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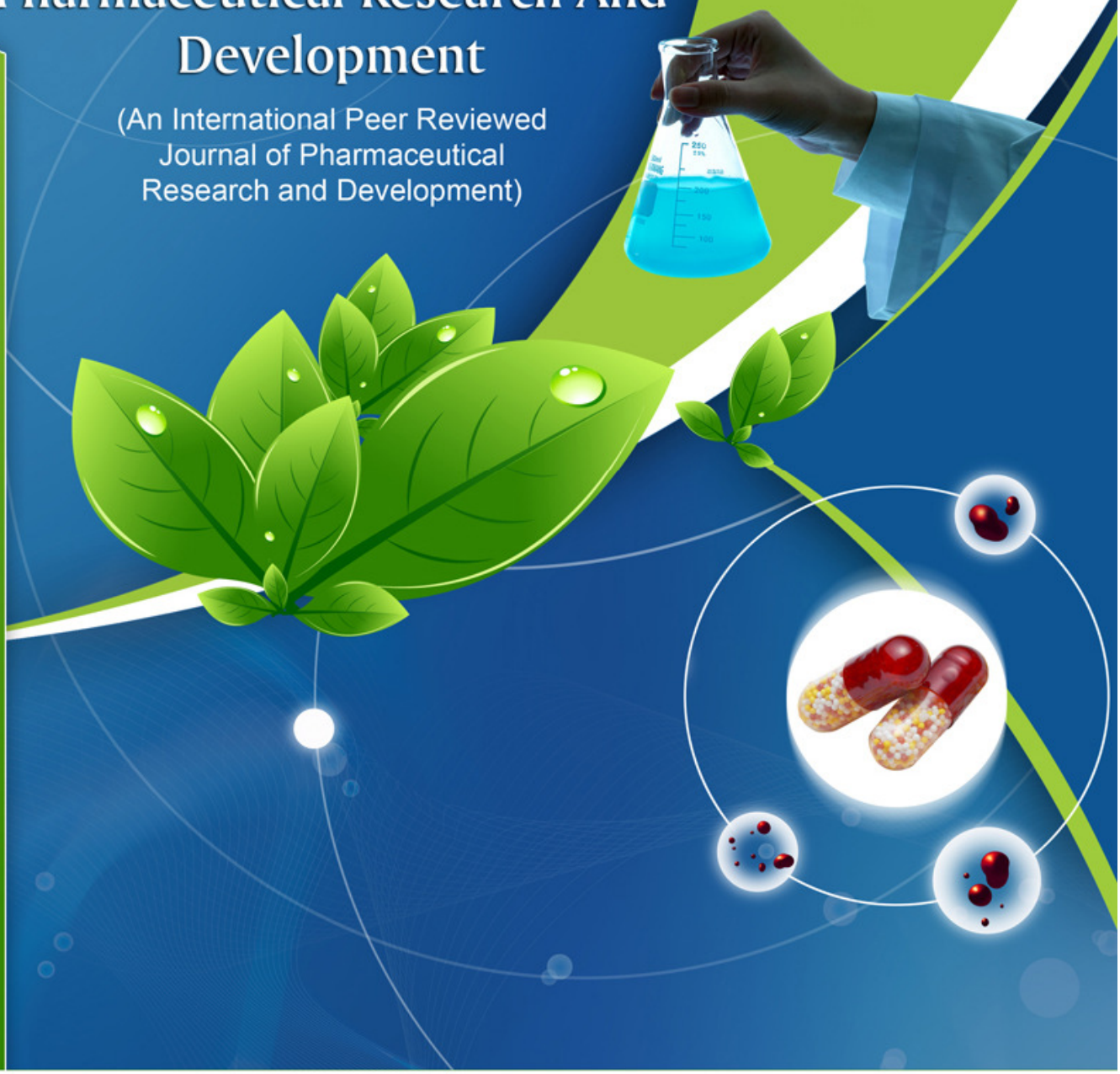

