in almost all the organs with similar histology to those found in human when carcinogens are used to induce tumor in ZF [153]. This is probably because many of the genes in human have at least an ortholog gene in ZF genome having the same cellular and molecular components which are involved in the disease initiation and development [146]. More importantly, the absence of any functional adaptive immune system during the early developmental stage in ZF until ~4 to 6 weeks old [154], allows for easy the formation of tumor within the model using either mouse or human cancer cells as there is nothing to actively suppress the immune system to avoid the rejection of injected human cells [154–156]. The transplanted tumors are established within 2 days post inoculation of the cancer cells. These properties make the ZF viable for cancer studies in vivo.

A more treasured and unique characteristic feature of the ZF model is the optical transparency of the embryo which allows for tissue imaging and monitoring down to the single cell level as a vertebrate research animal [151]. As such, the growth of tumors can be characterized by microscopic imaging at high resolution at time intervals in vivo in living organisms [149] which is a major advantage in cancer research. In time way, the growth of human cancer cells and the formation of solid tumor can be monitored as live phenomenon in real time with high spatial and transient resolution. Similarly, other fluorescent therapeutics such as NPs can be studied in vivo due to this optical transparency observed in ZF as further detailed in the next section.

12 NPs and the Zebrafish Model in Human Cancer Nanomedicines

Having highlighted the unique features of ZF as a model system for human diseases and tumor transplantation of human cancer cells into the ZF, the exceptional qualities of the ZF which allow for in vivo fluorescent microscopy coupled with its reduced immune system at the embryonic stage can be explored to study the mechanisms of action of cancer as well as to understand the possible treatment alternatives for cancer and other human diseases [157]. The importance of NPs both for diagnostic and drug delivery purposes is not easily understood in cancer nanomedicines. It is not readily easy to understand the dynamics of NP-host or NP-disease interactions within the biological system of most models after injection or other forms of delivery. Similarly, NPs' biodistribution and cellular internalization cannot be monitored easily in higher order vertebrate models such as mice and rats which require more complex and rigorous methods. Conversely, the ZF allows for an excellent view, monitoring and characterization of the interactions between NPs with the host as well as the disease in question [157]. In this way, both the benefits and potential complications of using NPs can be monitored. For instance, the undesirable characteristics exhibited by some NPs binding to the endothelial cells have been reported. Meanwhile, with very few exceptions, not many analyses have been done to understand the in vivo interactions of NPs with endothelial cells [158–160].

Usually, many of the studies that explored the use of nanotechnology and ZF mainly concentrate on the evaluation of the potential toxic effects of the NPs, with varied chemical makeup, have on normal systems [161, 162]. The report of Wagner and colleagues showed that prelabeled gold NPs with antibody specifically target and killed cancer cells when administered into the ZF embryo. Upon the application of heat through the laser pulse, the functionalized gold NPs generated a plasmonic nanobubble which killed the cancer cells preferentially [163]. However, in another development, previous report showed that NPs do not exhibit any toxic effect on the growth of cancer cells injected the ZF embryos [164]. Meanwhile, their reports do not validate the accumulation of NPs in the cancer tissues while the NPs were administered through bath-treatment.

13 Concluding Remarks

The increasing emergence of novel nanoparticulate systems has orchestrated the dire need of direct and accurate delivery of chemotherapeutics to tumor cells without harming the normal tissues. Central to the concept of passive targeting of NPs is the unique microvasculature of tumor. Various regulatory factors that control the blood pressure as well as maneuver the vasculature may shift the equilibrium towards the more captivating tumor environment for NPs. Based on the initial

success of an improved systemic delivery of proteins upon PEGylation, surface modification of NPs using PEG has also yield promising results for enhanced systemic delivery of therapeutic vectors. More importantly, we highlighted in this chapter how PEGylation of NPs and exploration of the EPR effect could increase the circulation times of NPs while escaping immediate elimination by the immune systems and rapid renal clearance in vivo. Similarly, the stealth properties of NPs can be explored for enhance therapeutic effects through surface modification with other nonfouling hydrophilic polymers in order to cover for the PEG dilemma. Furthermore, the zebrafish model presents a promising platform to monitor and characterize "magic" engineered nanocargoes for theranostic interventions. Overall, a matter of debate among the scientific community is that while passive targeting enhances the efficient accumulation of NPs in the neoplastic interstitium, it cannot facilitates their cellular uptake thereby requires the more promising and specific active targeting of NPs to overexpressed receptors on cancer cells. It is therefore our proposition that these two strategies be harnessed simultaneously in order to achieve an optimum therapeutic efficacy from prospective nanoengineered systems in cancer nanomedicines.

