1-1-2011

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Recommended Citation

Brewer, E., Coleman, J. & Lowman, A. (2011). Emerging Technologies of Polymeric Nanoparticles in Cancer Drug Delivery, Journal of Nanomaterials, 2011: 1-10.

Hindawi Publishing Corporation
Journal of Nanomaterials
Volume 2011, Article ID 408675, 10 pages
doi:10.1155/2011/408675

Review Article

Emerging Technologies of Polymeric Nanoparticles in Cancer Drug Delivery

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Received 2 June 2010; Accepted 2 August 2010

Academic Editor: Lu Sun

Polymeric nanomaterials have the potential to improve upon present chemotherapy delivery methods. They successfully reduce side effects while increasing dosage, increase residence time in the body, offer a sustained and tunable release, and have the ability to deliver multiple drugs in one carrier. However, traditional nanomaterial formulations have not produced highly therapeutic formulations to date due to their passive delivery methods and lack of rapid drug release at their intended site. In this paper, we have focused on a few "smart" technologies that further enhance the benefits of typical nanomaterials. Temperature and pH-responsive drug delivery devices were reviewed as methods for triggering release of encapsulating drugs, while aptamer and ligand conjugation were discussed as methods for targeted and intracellular delivery, with emphases on *in vitro* and *in vivo* works for each method.

1. Introduction

A major obstacle for chemotherapy is the inability to deliver adequate doses of drugs to the affected areas in the body. Systemic toxicity of these drugs limits their dose, while rapid clearance from circulation requires large doses in order to be effective. Doxorubicin, for instance, has a five to ten minute half life in the plasma [1].

Polymeric nanomaterials offer a promising solution by encapsulating chemotherapy drugs, and have been shown to reduce toxicity by providing a protective housing for the drug that limits its interaction with healthy cells [2–5]. As a result, the pharmacokinetic properties of the drug are based on the pharmacokinetic properties of the particle, as long as the drug can stay entrapped with the carrier until release is desired [6]. The potential benefits of such delivery devices also include controlled and long-term release rates, prolonged bioactivity, reduced side effects, increased patient compliance due to decreased administration frequency, and the ability to codeliver multiple drugs with synergistic effects to the same site [7–9].

Delivery devices made from erodible polymers are an attractive option over nonerodible ones because they degrade and gradually disappear after delivery [10]. Of these polymers, poly(ε-caprolactone), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers have been among the most extensively researched due to their biocompatibility, biodegradability, and regulatory approval [11–15]. For an anticancer drug carrier to prove effective, prolonged circulation times and controlled drug release at the tumor site are required [16, 17]. Various works have already been done to improve particle circulation time by limiting renal secretion and hindering uptake by the reticuloendothelial system (RES) [18–22]. This is often accomplished by pairing poly(ethylene glycol) (PEG) with the polymer of the nanoparticle. PEG has been shown to inhibit the binding of plasma proteins to the surface of polymeric drug carriers, preventing their recognition by the RES. This imparts "stealth" properties to the delivery device, increasing its systemic circulation time significantly [23].

Passive targeting is created because the size of the polymeric systems and their increased residence time make them suitable carriers to take advantage of the enhanced permeability and retention (EPR) effect in tumors [6, 24, 25]. The EPR effect is described as hyperpermeable tumor vessels that allow for the extravasation of circulating macromolecules, such as polymeric nanomaterials, that, combined with the lack of a lymphatic drainage system, results in their

gradual accumulation [26]. While this results in localized delivery gradually over time, there still remains the need for drugs that act intracellularly to release and permeate the cell walls. In addition, it has been shown that slow and passive drug release from drug-carrying particles reduces their effectiveness in cancer treatment [27]. Overall, passive polymer drug carriers have not demonstrated increased therapeutic efficacy due to lack of intercellular and localized, rapid drug delivery [28–30].

