Polymeric Nanoparticles as Carriers for Stimuli-responsive Drug Delivery Systems

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***Abstract* — Targeted drug delivery systems has emerged from the necessity to overcome the flaws of conventional therapeutics, such as poor targeting, lack of specificity, short circulation time, etc. Drug delivery systems are required to be nontoxic and biodegradable to normal tissue cells and, at the same time, toxic and lethal to the tumor cells. The fast release of drugs may lead to undesired results caused by systemic side effects, while a slow rate may reduce the efficiency of the drug at the site of action. Even more, the drug release should take place in a controlled manner upon arrival at the target site.**

Nanoparticles with optimal size and surface characteristics have been designed to increase their time in the bloodstream and, subsequently, to improve their distribution. They are able to selectively carry and deliver drugs to designated sites using unique features of physiology of the tumor and its environment. The use of nanoparticles as drug carriers may be a solution to overcome the drug resistance that limits the activity of therapeutic agents. At the same time, nanoparticles accumulate in cells without being recognized by mediators of multidrug resistance and, consequently, contribute to the increase of intracellular drug concentration.

Detailed information on polymers structure and characteristics are required in order to design polymeric nanoparticles for stimuli-responsive drug carriers. Typical stimuli include pH, temperature, light, redox potential, glucose gradient, magnetic field intensity and concentration of electrolytes. The responses of the drug carriers may be different phenomena such as: dissolution/precipitation, swelling/collapsing, hydrophilic/hydrophobic transition, bond cleavage, degradation, etc. This paper provides an overview of some recent data concerning polymeric nanoparticles used as carriers for stimuli-responsive drug delivery systems and future development directions.

***Index Terms* — drug carriers; polymeric nanoparticles; stimuli-responsive drug delivery systems.**

I. INTRODUCTION

Cancer, one of the diseases with the highest rates of death, may benefit from the recent advances in nanotechnology and targeted therapy. Classic chemotherapeutic agents are distributed nonspecifically in both neoplastic and normal cells, causing limitation of the optimum achievable dose within the tumor and creating local toxicity. Targeted drug delivery systems have emerged as a key approach to overcome the flaws of the conventional therapeutics, such as the low specificity or targeting, short circulation time, etc. [1].

Targeted drug delivery systems operate through two types of action: (1) passive targeting and (2) active targeting. Both of them enhance the intracellular concentration of drugs in cancer cells while avoiding toxicity in normal cells. ***Passive targeting*** leads to the accumulation of the drug delivery carriers in solid tumors at much higher concentrations due to the enhanced permeability and retention effect (EPR effect) [2-4], property most relevant for this type of action, by which certain size molecules (typically liposomes, nanoparticles and macromolecular drugs) tend to accumulate in tumor tissue much more than they do in normal tissues. This is due to the extensive tumor vasculature and ineffective lymphatic drainage system. ***Active targeting*** involves two major strategies: (a) stimuli-responsive drug carriers and (b) receptor recognition vehicles, based on the exterior microenvironment of tumor cells and specific ligand-receptor interactions, respectively [5].

Nanoparticles as drug carriers, polymeric nanoparticles included, can enhance the intracellular concentration of drugs in cancer cells, while avoiding toxicity in normal cells, by using both passive and active targeting strategies [6]. Although nanoparticles offer many advantages as drug carriers, there are still problems to be solved, such as poor oral bioavailability, instability in circulation, inadequate tissue distribution and toxicity [7].

In order to design appropriate drug delivery systems, there are several requirements [8]: high stability as to avoid the fast blood clearance, which will yield in longer intervals in circulation; accumulation in therapeutic dosage at target sites; efficient intracellular drug release at the target site; low toxicity; tolerability. Some of these features are strongly influenced by both physico-chemical characteristics of the encapsulated drug and the encapsulation method.

The design of drug delivery systems for cancer treatment has been focused on three main strategies: passive drug release [2,3], targeted delivery based on receptor recognition [9-12], and triggered release or stimuli-responsive release [13-16].