References

1. Masoudkabir, F., Sarrafzadegan, N., Gotay, C., Ignaszewski, A., Krahn, A. D., Davis, M. K., et al. (2017). Cardiovascular disease and cancer: Evidence for shared disease pathways and pharmacologic prevention. *Atherosclerosis, 263*, 343–351.
2. WHO|Cancer. WHO. Accessed November 24, 2014, from http://www.who.int/cancer/en/
3. Siegel, R., Ma, J., Zou, Z., & Jemal, A. (2014). Cancer statistics, 2014. *CA: a Cancer Journal for Clinicians, 64*(1), 9–29.
4. Sudhakar, A. (2009). History of Cancer, Ancient and Modern Treatment Methods. *Journal of Cancer Science and Therapy, 1*(2), 1–4.
5. Kreuter, J. (2007, February 22). Nanoparticles – A historical perspective. *International Journal of Pharmaceutics, 331*(1), 1–10.
6. Bazak, R., Houri, M., Se, A., Hussein, W., & Refaat, T. (2014). Passive targeting of nanoparticles to cancer: A comprehensive review of the literature. *Molecular and Clinical Oncology, 2*(6), 904–908.
7. Yu, X., Trase, I., Ren, M., Duval, K., Guo, X., & Chen, Z. (2016). Design of nanoparticle-based carriers for targeted drug delivery. *Journal of Nanomaterials*. https://doi.org/10.1155/2016/1087250.
8. Matsumura, Y., & Maeda, H. (1986). A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Research, 46*(12 I), 6387–6392.
9. Byrne, J. D., Betancourt, T., & Brannon-Peppas, L. (2008). Active targeting schemes for nanoparticle systems in cancer therapeutics. *Advanced Drug Delivery Reviews, 60*(15), 1615–1626.
10. Jones, A., & Harris, A. L. (1998). New developments in angiogenesis: A major mechanism for tumor growth and target for therapy. *The Cancer Journal from Scientific American, 4*(4), 209–217.
11. Hobbs, S. K., Monsky, W. L., Yuan, F., Roberts, W. G., Griffith, L., Torchilin, V. P., et al. (1998). Regulation of transport pathways in tumor vessels: Role of tumor type and

microenvironment. *Proceedings of the National Academy of Sciences of the United States of America, 95*(8), 4607–4612.
12. Haley, B., & Frenkel, E. (2008). Nanoparticles for drug delivery in cancer treatment. *Urologic Oncology: Seminars and Original Investigations, 26*(1), 57–64.
13. Krasnici, S., Werner, A., Eichhorn, M. E., Schmitt-Sody, M., Pahernik, S. A., Sauer, B., et al. (2003). Effect of the surface charge of liposomes on their uptake by angiogenic tumor vessels. *International Journal of Cancer, 105*(4), 561–567.
14. Kunstfeld, R., Wickenhauser, G., Michaelis, U., Teifel, M., Umek, W., Naujoks, K., et al. (2003). Paclitaxel encapsulated in cationic liposomes diminishes tumor angiogenesis and melanoma growth in a "humanized" SCID mouse model. *The Journal of Investigative Dermatology, 120*(3), 476–482.
15. Kuruppu, D., Christophi, C., Maeda, H., & O'Brien, P. E. (2002). Changes in the microvascular architecture of colorectal liver metastases following the administration of SMANCS/lipiodol. *The Journal of Surgical Research, 103*(1), 47–54.
16. Greish, K. (2007). Enhanced permeability and retention of macromolecular drugs in solid tumors: A royal gate for targeted anticancer nanomedicines. *Journal of Drug Targeting, 15*(7–8), 457–464.
17. Li, C. J., Miyamoto, Y., Kojima, Y., & Maeda, H. (1993). Augmentation of tumour delivery of macromolecular drugs with reduced bone marrow delivery by elevating blood pressure. *British Journal of Cancer, 67*(5), 975–980.
18. Seymour, L. W., & Fisher, K. D. (2016). Under pressure: Elevated blood pressure enhances targeting of tumors by oncolytic viruses. *Molecular Therapy, 24*(2), 204–205.
19. Iyer, A. K., Khaled, G., Fang, J., & Maeda, H. (2006). Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discovery Today, 11*(17–18), 812–818.
20. Wu, J., Akaike, T., & Maeda, H. (1998). Modulation of enhanced vascular permeability in tumors by a bradykinin antagonist, a cyclooxygenase inhibitor, and a nitric oxide scavenger. *Cancer Research, 58*(1), 159–165.
21. Maeda, H. (2001). The enhanced permeability and retention (EPR) effect in tumor vasculature: The key role of tumor-selective macromolecular drug targeting. *Advances in Enzyme Regulation, 41*, 189–207.
22. Stefanini, M. O., Wu, F. T. H., Mac Gabhann, F., & Popel, A. S. (2010). Increase of plasma VEGF after intravenous administration of bevacizumab is predicted by a pharmacokinetic model. *Cancer Research, 70*(23), 9886–9894.
23. Seki, T., Fang, J., & Maeda, H. (2009). Tumor-targeted macromolecular drug delivery based on the enhanced permeability and retention effect in solid tumor. In *Pharmaceutical perspectives of cancer therapeutics* (pp. 93–120). New York: Springer.
24. Chambers, A. F., & Matrisian, L. M. (1997). Changing views of the role of matrix metalloproteinases in metastasis. *Journal of the National Cancer Institute, 89*(17), 1260–1270.
25. Wu, J., Akaike, T., Hayashida, K., Okamoto, T., Okuyama, A., & Maeda, H. (2001). Enhanced vascular permeability in solid tumor involving peroxynitrite and matrix metalloproteinases. *Japanese Journal of Cancer Research, 92*(4), 439–451.
26. Wu, J., Akaike, T., Hayashida, K., Miyamoto, Y., Nakagawa, T., Miyakawa, K., et al. (2002). Identification of bradykinin receptors in clinical cancer specimens and murine tumor tissues. *International Journal of Cancer, 98*(1), 29–35.
27. Matsumura, Y., Kimura, M., Yamamoto, T., & Maeda, H. (1988). Involvement of the kinin-generating cascade in enhanced vascular permeability in tumor tissue. *Japanese Journal of Cancer Research, 79*(12), 1327–1334.
28. Wu, J., Akaike, T., & Maeda, H. (1998). Modulation of enhanced vascular permeability in tumors by a bradykinin antagonist, a cyclooxygenase inhibitor, and a nitric oxide scavenger. *Cancer Research, 58*(1), 159–165.
29. Papapetropoulos, A., García-Cardeña, G., Madri, J. A., & Sessa, W. C. (1997). Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. *The Journal of Clinical Investigation, 100*(12), 3131–3139.