Current research has thus focused on advancing these polymer vehicles with "smart" technologies that are responsive to environmental stimuli. These can be separated into two categories: (1) site-targeting, where particles actively search for and attach themselves to specific and diseased cells by the use of molecules such as ligands, antibodies, and aptamers; (2) site-triggering, where chemical or physical changes in the environment trigger the rapid release of the drug payload. This review focuses on a few selected "smart" technologies in each category: ligand and aptamer site-targeting particles, and pH and temperature-responsive particles.

2. Smart Nanomaterials

2.1. Site-Targeted Nanomaterials

2.1.1. Ligands. Attaching targeting ligands to the particle surface can take advantage of the overexpression of various receptors on tumor cell surfaces [31, 32]. Coupled with the passive accumulation at tumor sights caused by the EPR effect, targeted particles can increase the interaction time between particles and the tumor cell and increase the likelihood of the particles being taken up by the tumor cells via endocytosis [33].

Targeted delivery takes advantage of differences in the expression of cell surface receptors between healthy and tumor cells. For example, folate receptors are known to be vastly overexpressed in several human tumors [34–36]. Attaching folate to the outer shell of particles can create a targeted drug delivery carrier. Folate conjugation has shown success at creating targeted anticancer agents that can avoid nonspecific attacks on normal tissue and increase cellular uptake within target cells [31–33, 37, 38].

PEG is commonly associated with the surfaces of micelle-like particles and liposomes to increase particle circulation. By coupling ligands to polyethylene glycol (PEG), a targeted particle can be created where the ligand is expressed on the particle surface. Combining the benefits of prolonged particle circulation with the benefits of delaying drug release, an ideal system exists for targeted delivery [33, 39–45]. The increased residence time increases the likelihood of interaction between receptor and target for targeted delivery.

Yoo et al. developed folate-conjugated PEG-co-poly (lactic-co-glycolic acid) (PEG-PLGA) micelles loaded with the anti-cancer drug doxorubicin that expressed folate on the micelle surface [33]. Studies indicated increased cytotoxicity and decreased tumor growth for folate conjugated micelles as opposed to nontargeted micelles and free DOX [33]. Targeted particles also showed increased cellular uptake [33].

2.1.2. Aptamers. Aptamers are DNA and RNA sequences that recognize specific target analytes [46]. Aptamers can be selected to bind with high specificity and affinity to a wide range of molecules such as organic dyes, amino acids, biological cofactors, antibiotics, peptides, proteins, and whole cells [47]. Aptamers are often compared to antibodies for their affinity to select molecules, but despite their similarities, offer several important advantages: aptamers can be easily synthesized *in vitro* without the need for an induced immune response from animals [48], which makes them able to target nonimmunogenic molecules; the aptamer synthesis process, SELEX, can be carried out in nonphysiological settings [49]; they are more stable and can be obtained at a lower cost [50].

Since the targeted molecule can be uniquely associated with a particular disease, early research into aptamers has concentrated on early-stage disease diagnosis, particularly in cancer. Common cancer diagnostic methods involve somatic or visual techniques, such as self-examinations and localized X-rays. A major disadvantage of these methods is that they do not lead to diagnoses until advanced stages in the disease, a factor in cancers high death rates [51, 52]. However, cancer is a genetic disease, and aptamers provide a way for screening at the molecular level using selective cell binding [53].

Cancer-detecting assays using fluorescent imaging that are currently being developed utilize aptamers conjugated with dye-doped silica nanoparticles. These fluorescent nanoparticles are favored over direct dye conjugation due to their signal amplification and ability to immobilize biomolecules [54–56]. These particles have often combined with magnetic particles, which allows for convenient separation of bound cells, to make two-part aptamer-based assays [53, 57, 58]. Gold nanoparticles, which are ideal contrasting agents, have been conjugated with cancer-targeting aptamers to successfully create assays for detecting prostate and breast cancer cells [59, 60].

The ability of aptamers to bind directly with diseased cells has gained them recognition in site-specific drug delivery research. In particular, systems utilizing polymeric nanovehicle and aptamer conjugates are believed to create devices that can deliver high drug doses to diseased cells in a controlled fashion with minimal toxicity to healthy cells.