In this review, some recent data concerning polymeric nanoparticles used as carriers for stimuli-responsive drug delivery systems and future development directions are presented, with focus on types of nanoparticles used for such applications and types of polymers used as nanoparticles for stimuli-responsive drug carriers.

II. NANOPARTICLES AS CARRIERS FOR DRUG DELIVERY SYSTEMS

Ideal drugs, released from specific delivery systems, must reach the targeted tumor cells with minimal losses of their volume or activity in the blood circulation. Once on the target site, drugs should have the ability to selectively kill tumor cells, without affecting normal cells, through a controlled release mechanism of the active form. The aim is to increase the intracellular concentration of drugs and to simultaneously reduce toxicity. Interdisciplinary reports indicated that nanoparticles have the potential to satisfy both of these requirements, due to their nature and properties.

II.1. Characteristics of Nanoparticles. The efficacy of nanoparticles as carriers for drug delivery can be evaluated by their ability to remain in the bloodstream for intervals long enough to enable the drug release prior to their elimination. Conventional surface unmodified nanopar-ticles are, usually, captured by the mononuclear phagocyte system (also known as reticuloendothelial system), depending on their size and surface characteristics [17].

The time spent in the blood stream, drug release, level of toxicity, all these can be controlled by a thorough design of nanoparticles size and surface.

II.1.1. Nanoparticles Size. One of the main advantages of nanoparticles is their tunable size. Nanoparticles used in drug delivery systems should be large enough to prevent their rapid transfer to blood capillaries, but small enough to avoid macrophages that are lodged in the reticuloendo-thelial system, such as the liver and spleen. Since physiological dimensional limits are 150-200 nm [18] and 100-600 nm [19], by consequence, the nanoparticles size should be up to 100 nm in order to reach tumor cells.

II.1.2. Surface characteristics. The surface characteris-tics of nanoparticles are an important determining factor, influencing their life span in circulation and behaviour towards phagocites. In order to avoid macrophages capture, nanoparticles should have a hydrophilic surface [20], which can be achieved either by coating the nanoparticles surface with a hydrophilic polymer (such as polyethyleneglicol, PEG), able to protect them from opsonization by repelling plasma proteins, or by preparing nanoparticles from block copolymers with hydrophilic/hydrophobic domains [21,22].

II.2. Types of Nanoparticles Used as Carriers. Nanoparticles used as carriers for drug delivery systems have size of 3-200 nm and are obtained starting from various materials: polymers (polymeric nanoparticles, micelles, or dendrimers), lipid-based systems (liposomes), self assembled proteine/peptide cages (viral nanoparticles) and even materials with special properties (such as carbon nanotubes) [7].

Polymer-based Nanoparticles. Depending on the compatibility between macromolecular carrier and drug and on nanoparticles method of preparation, drugs can be either physically entrapped in or covalently bound to the polymer substrate [23]. The resulting compounds may be capsules (polymeric nanoparticles), amphiphilic core/shell structures (polymeric micelles) or hyperbranched macromolecules (dendrimers) (Fig. 1).

Polymers used as drug conjugates may be natural or synthetic. Polymer-drug conjugates based on natural polymers, such as albumin, chitosan, heparin, have been used for delivery of oligonucleotides, DNA, proteins, as well as drugs.

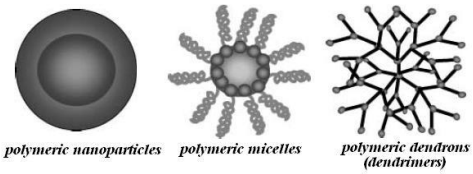


Fig. 1 Main types of polymer-based carriers

Recently, a nanoparticles formulation of paclitaxel with serum albumin has been reported [24] and subsequently evaluated in clinical trials, including non-small-cell lung cancer (phase II trial) and advanced non-hematologic malignancies (phase I and pharmacokinetics trials) [25,26].

