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Applications of Nanosystems to Anticancer Drug Therapy (Part I. Nanogels, Nanospheres, Nanocapsules)

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Abstract: One of the greatest challenges in cancer drug therapy is to maximize the effectiveness of the active agent while reducing its systemic adverse effects. To add more, many widely-used chemoterapeutic agents present unfavorable physicochemical properties (e.g. low solubility, lack of chemical or biological stability) that hamper or limit their therapeutic applications. All these issues may be overcome by designing adequate drug delivery systems; nanocarriers are particularly suitable for this purpose. Nanosystems can be used for targeted-drug release, treatment, diagnostic imaging and therapy monitoring. They allow the formulation of drug delivery systems with user-defined characteristics regarding solubility, biodegradability, particle size, release kinetics and active targeting, among others. This review (Part I) focuses on recent patents published between 2008 and the present day, related to nanospheres, nanocapsules and nanogels applied to anticancer drug therapy. Other nanosystems is covered in a second article (Part II).

Keywords: Anticancer drug therapy, drug delivery, hydrogels, nanocapsules, nanogels, nanoshells, nanospheres, patents.

1. INTRODUCTION

Despite the search of novel anticancer drugs is a very active, burgeoning research area which has provided many promising results in the last decade [1, 2], the biopharmaceutical and pharmacokinetical aspects of current and future anticancer drug therapies are particularly critical aspects to achieve favorable clinical outcome in the field of oncology. In other words, the development of novel chemotherapies should be complemented with innovative drug delivery devices.

Many commonly used chemotherapeutic agents such as the *Vinca* alkaloids, the anthracyclines, the epipodophyllotoxins, taxol and actinomycin D are substrates of ABC transporters involved in multi-drug resistance issues [3-5]. Several antineoplastic agents such as 5-fluorouracil, camptothecines, gemcitabine and curcumin are very rapidly metabolized or inactivated in the physiological environment [6-9]. This is not surprising: a number of anticancer agents are of lipophilic nature and plenty of them present aromatic rings or planar moieties in their molecular structures, features that have long been recognized as determinants for biotransformation by members of Cytochrome p450 superfamiliy [10, 11], among other metabolic enzymes. Last, but not least, the important side-effects of anticancer drug therapies might be greatly alleviated by reducing systemic free drug concentrations and assuring selective distribution to the specific site of action, either through passive or active targeting.

In relation to novel biopolymer-based treatments (e.g. gene, protein and polysaccharide therapies) [12-15], selective delivery to the malignant cells, solving absorption issues and preserving the intact macromolecule until delivery to the site of action (which implies avoiding enzymatic cleavage, i.e. DNAases, ribonucleases, proteases and glycosidases) are also crucial for the desired clinical outcome.

Nanoparticulated systems show promise in all fields of pharmacology but particularly as delivery vectors for anticancer treatment: their subcellular size allows relatively higher extravasation; they can be incorporated by active intracellular uptake; they can improve the stability of active ingredients within the organism and they are biocompatible when synthesized from biodegradable materials [16]. What is more, they can be designed for either passive or active, smart targeting of cancerous cells or tumors by biotechnological or physicochemical means, such as surface functionalization with appropriate biomolecules or pH-, magnetic-, temperature- or photo-sensitive vectors [17-22].

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In this article (Part I), we have focused on patents related to polymer-based organic nanospheres (NS), polymer-based organic nanocapsules (NC) and nanogels, published between 2008 and the present day. We have excluded from the analysis those inventions related to general drug delivery systems which are not specifically directed to cancer treatment, or which do not at least present particular examples/embodiments concerning cancer therapy, or which are not particularly valuable to cancer therapy applications from the authors' perspective. Other nanosystems (namely lipid-based nanocarriers and dendrimers) will be covered in a second article (Part II), currently in preparation.

2. HYDROGEL-BASED NANOSYSTEMS

Hydrogels are hydrophilic, three-dimensional, polymeric networks composed of either homopolymers or copolymers, which can entrap large amounts of water or biological fluids [23]. The hydrophilic polymer components are cross-linked into a network by either covalent (chemical cross-linking) or non covalent (physical cross-linking) interactions. The crosslinking provides dimensional stability which is critical to maintain the network structure of the hydrogels and to prevent dissolution of the hydrophilic chains [24] while the high solvent content gives rise to the fluid-like transport properties.