30. Seki, T., Fang, J., & Maeda, H. (2009). Tumor-targeted macromolecular drug delivery based on the enhanced permeability and retention effect in solid tumor. In *Pharmaceutical perspectives of cancer therapeutics* (pp. 93–120). New York: Springer.
31. Hori, K., Saito, S., Takahashi, H., Sato, H., Maeda, H., & Sato, Y. (2000). Tumor-selective blood flow decrease induced by an angiotensin converting enzyme inhibitor, temocapril hydrochloride. *Japanese Journal of Cancer Research, 91*(2), 261–269.
32. Noguchi, A., Takahashi, T., Yamaguchi, T., et al. (1992). Enhanced tumor localization of monoclonal antibody by treatment with kininase II inhibitor and angiotensin II. *Japanese Journal of Cancer Research, 83*(3), 240–243.
33. Greish, K. (2010). Enhanced permeability and retention (EPR) effect for anticancer nanomedicine drug targeting. *Methods in Molecular Biology, 624*, 25–37.
34. Maeda, H. (2012). Vascular permeability in cancer and infection as related to macromolecular drug delivery, with emphasis on the EPR effect for tumor-selective drug targeting. *Proceedings of the Japan Academy. Series B, Physical and Biological Sciences, 88*(3), 53–71.
35. Okamoto, T., Akaike, T., Sawa, T., Miyamoto, Y., van der Vliet, A., & Maeda, H. (2001). Activation of matrix metalloproteinases by peroxynitrite-induced protein S-glutathiolation via disulfide S-oxide formation. *The Journal of Biological Chemistry, 276*(31), 29596–29602.
36. Iyer, A. K., Khaled, G., Fang, J., & Maeda, H. (2006). Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discovery Today, 11*(17–18), 812–818.
37. Greish, K. (2007). Enhanced permeability and retention of macromolecular drugs in solid tumors: A royal gate for targeted anticancer nanomedicines. *Journal of Drug Targeting, 15*(7–8), 457–464.
38. Tanaka, S., Akaike, T., Wu, J., et al. (2003). Modulation of tumor-selective vascular blood flow and extravasation by the stable prostaglandin 12 analogue beraprost sodium. *Journal of Drug Targeting, 11*(1), 45–52.
39. Adeyemi, S. A., Choonara, Y. E., Kumar, P., Du Toit, C. L., & Pillay, V. (2017). Design and characterization of endostatin-loaded nanoparticles for in vitro antiangiogenesis in squamous cell carcinoma. *Journal of Nanomaterials, 2017*, 2539065. https://doi.org/10.1155/2017/2539065.
40. Tarhini, M., Greige-Gerges, H., & Elaissari, A. (2017). Protein-based nanoparticles: From preparation to encapsulation of active molecules. *International Journal of Pharmaceutics, 522*(1), 172–197.
41. Thomas, S. C., Harshita, Mishra, P. K., & Talegaonkar, S. (2015). Ceramic nanoparticles: Fabrication methods and applications in drug delivery. *Current Pharmaceutical Design, 21*(42), 6165–6188.
42. Cho, C. F., Sourabh, S., Simpson, E. J., Steinmetz, N. F., Luyt, L. G., & Lewis, J. D. (2014). Molecular targeted viral nanoparticles as tools for imaging cancer. *Methods in Molecular Biology, 1108*, 211–230.
43. Singla, R., Guliani, A., Kumari, A., & Yadav, S. K. (2016). Metallic nanoparticles, toxicity issues and applications in medicine. In *Nanoscale materials in targeted drug delivery, theragnosis and tissue regeneration* (pp. 41–80). Singapore: Springer.
44. Applications of Carbon Nanotubes. (2018). Accessed June 20, 2018, from https://www.azonano.com/article.aspx?ArticleID=4842
45. Sutton, D., Nasongkla, N., Blanco, E., & Gao, J. (2007). Functionalized micellar systems for cancer targeted drug delivery. *Pharmaceutical Research, 24*(6), 1029–1046.
46. Kukowska-Latallo, J. F., Candido, K. A., Cao, Z., Nigavekar, S. S., Majoros, I. J., Thomas, T. P., et al. (2005). Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. *Cancer Research, 65*(12), 5317–5324.
47. Allen, T. M., & Cullis, P. R. (2013). Liposomal drug delivery systems: From concept to clinical applications. *Advanced Drug Delivery Reviews, 65*(1), 36–48.
48. Byrne, J. D., Betancourt, T., & Brannon-Peppas, L. (2008). Active targeting schemes for nanoparticle systems in cancer therapeutics. *Advanced Drug Delivery Reviews, 60*(15), 1615–1626.