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Review Article

APPLICATION OF POLYMERIC NANOPARTICLES IN CANCER DRUG DELIVERY - A REVIEW

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ABSTRACT

Polymeric nanoparticles (PNPs) are defined as particulate dispersions or solid particles with size in the range of 10-1000nm. There has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. Polymeric nanoparticles have been extensively studied as particulate carriers in the pharmaceutical and medical fields, because they show promise as drug delivery systems as a result of their controlled and sustained release properties, subcellular size, biocompatibility with tissue and cells. Polymeric nanomaterial's have the potential to improve upon present chemotherapy delivery methods. They reduce side effects while increasing dosage, have the ability to deliver multiple drugs in one carrier, and offer a sustained release. 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 aptamers and ligand conjugation were discussed as methods for targeted and intracellular delivery, with emphases on in vivo and in vitro works for each method.

Key Words: *Nanoparticles, Polymeric nanoparticles, Aptamers, Combined Smart Technologies, Thermo responsive, Site-triggered Nanomaterial's, Site-Targeted Nanomaterial's.*

INTRODUCTION

The European and other International Committees have defined nanoparticle as: three dimensions structure, in the Order of 100nm or less. Important aspect to remember about the Nano scale (1–100 nm) which is used to describe nanoparticles should not be considered as strictly because of the variations that may exist while the measurement of nanoparticle as well as the appearance of Nano scale properties in particles slightly above or below the Nano scale limits. This may include other important properties such as surface area to mass ratio, shape, and composition to take into consideration [1].

Nanotechnology, has gained significant momentum in past years. Because of the recent advances in the last decade, in material science and Nano-engineering the nanoparticles have gained multiple applications in the fields of medicine (cardiovascular and orthopaedic) and biology. The size of nanostructure materials ranges from 1-100 nm, which explains its unique

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properties and functions due to their "size effect" [2]. Macromolecules and agents such as membranes, viruses and protein complexes are natural nanostructures which are most biologically active [3], it is assumed that they are capable of increasing the interaction with cell membrane and proteins. Their integration in to number of biomedical devices depends upon their size and structure. In medicine, Nanomaterials have been used in specific applications such as site specific drug delivery systems, tissue engineered scaffolds and devices, therapeutics, cancer therapy and clinical bio analytical diagnostics [4-6].

Polymeric Nanoparticles (PNPs):

In recent years, due to synergistic and hybrid properties of polymer–nanoparticle composite, which are derived from several components, they have gained the interest of a number of researchers. These materials have unique thermal, electrical, optical, and mechanical properties, and whether in solution or in bulk [7-10]. Such enhancements are caused by the physical presence of the nanoparticle and by the interaction of the polymer with the particle and the state of dispersion [11- 12]. The most important advantage of nanoparticles, as polymer additives is Loading requirements are quite low compared to traditional additives. Reducing light transmittance and optical clarity due to micronized particles which are used as reinforcing agents scatter light. Structural changes in fluids containing anisotropic species are caused due to shear which blocks copolymer melts and in particle solutions are often encountered in polymer solutions which are in liquid crystalline materials. As we known before about the influence of shear on combined polymer–nanoparticle systems. Here we will focus on some of the most recent results. Polymeric nanoparticles are submicron-sized polymeric colloidal particles in which a therapeutic agent can be encapsulated with in their polymeric matrix or adsorbed or conjugated onto the surface [13]. These nanoparticles act as an excellent vehicle for delivery of a number of bio molecules, genes, drugs, and vaccines to the site of interest in-vivo. During the 1980's and 1990's several drug delivery systems were developed to increase the efficiency of drugs and to reduce toxic side effects [14].

Polymers are macromolecules exhibiting a multiplicity of structures, compositions, and properties which are composed of number of repeating units which are organized in a chain-like molecular architecture. Because of the variety of compositions, structures, and properties of polymers they are being used in nanoparticle systems to produce nanoparticles which are suited for specific biomedical application. Main application of Polymeric nanoparticles is they are used in drug delivery system, although they are also used in bio imaging and bio sensing assays [15]. Polymeric nanoparticles are used in research studies which are related to production polymeric nanoparticles. They are tissue efficient, specific, and most importantly nontoxic. Depending on how the drug will be loaded onto the nanoparticle there are many methods for the preparation of nanoparticles for drug delivery. The resulting nanoparticle-drug compounds may have the structure of capsules, (polymeric nanoparticles), amphiphilic shell (polymeric micelles) [16].