As for synthetic polymers, there are many formulations responding to these application requirements. N-(2-hydroxypropyl)-methacrylamide (HPMA), polystyrene-maleic anhydride copolymer, polyethylene glycol (PEG), and poly-L-glutamic acid (PGA) are only a few of the most used (co)polymers. PGA, the first biodegradable polymer to be applied for conjugates synthesis, and its conjugates have been obtained and tested with promising results [27]. Among them, PGA-paclitaxel [28] and PGA-camptothecin [29] are now in trials. HPMA and PEG are the most widely used non-biodegradable synthetic polymers [30]. A conjugate of HPMA with doxorubicin was the synthetic polymer-drug conjugate reported to be evaluated in clinical trials as an anticancer agent [31].

Polymeric micelles are, basically, amphiphilic block copolymers and their properties enable them to assemble in aqueous media to form a nanosized core/shell structure. Their hydrophobic core is a reservoir for hydrophobic drugs, whereas the hydrophilic shell stabilizes the hydrophobic core and endows polymers water solubility. This behaviour makes the particle fit for intravenous administration [21]. Drugs can be loaded into polymeric micelles by two methods: physical encapsulation [32] and chemical attachment [33]. The first polymeric micelle formulation of paclitaxel was synthesized from PEG-poly(D,L-lactide)-paclitaxel and tested in patients with advanced refractory tumors [34]. Multifunctional polymeric micelles containing targeting ligands, as well as imaging and therapeutic agents, are being actively developed [35] and tested. Such complex structures will become the mainstream in terms of micelle model formulations in the near future.

Dendrimers are synthetic macromolecules of nanometer dimensions, composed of multiple highly branched monomers that emerge radially from the core. Their properties, such as monodisperse size, modifiable surface functionality, multivalency, water solubility, available internal cavity, make them highly attractive for controlled/targeted drug delivery systems [36]. Polyamido-amine dendrimer, most widely used as a scaffold, was conjugated with cisplatin [37]. The easy-to-alter surface of dendrimers is the feature that enables them to be simultaneously conjugated with several different molecules, such as imaging contrast agents, targeting ligands, therapeutic drugs, yielding in a dendrimer-based multifunctional drug delivery system [36].

A special mention has to be made concerning the carbon nanotubes (CNTs), members of fullerenes family. They are carbon-based cylinders composed of benzene rings (Fig. 2) and have been used in biology as sensors for protein and DNA detection, devices for the discrimination of different proteins, carriers for vaccines or protein delivery [38].

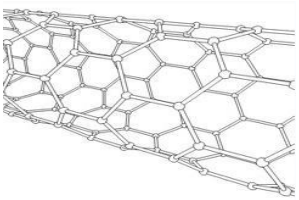


Fig. 2. CNTs structure

CNTs are completely insoluble in all solvents, generating some health issues connected to their tissue accumulation and associated toxicity. Despite these problems, chemical functionalization of CNTs may offer a solution in terms of granting CNTs water solubility and making them able to react with organic reagents so they can link a wide variety of active molecules (such as: peptides, proteins, nucleic acids, drugs) [39].

Antifungal agents (amphotericin B) or anticancer drugs (methotrexate) have been covalently linked to CNTs with a fluorescent agent and proved to be more effective into cells compared with free drugs alone and to have potent antifungal activity [40,41]. The multiple covalent functionalization of the sidewall or tips of CNTs allows them to simultaneously carry several different molecules and this strategy provides a fundamental advantage in the treatment of cancer.

III. STIMULI-RESPONSIVE POLYMER-BASED DRUG DELIVERY SYSTEMS

Active targeting concerns, basically, the attachment of antibodies, peptides or high affinity ligands to the surface of nanocarriers in order to increase their concentration in tumors environment or even in tumor cells. Active targeting may be obtained through two mechanisms: stimuli-responsive delivery and ligand targeting delivery. A combination of these two strategies might be considered an illustration of the Paul Ehrlich's "magic bullet" concept for diseases treatment [42,43]. Generally, there are three destinations used as target sites for anti-cancer drugs delivery: tumor vascular infrastructure, extracellular environment inside the tumor and tumor cells.