Hydrogel nanoparticles (HNP) or nanogels™ are colloidal stable particles with a size range between 100 nm and 1 μm [25] made from hydrogels with nanosized hydrophilic polymeric networks, being similar to nanoparticles (NP) after lyophilization [26]. These nanoscale particulate materials are self-assembled and have attracted an increasing interest due to their potential biopharmaceutical applications [27], since they would exhibit simultaneously the features of both hydrogels and NP.

Nanoscale hydrogels can be synthesized in the absence of the drug, and then loaded with it through self-assembly mechanisms involving non-covalent interactions between the drug and the polymer matrix, which results in relatively high drug loading capacity [28], along with the classical hydrogel advantages such as hydrophilicity, flexibility, versatility, high water absorptivity and biocompatibility. The formation of self-assembled NP is theorized by a free-energy minimized structure, sharing a common feature of self-assembly with polymeric micelles [29], but with a major advantage: while polymeric micelles possess only one hydrophobic internal core with a hydrophilic shell [30-33], the interior of HNP consists of dispersed multiple hydrophobic island domains in a hydrophilic sea domain due to the random association of hydrophobic moieties conjugated to soluble macromolecules.

In general, hydrogels can be classified according to several different criteria [34], such as the nature of side groups (neutral or ionic), mechanical and structural features, method of preparation (homo- or co-polymer), physical structure (amorphous, semicrystalline, hydrogen bonded, supermolecular and hydrocolloidal) and responsiveness to physiologic environment stimuli (pH, ionic strength, temperature, etc). The most commonly used classification of hydrogels is, however, based on the nature of the polymers used for their preparation, which are from natural, synthetic or semi-synthetic origin. Natural and synthetic polymers are the most frequently used in the pharmaceutical and biomedical fields [35].

Within the natural polymers category alginic acid, starch and chitosan hydrogels can be highlighted [36-38]. The well-defined structure of synthetic polymers is their main advantage, and lots of polymers belong to this category, including polyvinyl alcohol- (PVA), polyethylene oxide- (PEO), polyethyleneimine- (PEI), polyvinyl pyrrolidone- (PVP), poly-*N*-isopropylacrylamide- (PNIPAM) based hydrogels [39-43]. We can also include in this category the poloxamer-based systems (also known by the trade name Pluronic™), a non-ionic copolymer formed by ethylene oxide (EO) and propylene oxide (PO) blocks arranged in a triblock structure: EO_x–PO_y-EO_x, which appears to be very valuable for gene delivery applications [44, 45].

One of the main application areas of HNP is in controlled drug delivery. Chemotherapeutic agents with strong antitumor activity are often also severely toxic to rapidly proliferating normal cells. Therefore, the controlled distribution of drugs in the body will enhance their selective action as pharmaceuticals [46, 47], and targeted drug delivery systems offer a solution to this problem. By attaching receptor-specific molecules to the HNP surface, one can achieve selective targeting ability designed to reach specific tumor cells. Other special functions such as crossing the blood-brain barrier (BBB) and more sophisticated controlled release patterns may also be achieved [48].

A diversity of drugs can be entrapped into nanogels, for example, low-molecular weight chemotherapeutic drugs, cytotoxic nucleoside analogs or small interfering ribonucleic acid (siRNA) [49-51]. Because macromolecular drugs such as peptides and proteins need to be located in a hydrophilic environment to maintain their conformation and thus their activity, the particular hydrophilic property of nanogels would be of benefit. In addition to stimulus-triggered transitions, hydrogels loaded with a drug can interact with biological components or events (e.g. enzymatic processes) that would trigger the release of the drug in physiological conditions.

2.1. Recent Developments in Hydrogel-based Nanosystems

Many new developments of hydrogel polymers are continuously arising. The patents presented below were selected as examples of the latest advances regarding new materials and new preparation methods of hydrogel-based nanosystems. We also present patents that account for a breakthrough in terms of the drug release profile of the nanogel and/or the mechanism by which they are selectively targeted to the site of action.