49. Owens, D. E., & Peppas, N. A. (2006). Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *International Journal of Pharmaceutics, 307*(1), 93–102.
50. Gustafson, H. H., Holt-Casper, D., Grainger, D. W., & Ghandehari, H. (2015). Nanoparticle uptake: The phagocyte problem. *Nano Today, 10*(4), 487–510.
51. Gref, R., Lück, M., Quellec, P., et al. (2000). 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): Influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Colloids and Surfaces. B, Biointerfaces, 18*(3), 301–313.
52. Adebowale, A. S., Choonara, Y. E., Kumar, P., du Toit, L. C., & Pillay, V. (2015). Functionalized nanocarriers for enhanced bioactive delivery to squamous cell carcinomas: Targeting approaches and related biopharmaceutical aspects. *Current Pharmaceutical Design, 21*(22), 3167–3180.
53. Zhang, L., Gu, F. X., Chan, J. M., Wang, A. Z., Langer, R. S., & Farokhzad, O. C. (2008). Nanoparticles in medicine: Therapeutic applications and developments. *Clinical Pharmacology and Therapeutics, 83*(5), 761–769.
54. Canelas, D. A., Herlihy, K. P., & DeSimone, J. M. (2009). Top-down particle fabrication: Control of size and shape for diagnostic imaging and drug delivery. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 1*(4), 391–404.
55. Longmire, M. R., Ogawa, M., Choyke, P. L., & Kobayashi, H. (2011). Biologically optimized nanosized molecules and particles: More than just size. *Bioconjugate Chemistry, 22*(6), 993–1000.
56. Albanese, A., Sykes, E. A., & Chan, W. C. W. (2010). Rough around the edges: The inflammatory response of microglial cells to spiky nanoparticles. *ACS Nano, 4*(5), 2490–2493.
57. Yamamoto, Y., Nagasaki, Y., Kato, Y., Sugiyama, Y., & Kataoka, K. (2001). Long-circulating poly(ethylene glycol)-poly(D,L-lactide) block copolymer micelles with modulated surface charge. *Journal of Controlled Release, 77*(1–2), 27–38.
58. Dreaden, E. C., Austin, L. A., Mackey, M. A., & El-Sayed, M. A. (2012). Size matters: Gold nanoparticles in targeted cancer drug delivery. *Therapeutic Delivery, 3*(4), 457–478.
59. Hauert, S., & Bhatia, S. N. (2014). Mechanisms of cooperation in cancer nanomedicine: Towards systems nanotechnology. *Trends in Biotechnology, 32*(9), 448–455.
60. Sheng, Y., Yuan, Y., Liu, C., Tao, X., Shan, X., & Xu, F. (2009). In vitro macrophage uptake and in vivo biodistribution of PLA-PEG nanoparticles loaded with hemoglobin as blood substitutes: Effect of PEG content. *Journal of Materials Science. Materials in Medicine, 20*(9), 1881–1891.
61. Amoozgar, Z., & Yeo, Y. (2012). Recent advances in stealth coating of nanoparticle drug delivery systems. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 4*(2), 219–233.
62. Zhu, L., & Torchilin, V. P. (2013). Stimulus-responsive nanopreparations for tumor targeting. *Integrative Biology, 5*(1), 96–107.
63. Sailor, M. J., & Park, J.-H. (2012). Hybrid nanoparticles for detection and treatment of cancer. *Advanced Materials, 24*(28), 3779–3802.
64. Olson, E. S., Jiang, T., Aguilera, T. A., Nguyen, Q. T., Ellies, L. G., Scadeng, M., et al. (2010). Activatable cell penetrating peptides linked to nanoparticles as dual probes for in vivo fluorescence and MR imaging of proteases. *Proceedings of the National Academy of Sciences of the United States of America, 107*(9), 4311–4316.
65. Poon, Z., Chang, D., Zhao, X., & Hammond, P. T. (2011). Layer-by-layer nanoparticles with a pH-Sheddable layer for in vivo targeting of tumor hypoxia. *ACS Nano, 5*(6), 4284–4292.
66. Yaari, Z., da, S. D., Zinger, A., et al. (2016). Theranostic barcoded nanoparticles for personalized cancer medicine. *Nature Communications, 7*, 13325.
67. McCarthy, J. R., & Weissleder, R. (2008). Multifunctional magnetic nanoparticles for targeted imaging and therapy. *Advanced Drug Delivery Reviews, 60*(11), 1241–1251.
68. Soo Choi, H., Liu, W., Misra, P., et al. (2007). Renal clearance of quantum dots. *Nature Biotechnology, 25*(10), 1165–1170.