In vitro studies involving these systems often utilize the A10 2′-fluoropyrimidine RNA aptamer, which targets the prostate-specific membrane antigen (PSMA) found on the LNCaP cell line. This allows for comparison with control groups tested against PC3 cells, another prostate-cancer cell line that does not display the PSMA antigen, to prove that the drug carriers only have affinity for cells expressing the targeted antigen [61–63]. Using fluorescent imaging, this comparison was able to establish that drug vehicles conjugated with the PSMA-targeting aptamer were internalized by cells via receptor-mediated endocytosis [64]. PEG-PLGA nanoparticles carrying the chemotherapy drug docetaxel and targeting PSMA cells *in vivo* have produced dramatic reduction in tumor sizes in mice compared to free docetaxel and non-targeted particles [61]. The increase in cancer cell toxicity was credited to a combination of the intracellular delivery of the drug, increased retention time,

and reduced circulation clearance at the tumor site due to high-affinity binding with the antigen.

Polymeric micelles have proven to increase the overall affinity of aptamers that exhibit ones considered too low for drug-aptamer delivery systems [65]. They do this by taking advantage of multivalent binding effects, where multiple aptamers on the micelle surface link with the cell-surface antigens to produce an overall stronger bond. This allows for the targeting of unique cellular antigens that would otherwise be considered unsuitable for drug-aptamer conjugates.

Polymeric nanocarriers provide the benefit of being able to carry multiple drugs in the same vehicle. This, combined with aptamer targeting, can be used to selectively deliver dual-drug payloads to cancerous cells. Due to their different mechanisms of action, the drugs may provide additive or synergistic effects that can allow for lower doses, and reduce side effects [66, 67]. More importantly, this is thought to combat drug resistance, a major problem associated with cancer drug treatment [68]. Packaging the drugs in a nanocarrier, as opposed to a simple mixture, allows for their simultaneous delivery on a cell-by-cell basis, which has been proven to be more effective [69–71].

This can even be used to combine drugs with different water solubility properties, as was accomplished by Zhang et al. using PEG-PLGA [9]. In systems where the aptamer binding initiates endocytosis, such as with A10 RNA aptamer, combinations of drugs and genes that require delivery to intracellular compartments to properly function experience greater benefits [72]. This approach has been used successfully in aptamer-gene conjugates [73, 74], and is beginning to see promise in aptamer-nanoparticle conjugates. Polyethyleneimine-grafted-PEG (PEI-PEG) nanoparticles carrying doxorubicin and the hairpin shRNA, which suppresses the antiapoptotic gene Bc1-xl, produced significantly lower cell viability and enhanced therapeutic efficacy compared to single drug-loaded nanoparticle aptamer systems and free drug mixtures [75].

2.2. Site-triggered Nanomaterials

2.2.1. pH-Responsive Nanomaterials. One method to promote drug release at the tumor sight is by taking advantage of the lower pH of the tumor's microenvironment. Mildly acidic conditions exist in tumor and inflammatory tissues (pH 6.8) and in endosomes (pH 5-6) in comparison to the more neutral physiological condition (pH 7.4) [76, 77]. The ability of nanoparticles to accumulate in solid tumors has been shown by the enhanced permeation and retention (EPR) effect [6, 24, 25, 78]. In addition, it has also been demonstrated that nanoparticles can be taken up within cancer cells through a process called endocytosis [79, 80]. Many anticancer drugs, such as doxorubicin, work by inhibiting cell replication. Thus, for anticancer drugs to be effective, they must interact with intracellular components. If particles can gain access to the intracellular components through endocytosis, then it seems logical that the particle deliver its payload of anticancer drugs once inside the cell. Once the particle is taken up via endocytosis, the endocytic vesicles ultimately change to late endosomes and then to lysosomes in which the proton concentration is 100 times higher (pH 5.0) than the physiological condition (pH 7.4) [76]. Micelle forming polymer-drug conjugates and drug loaded liposomes provide the potential for drug release within a lower pH environment. Drug release from micelles can be targeted to these acidic environments by conjugating the polymer to the drug with an acid-cleavable linkage. Release can be targeted to acidic conditions in liposomes by causing destabilization of the liposome shell under acidic conditions.