APPLICATION IN CANCER DRUG DELIVERY

SMART NANOMATERIALS:

Site-Targeted Nanomaterials

Ligands:

Attaching targeting ligand to the particle surface can take advantage of the over expression of various receptors on tumour cell surfaces [17, 18]. Coupled with the passive accumulation at tumour sights caused by the EPR effect, targeted particles can increase the interaction time between particles and the tumour cell and increase the likelihood of the particles being taken up by the tumour cells via endocytosis [19]. Targeted delivery takes advantage of differences in the expression of cell surface receptors between healthy and tumour cells. For example, folate receptors are known to be vastly over expressed in several human tumours [20-22]. 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 [23, 24]. PEG is commonly associated with the surfaces of micelle like particles and liposomes to increase particle circulation. By coupling ligand 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 [24-30].

Aptamers:

Aptamers are DNA and RNA sequences that recognize specific target analytes [31]. Aptamers can be selected to bind with high specificity and affinity to a wide range of molecules such as organic dyes, amino acids, antibiotics, peptides, proteins, biological cofactors, and whole cells [32]. 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 [33], which makes them able to target non-immunogenic molecules; the aptamers synthesis process, can be carried out in nonphysiological settings [111]; they are more stable and can be obtained at a lower cost [34]. 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 [35, 36]. However, cancer is a genetic disease, and aptamers provide a way for screening at the molecular level using selective cell binding [37]. Cancer-detecting assays using fluorescent imaging that are currently being developed utilize aptamers conjugated with dye-doped silica nanoparticles. These fluorescent nanoparticles are favoured over direct dye conjugation due to their signal amplification and ability to immobilize biomolecules [38-40]. These particles have often combined with magnetic particles, which allows for convenient separation of bound cells, to make two-part aptamers-based assays [41, 42]. 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 [43, 44]. 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 nonvehicle and aptamers 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. 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 [45-47].

Using fluorescent imaging, this comparison was able to establish that drug vehicles conjugated with the PSMA-targeting aptamers were internalized by cells via receptor-mediated endocytosis [48]. 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 tumour 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-aptamers delivery systems [49]. 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-aptamers conjugates. Polymeric nanocarriers provide the benefit of being able to carry multiple drugs in the same vehicle. This, combined with aptamers 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 [50, 51]. More importantly, this is thought to combat drug resistance, a major problem associated with cancer drug treatment [52]. 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 [53-55]. This can even be used to combine drugs with different water solubility properties, as was accomplished by Zhang et al. using PEG-PLGA. In systems where the aptamers binding initiates endocytosis, such as with A10 RNA aptamers, combinations of drugs and genes that require delivery to intracellular compartments to properly function experience greater benefits [56]. This approach has been used successfully in aptamers-gene conjugates, and is beginning to see promise in aptamers-nanoparticle conjugates [57-58].

Site-triggered Nanomaterial's

pH-responsive Nanomaterial's:

One method to promote drug release at the tumour sight is by taking advantage of the lower pH of the tumour's microenvironment. Mildly acidic conditions exist in tumour and inflammatory tissues (pH 6.8) and in endosomes (pH 5-6) in comparison to the more neutral physiological condition (pH 7.4) [59, 60]. The ability of nanoparticles to accumulate in solid tumours has been shown by the enhanced permeation and retention (EPR) effect [61]. In addition, it has also been demonstrated that nanoparticles can be taken up within cancer cells through a process called endocytosis [62, 63]. 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). 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 tumours as a result of the EPR effect. 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 [64-66]. 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 [67, 68]. Previous work has been done to increase liposome stability by increasing circulation time and by preventing drug leakage until the target is reached [69-71]. Micelle like particles and liposomes with pH sensitivity have shown great promise as delivery vehicles for anticancer drugs, DNA, RNA, proteins, and peptides [72, 73]. 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 pro drug and allows the drug to remain inactive until cleavage from the polymer carrier. When used in the formation of micelles, polymeric pro drugs 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 [74]. In order to prolong drug release, an active substance can be linked to a polymeric molecule via a covalent bond which is naturally hydrolysed *in vivo* [75, 76]. For pH responsiveness of