The 2011 patent of Muratoglu *et al.* [52] provides methods for obtaining covalently cross-linked PVA hydrogels with the ability to achieve a pre-designed drug delivery rate. These gels are prepared by exposing a physically associated vinyl polymer hydrogel to ionizing radiation in sufficient dose to form covalent crosslinks. The physical properties of the produced hydrogels can be adjusted by varying controlled parameters such as the proportion of physical associa-

tions, the concentration of polymer and the amount of radiation applied. The resulting hydrogel has many advantageous physical properties mentioned in the original work; a very interesting one is the possibility of generating a gradient in the pore size of the gel since the drug release rate often depends on the pore size of the pharmaceutical vehicle. In tumor treatment, the drug release pattern is of essential importance, because it can help maintaining tumor tissue drug concentration at a stable, effective level for a long time [53]. Typically a zero-order or constant drug release rate is desired. Muratoglu *et al.* hydrogels can provide the ability to modulate not only pore size but gradients in pore size.

In another 2011 patent [54], Kazakov *et al.* describe an original method for the preparation of poly(acrylamide), poly(*N*-isopropylacrylamide), and poly(*N*-isopropylacrylamide-co-1-vinylimidazole) hydrogel particles: obtention inside liposomes. The proposed method is schematically represented in Fig. (**1**) and it includes encapsulating hydrogel-forming components into liposomes, diluting the large unilamellar liposomes suspension to prevent polymerization outside the liposomes, and polymerizing the encapsulated hydrogel-forming components. After that, the lipid bilayer may be solubilized with detergent and the phospholipid and tensioactive molecules and their micelles may be removed by dialysis. The resulting nanogels may then be dried by evaporation, resulting in hydrogel particles with a diameter from 30 to 300 nm.

An interesting patent application of 2012 by Sinko *et al.* [55] describes a lung-targeting delivery system for the treatment of non-small cell lung cancer (NSCLC). These cells are treated with gel micro-particles (GMP) that accumulate in the lungs and contain NP conjugated to one or more therapeutic agents that are cytotoxic to the NSCLC (e.g. camptothecin -CPT-, paclitaxel) with one or more chemopotentiators (e.g. alpha-lipoic acid -ALA- or its analogues). Two levels of targeting are proposed for the delivery system. The first level is passive targeting, with GMP selectively accumulating in the lungs after IV administration, and the second level is active targeting. Two types of NP are used to achieve active targeting: drug loaded NP with their surface functionalized with ligands that selectively target cancer cells and a second NP group also functionalized with cell surface ligands. These second NP group are engineered to tightly bind to cancer cell surface receptors and remain there in order to inhibit the metastatic signaling cascade. Once GMP passively accumulate in the lung, NP imbedded in the GMP matrix diffuse out and seek cancer cells. The authors declared that while the passive targeting achieves a 10-fold increase in anti-cancer drug potency in the lung and 10-fold lower peak systemic drug concentrations, the dual targeting approach results in a remarkably specificity of treatment and an additional 10-fold reduction in effective drug concentrations. The methods by which the loaded NP may be prepared can be found in a previous patent application of the authors [56].