69. Maeda, H., Nakamura, H., & Fang, J. (2013). The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. *Advanced Drug Delivery Reviews, 65*(1), 71–79.
70. Wang, J., Byrne, J. D., Napier, M. E., & DeSimone, J. M. (2011). More effective nanomedicines through particle design. *Small, 7*(14), 1919–1931.
71. Venkataraman, S., Hedrick, J. L., Ong, Z. Y., et al. (2011, November). The effects of polymeric nanostructure shape on drug delivery. *Advanced Drug Delivery Reviews, 63*(14–15), 1228–1246.
72. Owens, D. E., III, & Peppas, N. A. (2006). Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *International Journal of Pharmaceutics, 307*(1), 93–102.
73. Marquez, M., Nilsson, S., Lennartsson, L., et al. (2004). Charge-dependent targeting: Results in six tumor cell lines. *Anticancer Research, 24*(3A), 1347–1352.
74. Romberg, B., Hennink, W. E., & Storm, G. (2008). Sheddable coatings for long-circulating nanoparticles. *Pharmaceutical Research, 25*(1), 55–71.
75. Petros, R. A., & DeSimone, J. M. (2010). Strategies in the design of nanoparticles for therapeutic applications. *Nature Reviews. Drug Discovery, 9*(8), 615–627.
76. Rodriguez, P. L., Harada, T., Christian, D. A., Pantano, D. A., Tsai, R. K., & Discher, D. E. (2013). Minimal "self" peptides that inhibit phagocytic clearance and enhance delivery of nanoparticles. *Science, 339*(6122), 971–975.
77. Mishra, P., Nayak, B., & Dey, R. K. (2016). PEGylation in anti-cancer therapy: An overview. *Asian Journal of Pharmaceutical Sciences, 11*(3), 337–348.
78. Abuchowski, A., van Es, T., Palczuk, N. C., & Davis, F. F. (1977). Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. *The Journal of Biological Chemistry, 252*(11), 3578–3581.
79. Abuchowski, A., McCoy, J. R., Palczuk, N. C., van Es, T., & Davis, F. F. (1977). Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. *The Journal of Biological Chemistry, 252*(11), 3582–3586.
80. Matsushima, A., Kodera, Y., Hiroto, M., Nishimura, H., & Inada, Y. (1996). Bioconjugates of proteins and polyethylene glycol: Potent tools in biotechnological processes. *Journal of Molecular Catalysis B: Enzymatic, 2*(1), 1–17.
81. Giorgi, M. E., Agusti, R., & de Lederkremer, R. M. (2014). Carbohydrate PEGylation, an approach to improve pharmacological potency. *Beilstein Journal of Organic Chemistry, 10*, 1433–1444.
82. Riley, T, & Riggs-Sauthier, J. (2008) The benefits and challenges of PEGylating small molecules. Accessed July 2, 2018, from http://www.pharmtech.com/benefits-and-challenges-pegylating-small-molecules
83. Roberts, M. J., Bentley, M. D., & Harris, J. M. (2002). Chemistry for peptide and protein PEGylation. *Advanced Drug Delivery Reviews, 54*(4), 459–476.
84. Dozier, J. K., & Distefano, M. D. (2015). Site-specific PEGylation of therapeutic proteins. *International Journal of Molecular Sciences, 16*(10), 25831–25864.
85. Veronese, F. M., & Pasut, G. (2008). PEGylation: Posttranslational bioengineering of protein biotherapeutics. *Drug Discovery Today: Technologies, 5*(2–3), e57–e64.
86. Caliceti, P., & Veronese, F. M. (2003). Pharmacokinetic and biodistribution properties of poly(ethylene glycol)-protein conjugates. *Advanced Drug Delivery Reviews, 55*(10), 1261–1277.
87. Bailon, P., & Berthold, W. (1998). Polyethylene glycol-conjugated pharmaceutical proteins. *Pharmaceutical Science & Technology Today, 1*(8), 352–356.
88. Yamaoka, T., Tabata, Y., & Ikada, Y. (1994). Distribution and tissue uptake of poly(ethylene glycol) with different molecular weights after intravenous administration to mice. *Journal of Pharmaceutical Sciences, 83*(4), 601–606.
89. Remaut, K., Lucas, B., Braeckmans, K., Demeester, J., & De Smedt, S. C. (2007). Pegylation of liposomes favours the endosomal degradation of the delivered phosphodiester oligonucleotides. *Journal of Controlled Release, 117*(2), 256–266.

90. Hatakeyama, H., Akita, H., & Harashima, H. (2013). The polyethyleneglycol dilemma: Advantage and disadvantage of PEGylation of liposomes for systemic genes and nucleic acids delivery to tumors. *Biological & Pharmaceutical Bulletin, 36*(6), 892–899.
91. Sato, Y., Hatakeyama, H., Sakurai, Y., Hyodo, M., Akita, H., & Harashima, H. (2012). A pH-sensitive cationic lipid facilitates the delivery of liposomal siRNA and gene silencing activity in vitro and in vivo. *Journal of Controlled Release, 163*(3), 267–276.
92. Hatakeyama, H., Akita, H., & Harashima, H. (2011). A multifunctional envelope type nano device (MEND) for gene delivery to tumours based on the EPR effect: A strategy for overcoming the PEG dilemma. *Advanced Drug Delivery Reviews, 63*(3), 152–160.
93. Suk, J. S., Xu, Q., Kim, N., Hanes, J., & Ensign, L. M. (2016). PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Advanced Drug Delivery Reviews, 99*. (Pt A, 28–51.
94. Vittaz, M., Bazile, D., Spenlehauer, G., et al. (1996). Effect of PEO surface density on long-circulating PLA-PEO nanoparticles which are very low complement activators. *Biomaterials, 17*(16), 1575–1581.
95. Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Molecular Pharmaceutics, 5*(4), 505–515.
96. Yang, M., Lai, S. K., Wang, Y.-Y., et al. (2011). Biodegradable nanoparticles composed entirely of safe materials that rapidly penetrate human mucus. *Angewandte Chemie (International Ed. in English), 50*(11), 2597–2600.
97. Chan, J. M., Zhang, L., Yuet, K. P., et al. (2009). PLGA-lecithin-PEG core-shell nanoparticles for controlled drug delivery. *Biomaterials, 30*(8), 1627–1634.
98. Lin, J.-J., Chen, J.-S., Huang, S.-J., et al. (2009). Folic acid-Pluronic F127 magnetic nanoparticle clusters for combined targeting, diagnosis, and therapy applications. *Biomaterials, 30*(28), 5114–5124.
99. Mi, Y., Liu, Y., & Feng, S.-S. (2011). Formulation of Docetaxel by folic acid-conjugated d-α-tocopheryl polyethylene glycol succinate 2000 (Vitamin E TPGS(2k)) micelles for targeted and synergistic chemotherapy. *Biomaterials, 32*(16), 4058–4066.
100. Wang, H., Zhao, P., Su, W., et al. (2010). PLGA/polymeric liposome for targeted drug and gene co-delivery. *Biomaterials, 31*(33), 8741–8748.
101. Cu, Y., & Saltzman, W. M. (2009). Controlled surface modification with poly(ethylene) glycol enhances diffusion of PLGA nanoparticles in human cervical mucus. *Molecular Pharmaceutics, 6*(1), 173–181.
102. Nuruzatulifah, A. M., Nizam, A. A., & Ain, N. M. N. (2016). Synthesis and characterization of polystyrene nanoparticles with covalently attached fluorescent dye. *Materials Today Proceedings, 3*, S112–S119.
103. Perrault, S. D., & Chan, W. C. W. (2009). Synthesis and surface modification of highly monodispersed, spherical gold nanoparticles of 50–200 nm. *Journal of the American Chemical Society, 131*(47), 17042–17043.
104. Kim, A. J., Boylan, N. J., Suk, J. S., et al. (2013). Use of single-site-functionalized PEG dendrons to prepare gene vectors that penetrate human mucus barriers. *Angewandte Chemie (International Ed. in English), 52*(14), 3985–3988.
105. Nance, E. A., Woodworth, G. F., Sailor, K. A., et al. (2012). A dense poly(ethylene glycol) coating improves penetration of large polymeric nanoparticles within brain tissue. *Science Translational Medicine, 4*(149), 149ra119.
106. Uster, P. S., Allen, T. M., Daniel, B. E., Mendez, C. J., Newman, M. S., & Zhu, G. Z. (1996). Insertion of poly(ethylene glycol) derivatized phospholipid into pre-formed liposomes results in prolonged in vivo circulation time. *FEBS Letters, 386*(2–3), 243–246.
107. Nakamura, K., Yamashita, K., Itoh, Y., Yoshino, K., Nozawa, S., & Kasukawa, H. (2012). Comparative studies of polyethylene glycol-modified liposomes prepared using different PEG-modification methods. *Biochimica et Biophysica Acta, 1818*(11), 2801–2807.
108. Torchilin, V. P. (2005). Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews. Drug Discovery, 4*(2), 145–160.