Nanomaterials such as liposomes and micelles are examples of particles that can accumulate in solid tumors as a result of the EPR effect [3, 9–11]. Micelles consist of a hydrophobic core and a hydrophilic corona or shell and are well suited to entrap and solubilize hydrophobic drugs within their core. Because some of the most commonly used cancer drugs are hydrophobic, micelles have gained widespread use for the delivery of cancer therapeutics [39, 41, 42, 45, 80–83]. Liposomes typically involve a bimolecular phospholipid membrane that encloses an aqueous compartment. Because liposomes contain a phospholipid membrane they can entrap hydrophobic drugs, but they can also encapsulate various hydrophilic drugs such as peptides, proteins, and nucleic acids within their aqueous compartment [84, 85]. Previous work has been done to increase liposome stability by increasing circulation time and by preventing drug leakage until the target is reached [86–88]. Micelle like particles and liposomes with pH sensitivity have shown great promise as delivery vehicles for anticancer drugs, DNA, RNA, proteins, and peptides [76, 82, 89–98].

In order for micelles to take on pH responsibilities, the drug is typically conjugated to the polymer that makes up the core of the micelle by an acid cleavable linkage. The creation of a polymer-drug conjugation is referred to as a polymeric prodrug and allows the drug to remain inactive until cleavage from the polymer carrier. When used in the formation of micelles, polymeric prodrugs can control release by chemically attaching the drug within the core of the micelle or by increasing the thermodynamic stability of the micelle in order to delay micelle degradation [39, 99]. In order to prolong drug release, an active substance can be linked to a polymeric molecule via a covalent bond which is naturally hydrolyzed *in vivo* [100–102]. For pH-responsiveness of polymeric prodrug micelles, the linkage between drug and polymer is more readily hydrolyzed at a lower pH.

If taken up via endocytosis, drug association with a polymer carrier can help avoid the multidrug resistance (MDR) effect (i.e. recycling of chemotherapy drugs). Drug association with a polymer carrier, either through conjugation or entrapment within the micelle core, can help limit free drug being outfluxed from the cancer cell through the *p*-glycoprotein pump.

Various works have been done involving the conjugation of the anti-cancer drug doxorubicin (DOX) to the hydrophobic core forming polymer of the micelle [82, 89, 93, 94]. The conjugation of drug to polymer was performed via a hydrazone linkage and ultimately resulted in enhanced DOX accumulation and cytotoxicity within tumor cells as opposed

to free DOX. One of the more promising aspects for this type of pH-responsive release is the ability of the DOX-conjugated micelles to circumvent the multi-drug resistant effect once taken up by endocytosis [82].

One of the main disadvantages of conjugating the drug to the polymer to get pH responsiveness is the need to maintain drug bioactivity throughout the conjugation scheme. Liposomes that are pH responsive overcome this barrier because the shell of the liposomes is what can be tailored to exhibit pH effects. Because of previous work to increase liposome stability and circulation, the liposome can circulate long enough to passively reach the target sight (EPR effect), and the drug can stay associated with the liposome until the proper pH environment is reached [85–88, 91].

In order for liposomes to deliver their payload at the intracellular layer, the liposomes must first be taken up by endocytosis. Once taken up, the liposomes need to destabilize at the lower endosomal pH. This destabilization can allow the liposome to break down and deliver its contents into the cell cytoplasm. Modification by the inclusion of lipids with pH sensitivity can give the liposome "fusogenic" properties[91]. The term fusogenic refers to the ability of liposomes to destabilize at the lower endosomal pH and "fuse" with the endosomal bilayer to allow for access to the cell cytoplasm. This first became a desired intracellular release mechanism by the observation that certain viruses take advantage of the endosomal acidification to infect cells[91]. Acidic environments within the body also occur at tumors, inflamed or infected tissue, where pH sensitive delivery may also be desirable.