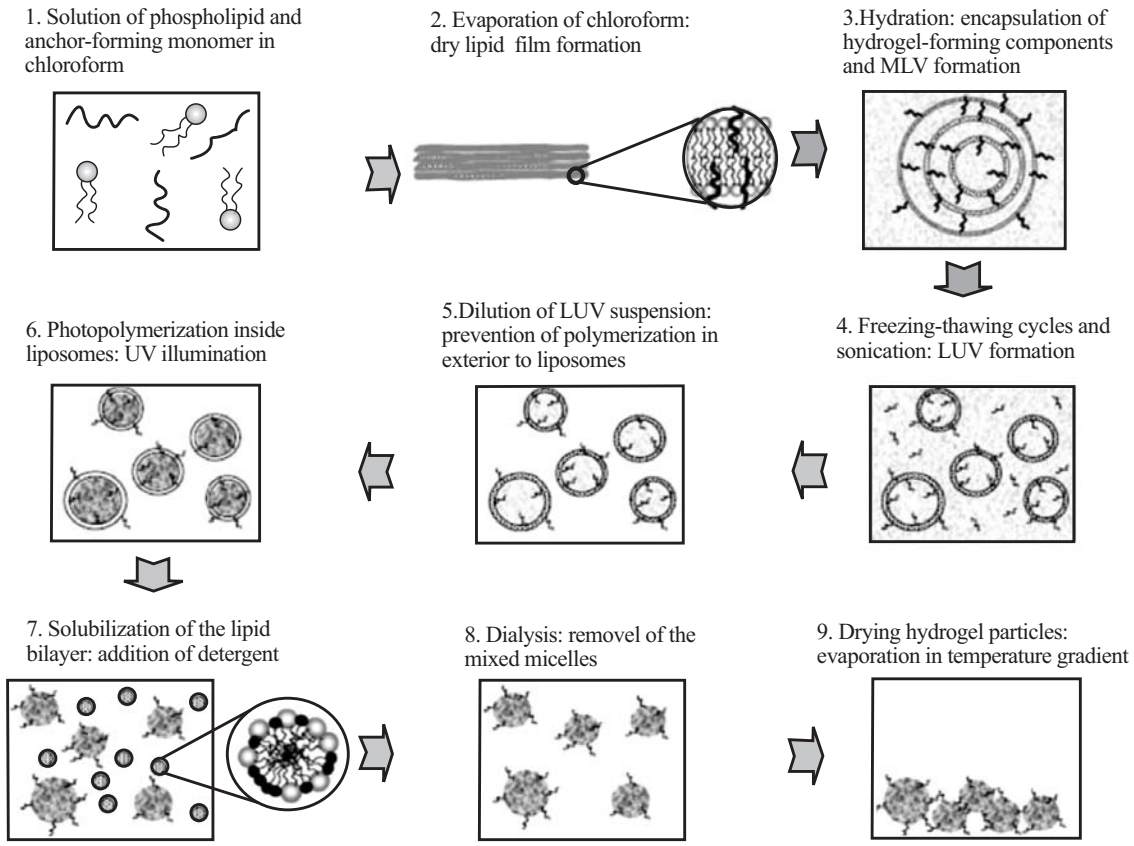

Fig. (1). Scheme of the steps of hydrogel nanoparticle (HNP) preparation. Reprinted with permission from Kazakov *et al.* Poly(N-isopropylacrylamide-co-1-vinylimidazole) HNP prepared and hydrophobically modified in liposome reactors: atomic force microscopy and dynamic light scattering study. Langmuir 19, 8086. Copyright 2003 American Chemical Society.

On the other hand, another patent application of the group discloses the method by which the dual level targeting system may be obtained [57]. Hydrophobic drugs such as CPT can be loaded into NP and their release controlled using Flash Nanoprecipitation (FNP), and the NP could include polyethylene glycol (PEG) protective coatings with the PEG's ends functionalized for targeting [58]. As an example of the system performance, the authors included the results of an *in vivo* study with rats receiving a single bolus IV injection of PEGylated polystyrene particles with a prodrug of CPT. The results showed that the passive pulmonary targeting of CPT resulted in low systemic CPT exposure (equivalent to CPT blood levels after 7 elimination half-lives), a significant reduction in cancerous areas in the lung, and allowed for a 10-fold lower dose as compared to IV administration of free CPT [59, 60].

The work of Sinko *et al.* is relevant for many reasons: (1) unlike current treatment approaches, the authors propose chemotherapy for earlier stage disease specifically to minimize the probability of metastatic spread (i.e. keeping and treating the disease locally in the lung), (2) the dual targeting approach is a novel method to achieve effective lung drug concentrations while minimizing systemic exposure, and (3) embedding NP into GMP using microfluidics has not been previously reported.

The patent application of Bloembergen *et al.* presented in 2012 [61] introduces a delivery device including a nanoparticle that was originally developed as industrial latex. The NP production is described in a previous patent [62] and involves a biopolymer, such a starch, combined with a plasticizer. The combination is mixed under high shear forces, preferably in a twin screw fully intermeshing co-rotating extruder, to plasticize the biopolymer and create a thermoplastic melt phase in which the crystalline structure of the biopolymer is removed. A cross-linking agent is then added, while mixing continues, to form cross-linked NP. The NP exit the extruder as a strand, which is ground to a fine dry powder. The starch-based NP are present in the powder in an agglomerated and non-water soluble form, but they can be dispersed in an aqueous medium to produce a stable latex dispersion of cross-linked HNP. One useful attribute of these biocompatible NP is that they can be broken down by chemical and enzymatic elements, but they persist in the body long enough to give a sustained drug release. While native starch particles would survive for less than 30 minutes in the body, the cross-linked starch NP has a considerably longer half- life.