109. Cavalli, S., Tipton, A. R., Overhand, M., & Kros, A. (2006). The chemical modification of liposome surfaces via a copper-mediated [3+2] azide–alkyne cycloaddition monitored by a colorimetric assay. *Chemical Communications, 0*(30), 3193–3195.
110. Kumar, A., Erasquin, U. J., Qin, G., Li, K., & Cai, C. (2010). "Clickable", polymerized liposomes as a versatile and stable platform for rapid optimization of their peripheral compositions. *Chemical Communications, 46*(31), 5746–5748.
111. Manzenrieder, F., Luxenhofer, R., Retzlaff, M., Jordan, R., & Finn, M. G. (2011). Stabilization of virus-like particles with poly(2-oxazoline)s. *Angewandte Chemie, International Edition, 50*(11), 2601–2605.
112. Zhang, N., Pompe, T., Amin, I., Luxenhofer, R., Werner, C., & Jordan, R. (2012). Tailored poly(2-oxazoline) polymer brushes to control protein adsorption and cell adhesion. *Macromolecular Bioscience, 12*(7), 926–936.
113. Viegas, T. X., Bentley, M. D., Harris, J. M., et al. (2011). Polyoxazoline: Chemistry, properties, and applications in drug delivery. *Bioconjugate Chemistry, 22*(5), 976–986.
114. Tong, J., Yi, X., Luxenhofer, R., et al. (2013). Conjugates of superoxide dismutase 1 with amphiphilic poly(2-oxazoline) block copolymers for enhanced brain delivery: Synthesis, characterization and evaluation in vitro and in vivo. *Molecular Pharmaceutics, 10*(1), 360–377.
115. Pidhatika, B., Rodenstein, M., Chen, Y., et al. (2012). Comparative stability studies of poly(2-methyl-2-oxazoline) and poly(ethylene glycol) brush coatings. *Biointerphases, 7*(1), 1.
116. Foreman, M. B., Coffman, J. P., Murcia, M. J., et al. (2003). Gelation of amphiphilic lipopolymers at the air-water interface: 2D analogue to 3D gelation of colloidal systems with grafted polymer chains? *Langmuir, 19*(2), 326–332.
117. Luxenhofer, R., Schulz, A., Roques, C., et al. (2010). Doubly amphiphilic poly(2-oxazoline)s as high-capacity delivery systems for hydrophobic drugs. *Biomaterials, 31*(18), 4972–4979.
118. von Erlach, T., Zwicker, S., Pidhatika, B., et al. (2011). Formation and characterization of DNA-polymer-condensates based on poly(2-methyl-2-oxazoline) grafted poly(L-lysine) for non-viral delivery of therapeutic DNA. *Biomaterials, 32*(22), 5291–5303.
119. Cheon Lee, S., Kim, C., Chan Kwon, I., Chung, H., & Young Jeong, S. (2003). Polymeric micelles of poly(2-ethyl-2-oxazoline)-block-poly(epsilon-caprolactone) copolymer as a carrier for paclitaxel. *Journal of Controlled Release, 89*(3), 437–446.
120. Kim, C., Lee, S. C., Shin, J. H., Yoon, J.-S., Kwon, I. C., & Jeong, S. Y. (2000). Amphiphilic diblock copolymers based on poly(2-ethyl-2-oxazoline) and poly(1,3-trimethylene carbonate): Synthesis and micellar characteristics. *Macromolecules, 33*(20), 7448–7452.
121. Wang, C.-H., Wang, W.-T., & Hsiue, G.-H. (2009). Development of polyion complex micelles for encapsulating and delivering amphotericin B. *Biomaterials, 30*(19), 3352–3358.
122. Kainthan, R. K., & Brooks, D. E. (2007). In vivo biological evaluation of high molecular weight hyperbranched polyglycerols. *Biomaterials, 28*(32), 4779–4787.
123. Siegers, C., Biesalski, M., & Haag, R. (2004). Self-assembled monolayers of dendritic polyglycerol derivatives on gold that resist the adsorption of proteins. *Chemistry, 10*(11), 2831–2838.
124. Kainthan, R. K., Hester, S. R., Levin, E., Devine, D. V., & Brooks, D. E. (2007). *In vitro* biological evaluation of high molecular weight hyperbranched polyglycerols. *Biomaterials, 28*(31), 4581–4590.
125. Maruyama, K., Okuizumi, S., Ishida, O., Yamauchi, H., Kikuchi, H., & Iwatsuru, M. (1994). Phosphatidyl polyglycerols prolong liposome circulation in vivo. *International Journal of Pharmaceutics, 111*(1), 103–107.
126. Hofmann, A. M., Wurm, F., Hühn, E., Nawroth, T., Langguth, P., & Frey, H. (2010). Hyperbranched polyglycerol-based lipids via oxyanionic polymerization: Toward multifunctional stealth liposomes. *Biomacromolecules, 11*(3), 568–574.
127. Romberg, B., Metselaar, J. M., Baranyi, L., et al. (2007). Poly(amino acid)s: Promising enzymatically degradable stealth coatings for liposomes. *International Journal of Pharmaceutics, 331*(2), 186–189.