Drug delivery systems based on polymers and polymeric materials are required to be biodegradable and non-toxic toward normal tissue cells, but decisively harmful to tumor cells. At the same time, the fast release of the drug may lead to systemic side effects, while a slow rate of discharge may reduce the drug efficiency at the site of action. For medium and long term, this lack of control may induce multiple-drug resistance (MDR). Therefore, controlled drug release is a key factor to be considered in the design of polymer-based carriers, as it affects the drug bioavailability and antitumor activity. Typical stimuli currently under consideration are the pH, temperature, light, redox potential, glucose concentration, magnetic or electric field, concentration of electrolytes. Polymer-based drug carriers may respond to such stimuli through various phenomena: dissolution/precipitation, swelling/collapsing, hydrophilic/hydrophobic transition, bond cleavage, degradation [44]. Such carriers that readily respond to internal stimuli (such as pH, temperature, redox potential) attract special attention as they are more interesting from the clinic point of view.

III.1. pH-Responsive Drug Delivery Systems. The pH profile of tissues having certain pathology, such as inflammation, infection, carcinoma, is significantly different from that normal tissues (Table I) [45, 46].

TABLE I. pH VALUES FOR SPECIFIC CELLS AND TISSUES

Cell/tissue	**pH value**
Blood	7.35-7.45
Stomach	1.0-3.0
Colon	7.0-7.5
Early endosome	6.0-6.5
Late endosome	5.0-6.0
Lysosome	4.5-5.0
Golgi apparatus	6.4
Tumor and tumor extracellular	7.2-6.5

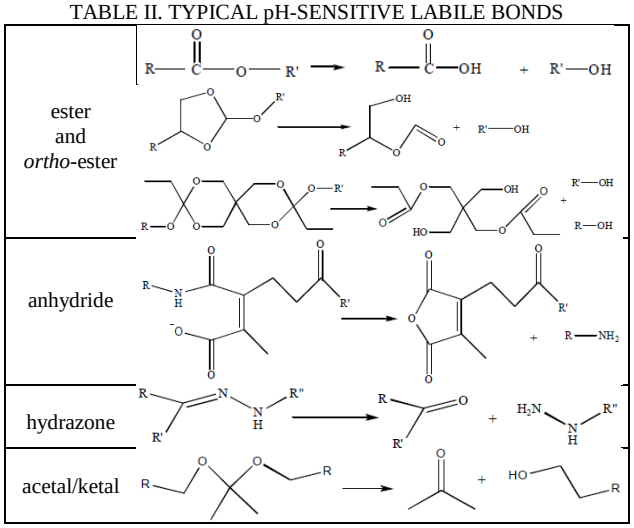
In general, the surrounding environment of tumor cells tends to display an enhanced acidity (pH=6.5) [47] compared to normal tissue cells (pH=7.4) [48]. Even more, according to literature data, the drug could be released in early or secondary endosomes by (1) pH-controlled hydrolysis, due to the fact that the pH drops from physiological 7.4 to endosomes (pH=5÷6) or to lysosomes (pH=4÷5), or, specifically, by (2) enzymolysis inside lysosomes [49].

Therefore, researchers have devoted considerable efforts to design polymer-based pH-responsive drug delivery systems able to respond in a controlled manner to pH variations and to specifically release the drug to targeted intracellular or extracellular sites. Ionisable polymers with pKa=3÷10 are good candidates for pH-responsive systems [50]. The pH-sensitive polymers have, usually, reactive groups able to be submitted to titration and corresponding typical polymers are polyacids and polybases. Polyacids generally have pendant weak acidic groups, (*e. g.*, carboxylic acids, sulfonic acids), while polybases have pendant weak basic groups (such as primary, secondary, or tertiary amine groups, like poly(*β*-amino ester), etc.) [51]. pH-sensitivity leads to a conformational change for the soluble polymers and a

change in the swelling behaviour of the hydrogels when these ionisable groups are linked to the polymer structure. There are mainly two different mechanisms: (a) pH-dependent hydrophobic-to-hydrophilic transitions and (b) labile bonds collapse upon pH-variation. The mechanism (a) evolves through the ionization (protonation or deprotonation) of the pendant groups upon pH change, which alters molecules character from soluble to insoluble (or vice-versa). In the case (b), the mechanism is based on the break of the weak bonds, such as ester, anhydride, hydrazone, acetal/ketal (Table II) [5].