The drug delivery device described in the work of Bloembergen involves the above described NP conjugated to an active agent such as a chemotherapeutic drug, with an average particle size between 50-150nm. The cross-linked polymers are further conjugated to a targeting molecule such as an aptamer, which targets the NP for delivery of the active agent to cancer cells. The authors presented the results of the encapsulation of doxorubicin (Dox) in the NP and the biphasic release profile observed, with suitable release kinetics spanning multiple hours of sustained release of the active agent. The estimate time for complete release is 24 hours. In an animal study also presented in the application, Dox loaded NS were used to treat glioblastoma multiforme, a primary brain tumor in athymic mice. The results demonstrated a 30% increase in survival for the mice treated with Dox-loaded NS relative to the appropriate controls. Aptamers are capable of binding to a target molecule that is located in a specific site which may include cancer cells. For example, AS141 1 has been shown to bind to nucleolin [63]. Binding to nucleolin receptors is useful in the treatment of a wide array of cancers such as renal cell carcinoma, breast cancer, prostate cancer and others. AS141 1 may also be tagged with, for example, a Cy3 fluorescent tag for imaging purposes. Another potentially useful aptamer is sgc4, which was developed by way of the *Systemic Evolution of Ligands by Exponential Enrichment (SELEX)* process from T-cell leukemia cell lines and is able to recognize leukemia cells [64].

2.2. Nanosystems Based in Stimulus-responsive Hydrogels

Some of the main applications of HNP arise from their stimulus-responsive nature, that is, their ability to undergo reversible volume phase transitions in response to environmental stimuli such as pH, temperature, ionic strength, quality of solvent, and/or the action of the external electromagnetic field [65-67]. This behavior is governed by the balance between repulsive and attractive forces acting in the particles: swelling occurs when ionic repulsion and osmotic forces exceed attractive forces, such as hydrogen bonding, hydrophobic interactions and van der Waals interactions, among others [68].

The patent application of Matyjaszewski *et al.* [69] proposes the preparation of a functional gel material formed from a dual cross-linked polymeric network including a fraction of stable cross-links and a second fraction of cleavable cross-links. Active ingredients may be chemically or physically encapsulated within and/or released from the gel particle by selective cleavage of the cleavable cross-links, and they may be delivered and released to a pre-selected target site. Peripheral or other accessible functionality on the surface of the gel particle allows attachment of a surface reactive agent, thereby modifying surface properties of the gel particle.

The interesting characteristic about the double-crosslinking system is that it can predictably open and close the polymeric network upon exposure to different redox environments, and because of the addition of an excess of permanent cross-linking agent, the gel particle retains a gel structure even after opening the reversible cross-links. Therefore, after releasing the active ingredient at the target site, the more stable cross-link network can slowly degrade. The molecular fragments of the gel particle formed after complete degradation have a molecular weight lower than the renal threshold allowing their elimination from the body. The authors prepared several microgels with particles size of around 200 nm, all with the property of increasing their particle size in the presence of a reducing agent (such as tributhylphosphine or glutathione) that broke the cleavable links, demonstrating that the systems can be "opened" by exposing the particle to a reducing atmosphere.

Among those microgels, stable biodegradable nanogels cross-linked solely with disulfide linkages, which possess useful features as targeting drug delivery scaffolds for bio-

medical applications, were prepared. They have a uniformly cross-linked network which can improve control over the release of encapsulated agents, and they are biodegraded into water-soluble polymers in the presence of a biocompatible glutathione tripeptide commonly found within cells. This biodegradation process triggers the release of encapsulated molecules, exemplified in the original work by Rhodamine 6G, a fluorescent dye, and Dox. The patent application includes results obtained from optical fluorescence microscope images and cytotoxicity assays in HeLa cancer cells, which suggested that the released Dox molecules could penetrate cell membranes to suppress the growth of cancer cells. Further experiments with glutathione in the reaction media showed that the Dox-loaded nanogels are essentially non-toxic before addition of the reducing agent (see Fig. (**2**), 48 h mark), but after the reducing agent is added, the drug is released, and the cell growth is significantly inhibited due to the contact with the drug (96 h in Fig. (**2**)).