The most common pH-sensitive liposomes are composed of phosphatidylethanolamine (PE) as the primary bilayer component combined with compounds that are stable at a neutral pH, but unstable under acidic conditions [91]. Altering pH sensitivity is typically done by including pH-sensitive lipids, synthetic peptides/proteins, or pH-sensitive polymers within the lipid bilayer or the liposome aqueous compartment [91, 103–108]. With PE liposomes destabilization occurs by intercalation of amphiphilic molecules that contain a protonatable acidic group (i.e. a carboxylic group) that becomes protonated under acidic conditions and causes the PE molecule to revert to an inverted and unstable hexagonal phase [91, 109, 110]. Some of the most effective molecules included within the bilayer that induce pH sensitivity and ultimately, increase drug delivery to the cytoplasm, consist of combinations of dioleoylphosphatidylethanolamine (DOPE) and cholesteryl hemisuccinate (CHEMs) [91, 111, 112].

2.2.2. Thermoresponsive. Hyperthermia has been investigated as a method for triggered drug release to targeted areas in thermoresponsive liposomes. Here, *in vivo* temperatures are achieved through either older and more general methods, such as warmed baths or perfusates [113], or through more advances and localized methods, requiring ultrasonic and microwave units [114, 115]. Since most mammalian cells begin to show damage at 42°C [116], hyperthermia is defined as temperatures between this and physiological temperature (37°C). When the liposomes pass through the area with increased temperature, they release their encapsulated drugs.

In addition to localized drug release, hyperthermia offers other indirect benefits, such as increased microvessel permeability in tumors, which causes more liposomes to accumulate at the intended site [117, 118] while healthy microvessels are not significantly altered [119]; increased cell permeability, which allows the released drugs to diffuse through the cell walls more easily [120]; and increased sensitivity to thermal injury compared to healthy cells [121].

To take advantage of this triggering mechanism, liposomes must have a liquid-crystalline transition temperature (T_c) within the accepted temperature range. Upon reaching this temperature, they become highly permeable to their water-soluble contents, causing hydrophilic drugs to release in the intended location [122, 123]. T_c is a material property of the liposome polymer and is primarily determined by the length of its fatty acid chains [124]. This allows for the addition of other polymers to the liposome, notably polyethylene glycol (PEG), to increase the retention time and stability [125] and alter the release kinetics [126, 127], without significantly changing its transition temperature. To achieve a desirable T_c, it is possible to combine polymers with different transition temperatures in ratios that result in one in the hyperthermic range [128].

In order for a thermosensitive liposome to be considered for a drug-delivery device, it must be stable in plasma circulation, release minimal amount of drug at physiological temperatures, and then release its payload quickly in hyperthermia conditions. Common phospholipids include 1,2-dihexadecanoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), and 1,2-dipalmitoyl-sn-glycero-3-phosphoglyceroglycerol (DPPGOG), often in combination and with varying amounts of PEG [27, 113, 129–131].

In vivo experimentation has proven promising for these thermo-sensitive devices. The chemotherapy drug carboxyfluorescein (CF) produced a sixfold bioavailability increase in cancerous hamsters when packaged in a thermosensitive liposome under hyperthermia compared to free CF [131]. Similar nanovehicles carrying DOX successfully eliminated tumors in six out of nine cancerous mice after 60 days [132]. In a phase I clinical trial, temperature-sensitive liposomes carrying DOX were given to dogs with solid tumors in conjunction with localized hyperthermia. The study reported a 17-fold decrease in drug clearance rate when using the liposomes compared to the free drug, resulting in a higher bioavailability [133].

Alternatively, copolymers have been designed that have a different thermo-sensitive property, called the cloud point (CP), which changes over time, eliminating the need for hyperthermic conditions. Above the CP, the co-polymer precipitates out of solution and freely forms micelles that can encapsulate their drug; below the CP, the polymer dissolves into solution, causing the micelle to destabilize and release its payload [134]. Thus, these co-polymers are designed to begin with a CP that is below ambient conditions so that a drug vehicle can be made, and then end with a CP that is above physiological temperature after the micelles have been delivered to the target cells [135].