Classical monomers are acrylic acid (AAc), methacrylic acid (MAAc), maleic anhydride (MA), N,N-dimethyl-aminoethyl methacrylate (DMAEMA), but polymers containing phosphoric acid derivatives have been also reported [52,53]. The pH-responsive swelling and collapsing behavior has been used to induce controlled elease of model compounds like caffeine [54], drugs like indomethacin [55], or cationic proteins like lysozome [56].

TABLE II. TYPICAL pH-SENSITIVE LABILE BONDS



The poly(amido-amine)s [57] are different since they combine positive and negative charges within the polymer backbone. A weak acid sulfonamide was investigated as trigger for extracellular delivery of doxorubicin [58]. Poly(L-histidine)-b-PEG in combination with PLLA-b-PEG and adriamycin as drug was also studied for an extracellular tumour targeting [59]. Most prominent acid-labile linkers, which have been used in a pH-triggered release mechanism, are cis-aconityl acid, Schiff's base derivatives [60]. For example, adriamycin has been conjugated to IgM *via* a cis-aconityl linker [61].

Extensive studies regarding pH-sensitive liposomes [62], nanoparticles [63], capsules and hydro/nano-gels [64], micelles [65], and dendrimers [66] used as polymeric carriers for pH-controlled drug delivery systems were reported.

Cationic polymers are also used in ***non-viral gene therapy*** [67]. The polycations can bind nucleotides by complexation through electrostatic interactions. The pH-responsive character of the polymer is important when the pH drops during cellular uptake as the polymer becomes more and more charged and triggers osmotic, endosomolytic or other events subsequently. Various amine-based polymers are currently under investigation (Fig. 3). The most important feature is the transfection (process of deliberately introducing nucleic acids into cells) efficiency, which is still below than that of viral vectors. In addition, the studied polycations are still too toxic.

Therefore, the research is still focused on appropriate synthetic vectors with high transfection efficiency, whilst having a tolerably low toxicity. Poly(ethylene imine) (PEI) is the standard for new polymers [68,69], even though a large number of investigated polymers perform better in terms of cytotoxicity and transfection efficiency. Some other candidates are PAMAM and dendrimers [70–73], poly(N,N-dimethylaminoethyl methacrylate) [74,75], poly(amido-amine)s [76,77], poly(L-lysine) [78], modified chitosan [79].

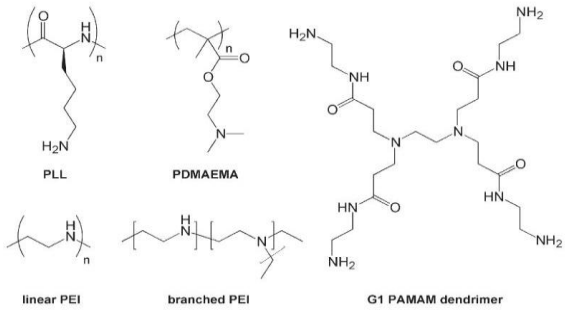


Fig. 3. Amine-based polymers

III.2. Thermo-Responsive Drug Delivery Systems. Hyperthermia has been extensively applied to various types of solid tumors, as an alternate choice to radio- or chemotherapy [5], due to the fact that tumor cells seem to be more sensitive to heat than normal cells. Therefore, temperature has been widely investigated as a criterion for stimuli responsive drug delivery systems [80]. Polymers with lower critical solution temperature (LCST), as well as upper critical solution temperature (UCST), have been considered. The significant changes in the hydration state finally lead to the volume phase transition due to the coil-to-particle transition. Thermodynamically, these transitions are governed by entropic effects (due to the release of water molecules in the vicinity of the polymer and the dissolution process itself) and enthalpy effects (due to intra- and intermolecular forces and solvation, *e. g.*,, hydrogen bonding and hydrophobic interactions) [80].