128. Romberg, B., Oussoren, C., Snel, C. J., Carstens, M. G., Hennink, W. E., & Storm, G. (2007). Pharmacokinetics of poly(hydroxyethyl-l-asparagine)-coated liposomes is superior over that of PEG-coated liposomes at low lipid dose and upon repeated administration. *Biochimica et Biophysica Acta, 1768*(3), 737–743.
129. Shao, Q., He, Y., White, A. D., & Jiang, S. (2010). Difference in hydration between carboxybetaine and sulfobetaine. *The Journal of Physical Chemistry. B, 114*(49), 16625–16631.
130. Chen, S., Chen, S., Jiang, S., et al. (2011). Study of zwitterionic sulfopropylbetaine containing reactive siloxanes for application in antibacterial materials. *Colloids and Surfaces. B, Biointerfaces, 85*(2), 323–329.
131. Zhang, Z., Vaisocherová, H., Cheng, G., Yang, W., Xue, H., & Jiang, S. (2008). Nonfouling behavior of polycarboxybetaine-grafted surfaces: Structural and environmental effects. *Biomacromolecules, 9*(10), 2686–2692.
132. Cheng, G., Li, G., Xue, H., Chen, S., Bryers, J. D., & Jiang, S. (2009). Zwitterionic carboxybetaine polymer surfaces and their resistance to long-term biofilm formation. *Biomaterials, 30*(28), 5234–5240.
133. Jiang, S., & Cao, Z. (2010). Ultralow-fouling, functionalizable, and hydrolyzable zwitterionic materials and their derivatives for biological applications. *Advanced Materials, 22*(9), 920–932.
134. Zhang, L., Xue, H., Gao, C., et al. (2010). Imaging and cell targeting characteristics of magnetic nanoparticles modified by a functionalizable zwitterionic polymer with adhesive 3,4-dihydroxyphenyl-l-alanine linkages. *Biomaterials, 31*(25), 6582–6588.
135. Jia, G., Cao, Z., Xue, H., Xu, Y., & Jiang, S. (2009). Novel zwitterionic-polymer-coated silica nanoparticles. *Langmuir, 25*(5), 3196–3199.
136. Yang, W., Zhang, L., Wang, S., White, A. D., & Jiang, S. (2009). Functionalizable and ultra stable nanoparticles coated with zwitterionic poly(carboxybetaine) in undiluted blood serum. *Biomaterials, 30*(29), 5617–5621.
137. Cao, Z., Yu, Q., Xue, H., Cheng, G., & Jiang, S. (2010). Nanoparticles for drug delivery prepared from amphiphilic PLGA zwitterionic block copolymers with sharp contrast in polarity between two blocks. *Angewandte Chemie (International Ed. in English), 49*(22), 3771–3776.
138. Kim, K., Kim, J. H., Park, H., Kim, Y.-S., Park, K., Nam, H., et al. (2010). Tumor-homing multifunctional nanoparticles for cancer theragnosis: Simultaneous diagnosis, drug delivery, and therapeutic monitoring. *Journal of Controlled Release, 146*(2), 219–227.
139. Choi, K. Y., Chung, H., Min, K. H., et al. (2010). Self-assembled hyaluronic acid nanoparticles for active tumor targeting. *Biomaterials, 31*(1), 106–114.
140. Li, Y.-L., Zhu, L., Liu, Z., et al. (2009). Reversibly stabilized multifunctional dextran nanoparticles efficiently deliver doxorubicin into the nuclei of cancer cells. *Angewandte Chemie (International Ed. in English), 48*(52), 9914–9918.
141. Hou, L., Fan, Y., Yao, J., et al. (2011). Low molecular weight heparin-all-trans-retinoid acid conjugate as a drug carrier for combination cancer chemotherapy of paclitaxel and all-trans-retinoid acid. *Carbohydrate Polymers, 86*(3), 1157–1166.
142. Kean, T., & Thanou, M. (2010). Biodegradation, biodistribution and toxicity of chitosan. *Advanced Drug Delivery Reviews, 62*(1), 3–11.
143. Adeyemi, S. A., Choonara, Y. E., Kumar, P., du Toit, L. C., & Pillay, V. (2017). Synthesis and in vitro characterization of a pH-responsive chitosan- polyethylenimine nanosystem for the delivery of therapeutic proteins. *Journal of Drug Delivery Science and Technology, 39*, 266–276.
144. Papisov, M. I. (2001). Biopolymers from polysaccharides and agroproteins. *American Chemical Society, 786*. https://doi.org/10.1021/bk-2001-0786.
145. Dahm, R., & Geisler, R. (2006). Learning from small fry: The zebrafish as a genetic model organism for aquaculture fish species. *Marine Biotechnology, 8*(4), 329–345.
146. Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., et al. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature, 496*(7446), 498–503.