This has been achieved through the use of a novel class of hydrophobic lactate-containing polymers, notably poly(*N*-(2-hydroxypropyl) methacrylamide oligolactates) (pHPMAm-Lac$_n$) and poly(*N*-(2-hydroxyethyl) methacrylamide)-oligolactates (pHEMAm-Lac$_n$). The change in CP over time is caused by the hydrolysis of the lactate side group: as the polymer degrades and the lactate hydrolyzes, the polymer becomes more hydrophilic, causing an increase in the CP [134]. In both polymers, the initial CP is dependent on the length of the lactate chain, and can thus be tailored, though pHPMAm-Lac$_2$ and pHEMAm-Lac$_2$ provide the most convenient CPs of 10°C [136] and 22°C [137], respectively. To create an amphiphilic block copolymer, PEG is most commonly used as the hydrophilic segment to take advantage of its stealth properties and longer circulation times [138], as previously described.

These micelles have encountered obstacles in preliminary *in vitro* and *in* vivo experimentation, as release kinetics of encapsulated paclitaxel have been in large part due to diffusion rather than micelle destabilization [139]. In addition, fast degradation kinetics of the lactate chains, causing quick micelle destabilization, resulted in no measurable accumulation in mice 24 h after i.v. injection [140]. However, mPEG-*b*-p(HEMAm-Lac$_n$) polymers modified with methacryloxy-chloride in the micelle core have displayed prolonged circulation times *in vivo* and increased tumor accumulation compared to unmodified micelles [141]. This new class of thermosensitive polymers shows promise for future chemotherapy work.

2.3. Combined Smart Technologies. Because targeted particles can increase uptake by endocytosis, pH-sensitive release is desirable. Combining the benefits of a receptor-targeted micelle and a pH-responsive drug conjugate was performed by Bae et al. [92–95]. Targeting a surface receptor on cancer cells can cause increased cellular uptake, and a pH-responsive degradable bond between drug and polymer can cause release in the low pH environment of the lysosome. Folate was used as the targeting molecule and the pH-responsive hydrazone bond was used to conjugate DOX to the polymer. The self-assembling block copolymers required to prepare the targeted and pH-responsive micelles (approximately 60 nm), consisted of folate-PEG-poly(aspartate hydrazone doxorubicin) [FOL-PEG-P(Asp-Hyd-DOX)]. Delivery to tumor cells known to overexpress folate receptors has been shown with micelles using folate as the targeting moiety to cause increased endocytotic cellular uptake into the intracellular acidic compartments known as endosomes (pH 5-6) [33]. Drugs conjugated within a micelle by a hydrazone linkage show selective release within the low pH environment of endosomes [76, 82, 89].

In terms of effective dose (ED), the effective doses for free DOX and micelles without folate were similar, but the ED for folate conjugated micelles was lowered 2-fold compared to the free DOX micelles [93]. The overall findings by Bae *et al.* suggest that an intracellular, environment-targeting micelle drug carrier is one of the most effective approaches for cancer treatment [92]. Liposomes with pH-sensitivity and targeting ligands have also been effectively used to increase residence time at the target cells, increase uptake, and increase intracellular delivery [37, 96, 98].

3. Conclusion

Smart technologies in polymer nanomaterials offer a unique way to deliver chemotherapy drugs to their intended target without affecting healthy cells. By utilizing the naturally low pH environment found in tumors and endosomes, these drug carriers are free to circulate in the body, only releasing their drugs at their intended location. Thermosensitive polymer vehicles, when combined with localized hyperthermia, can be triggered to release their payload at the desired site. Ligands and aptamers, on the other hand, provide a way for these vehicles to actively target cancerous cells and then induce receptor-mediated endocytosis for intracellular delivery. Compared to free drug and passive nanomaterial systems, these smart devices have proven to increase therapeutic effects and efficacy in a variety of cellular and animal models. Progression of these techniques will eventually lead to increased accuracy in delivering higher doses and more toxic drugs, which will require challenges like premature drug release and false cell targeting to be addressed. As these technologies are further developed and other methods of triggering and targeting emerge, smart polymer nanomaterials will be able to provide improved cancer treatment methods.

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