The literature is not abundant in data on thermo-responsive polymers or copolymers synthesized for controlled drug release in cancer therapy [81-84]. Poly (*N*-isopropyl acrylamide) (PNIPAAm), the polymer most extensively investigated, is based on poly (*N*-alkylacrylamide) and exhibits a LCST of approximately 33°C in aqueous solution (below 33°C is water soluble, but above it becomes insoluble) [85]. Two categories of PNIPAAm-based block copolymers are currently applied for thermo-responsive drug delivery systems: (1) PNIPAAm as hydrophilic shell-forming segments below the LCST (increasing the temperature slightly above the LCST, the system reacts towards an accelerated drug

release) [86]; (2) PNIPAAm as hydrophobic core-forming segments above the LCST (lowering the temperature slightly below the LCST, the same effect of accelerated drug delivery at the targeted site is obtained) [87]. Other studies have been focused on the increase of LCST of the PNIPAAm-based systems in order to adapt to the normal body temperature (37°C): cholesteryl end-capped thermo-sensitive amphiphilic polymers [88], elastin-like poly-peptides [89], temperature-sensitive liposomes with doxo-rubicin *via* incorporation of poly[2-(2-ethoxy) ethoxyethyl vinyl ether] [90].

Polymers with UCST, though not yet reported [5], could also be extremely interesting and promising for tumor-specific intracellular drug delivery systems. The main disadvantage of thermo-responsive polymeric drug nanocarriers is their inherent inability to treat metastatic cancer.

III.3. Redox-Responsive Drug Delivery Systems. Polymeric drug delivery systems containing redox-labile linkages are an attractive option to consider when it comes to intracellular factors as trigger for cytoplasmic degradation of polymer carriers [5]. There is a high redox potential difference (about 100-1000 fold) between the reducing intracellular space and oxidizing extracellular environment. Redox-responsive nanocarriers rely on the higher intracellular reduction capacity compared to the extracellular medium [91]. Disulfide linkages, unstable in a reductive environment as the disulfide bond is readily cleaved, are most investigated for biomedical applications as redox-responsive drug delivery systems [92]. Disulfide bonds tend to be reduced under a low reducing potential due to an excess of reduced glutathione (GSH) inside the cell, and subsequently release the drug. Oxidation-responsive polymersomes with an intervening disulfide bond [93,94], drug loaded disulfide-linked micelles [95] that can undergo destructuration under glutathione effect, resulting in rapid drug release, redox responsive micelles composed of diselenide block copolymers [96], quite stable under ambient conditions, have been reported.

IV. CONCLUSION

Expansion of concepts of stimuli-responsiveness is the next step the research has to take [97]. Double-responsive systems, some of which assembled into micelles, have been already reported. Double- or multi-responsive systems can be distinguished based, generally, on the polymer architecture. Random copolymers may be used to tailor the transition point depending on two independent parameters, *e.g.* pH and temperature. In contrast, block-copolymers tend to self-assemble reversibly and form micelles depending on the environmental conditions. Other directions are stimuli-responsive polymer–protein conjugates, hydrogels, etc.

Similar to the ongoing trend towards site-specific protein conjugation, scientists aim for monodisperse systems with all reaction sites known. Besides the better control over the purity of products, this may lead to more reliable structure–property relationships and potentially increases the patient's safety. Synthetic strategies, like dendrimer synthesis and controlled polymerisation techniques, are now quite well established and ready-to-employ for achieving these goals.

All these will allow researchers to design tailor-made polymer-based drug delivery systems with superior pharmacokinetics, while having all safety questions addressed.

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