polymeric pro drug micelles, the linkage between drug and polymer is more readily hydrolysed 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 out fluxed 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. The conjugation of drug to polymer was performed via a hydrazone linkage and ultimately resulted in enhanced DOX accumulation and cytotoxicity within tumour 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 [77]. 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. 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. 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. Acidic environments within the body also occur at tumours, 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. 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 [78-81]. With PE liposomes destabilization occurs by intercalation of amphiphilic molecules that contain a proton table 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 [82-85].

Thermo responsive:

Hyperthermia has been investigated as a method for triggered drug release to targeted areas in thermo responsive liposomes. Here, *in vivo* temperatures are achieved through either older or more general methods, such as warmed baths or perfusates [86], or through more advances and localized methods, requiring ultrasonic and microwave units [87, 88]. Since most mammalian cells begin to show damage at 42°C [89], 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 micro vessel permeability in tumours, which causes more liposomes to accumulate at the intended site [90, 91] while healthy micro vessels are not significantly altered [92]; increased cell permeability, which allows the released drugs to diffuse through the cell walls more easily [93]; and increased sensitivity to thermal injury compared to healthy cells [94]. To take advantage of this triggering mechanism, liposomes must have a liquid-crystalline transition temperature 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 [95, 96]. Temperature is a material property of the liposome polymer and is primarily determined by the length of its fatty acid chains [97]. This allows for the addition of other polymers to the liposome, notably polyethylene glycol (PEG), to increase the retention time and stability [98] and alter the release kinetics [99, 100], without significantly changing its transition temperature. To achieve a desirable Temperature, it is possible to combine polymers with different transition temperatures in ratios that result in one in the hyper thermic range [101]. In order for a thermo sensitive 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 [102, 103]. *In vivo* experimentation has proven promising for these thermo-sensitive devices. The chemotherapy drug carboxyl fluorescein (CF) produced a six fold bioavailability increase in cancerous hamsters when packaged in a thermo sensitive liposome under hyperthermia compared to free CF [104]. Similar Nano vehicles carrying DOX successfully eliminated tumours in six out of nine cancerous mice after 60 days [105]. In a phase I clinical trial, temperature-sensitive liposomes carrying DOX were given to dogs with solid tumours 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 [106, 107]. 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 [108]. This has been achieved through the use of a novel class of hydrophobic lactate-containing polymers, notably poly (*N*-(2-hydroxypropyl) meth acrylamide oligolactates). The change in CP over time is caused by the hydrolysis of the lactate side group: as the polymer degrades and the lactate hydrolyses, the polymer becomes more hydrophilic, causing an increase in the CP. In both polymers, the initial CP is dependent on the length of the lactate chain, and can thus be tailored, though pHPMAm-Lac2 and pHEMAm-Lac2 provide the most convenient CPs of 10°C [109] and 22°C [110], 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 [111], 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 [112]. 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 [113]. 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 tumour accumulation compared to unmodified micelles [114]. This new class of thermo sensitive polymers shows promise for future chemotherapy work.

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. [115, 116]. 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 tumour cells known to overexpress folate receptors has been shown with micelles using folate as the targeting moiety to cause increased endocytosis cellular uptake into the intracellular acidic compartments known as endosomes (pH 5-6) [23]. Drugs conjugated within a micelle by a hydrazone linkage show selective release within the low pH environment of endosomes [137,143,150]. 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 [117]. 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 [118]. 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 [24, 120, and 121]

CONCLUSION

The main goal of this review was to describe the different preparation techniques available for production of polymeric nanoparticles 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 tumours and endosomes, these drug carriers are free to circulate in the body, only releasing their drugs at their intended location. Thermo sensitive 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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