147. Corey, D. R., & Abrams, J. M. (2001). Morpholino antisense oligonucleotides: Tools for investigating vertebrate development. *Genome Biology, 2*(5), 1015.1.
148. Meng, X., Noyes, M. B., Zhu, L. J., Lawson, N. D., & Wolfe, S. A. (2008). Targeted gene inactivation in zebrafish using engineered zinc-finger nucleases. *Nature Biotechnology, 26*(6), 695–701.
149. Zon, L. I., & Peterson, R. T. (2005). In vivo drug discovery in the zebrafish. *Nature Reviews. Drug Discovery, 4*(1), 35–44.
150. Etchin, J., Kanki, J. P., & Look, A. T. (2011). Zebrafish as a model for the study of human cancer. *Methods in Cell Biology, 105*, 309–337.
151. He, S., Lamers, G. E., Beenakker, J.-W. M., et al. (2012). Neutrophil-mediated experimental metastasis is enhanced by VEGFR inhibition in a zebrafish xenograft model. *The Journal of Pathology, 227*(4), 431–445.
152. Teng, Y., Xie, X., Walker, S., White, D. T., Mumm, J. S., & Cowell, J. K. (2013). Evaluating human cancer cell metastasis in zebrafish. *BMC Cancer, 13*, 453.
153. Amatruda, J. F., Shepard, J. L., Stern, H. M., & Zon, L. I. (2002). Zebrafish as a cancer model system. *Cancer Cell, 1*(3), 229–231.
154. Tobin, D. M., May, R. C., & Wheeler, R. T. (2012). Zebrafish: A see-through host and a fluorescent toolbox to probe host–pathogen interaction. *PLoS Pathogens, 8*(1), e1002349.
155. Yang, X., Cui, W., Gu, A., et al. (2013). A novel zebrafish xenotransplantation model for study of glioma stem cell invasion. *PLoS One, 8*(4), e61801.
156. Konantz, M., Balci, T. B., Hartwig, U. F., et al. (2012). Zebrafish xenografts as a tool for in vivo studies on human cancer. *Annals of the New York Academy of Sciences, 1266*, 124–137.
157. Fenaroli, F., Westmoreland, D., Benjaminsen, J., et al. (2014). Nanoparticles as drug delivery system against tuberculosis in zebrafish embryos: Direct visualization and treatment. *ACS Nano, 8*(7), 7014–7026.
158. Moghimi, S. M., Hunter, A. C., & Murray, J. C. (2001). Long-circulating and target-specific nanoparticles: Theory to practice. *Pharmacological Reviews, 53*(2), 283–318.
159. Coradeghini, R., Gioria, S., García, C. P., et al. (2013). Size-dependent toxicity and cell interaction mechanisms of gold nanoparticles on mouse fibroblasts. *Toxicology Letters, 217*(3), 205–216.
160. Palko, H. A., Fung, J. Y., & Louie, A. Y. (2010). Positron emission tomography: A novel technique for investigating the biodistribution and transport of nanoparticles. *Inhalation Toxicology, 22*(8), 657–688.
161. Rocco, L., Santonastaso, M., Mottola, F., et al. (2015). Genotoxicity assessment of TiO2 nanoparticles in the teleost *Danio rerio*. *Ecotoxicology and Environmental Safety, 113*, 223–230.
162. Gao, J., Sepúlveda, M. S., Klinkhamer, C., Wei, A., Gao, Y., & Mahapatra, C. T. (2015). Nanosilver-coated socks and their toxicity to zebrafish (*Danio rerio*) embryos. *Chemosphere, 119*, 948–952.
163. Wagner, D. S., Delk, N. A., Lukianova-Hleb, E. Y., Hafner, J. H., Farach-Carson, M. C., & Lapotko, D. O. (2010). The in vivo performance of plasmonic nanobubbles as cell theranostic agents in zebrafish hosting prostate cancer xenografts. *Biomaterials, 31*(29), 7567–7574.
164. Wu, Q., Deng, S., Li, L., et al. (2013). Biodegradable polymeric micelle-encapsulated quercetin suppresses tumor growth and metastasis in both transgenic zebrafish and mouse models. *Nanoscale, 5*(24), 12480–12493.