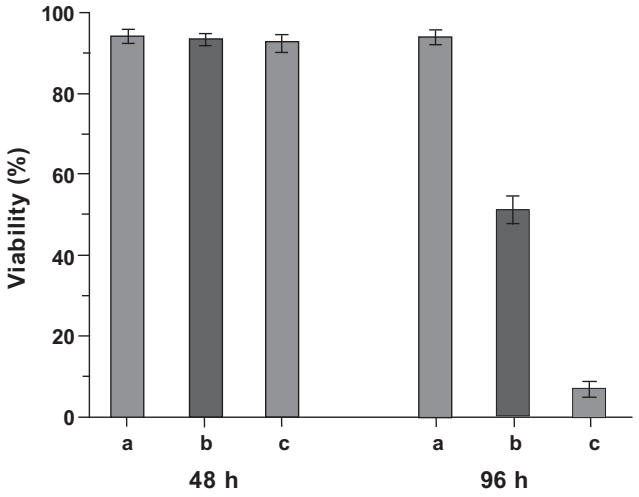

Fig. (2). Viability of HeLa cells in the presence of Dox-loaded nanogels before and after the addition of glutathione to release Dox from Dox-loaded nanogels. Glutathione (**b**) and Dox (**c**) were added after 48 h incubation. Control (**a**), Dox-loaded nanogels (**b**), and Dox (**c**). Adapted with permission from Oh *et al.* Biodegradable nanogels prepared by atom transfer radical polymerization as potential drug delivery carriers: synthesis, biodegradation, *in vitro* release, and bioconjugation. J Am Chem Soc 129, 5939. Copyright 2007 American Chemical Society.

The hydrogel-based nanosystem developed by Matyjaszewski *et al.* may also be targeted by its conjugation with biotin. The data presented show that each nanogel particle may bond 142,000 biotin molecules, which are promising results, since previous works have shown that the biotin-conjugated NP could improve the selective delivery of drugs into cancer cells via interactions with overexpressed biotin receptors on the cells surfaces [70].

Another stimulus-responsive, site-targeted hydrogel applicable to drug delivery is presented in a 2012 patent application by Chirwa *et al.* [71], in which a pharmaceutical system for intraperitoneal delivery of an anti-neoplastic agent is provided for treating cancers associated with aberrant mucin expression, preferably ovarian cancer as well as pancreatic, prostate, metastatic breast, bladder and lung cancers.

The developed system includes nanomicelles loaded with the anti-neoplastic agent (typically a combination of taxanes and platinum analogs), and conjugated with antibodies such as anti-MUC16, anti-MUC1 or anti-MUC4. The antibody-bound nanomicelles are embedded in a biodegradable pH- and thermo-responsive hydrogel capable of sol-gel transition at body temperature. The hydrogel composition may be formed from one or more biodegradable polymers. This pharmaceutical composition is conceived to be implanted in the peritoneum, where the hydrogel would swell and release the antibody-bound nanomicelles. These nanomicelles (formulated to circulate for a long time in the peritoneal fluid) specifically target mucin 1, 4 and/or 16 antigens significantly over-expressed on cancer cells at the primary tumor site (tumors confined to the ovary in stage I and II), those circulating in the peritoneal fluid during stage III and IV (when patients are usually diagnosed) and lastly, cancer cells forming nodules at distant sites in the peritoneal cavity.

3. POLYMERIC NANOSPHERES

Polymeric nanospheres (NS) are generally defined as a matrix-type, insoluble solid-colloidal NP in which drugs are dispersed, entrapped, encapsulated, chemically bounded or adsorbed to the constituent polymer matrix. These particles usually have sizes ranging from about 100 to 1000 nm [72, 73]. NS formulation of anticancer drugs aroused intensive research in the past decades and has become an important area in cancer-oriented nanotechnology applications. NS (and NP in general) can accumulate in tumors cells after systemic administration by penetration through cancers leaky blood capillaries followed by uptake of cancer cells via endocytosis/phagocytosis [72, 74-79]. The pore cutoff size of cancer's blood capillaries has been reported in the range from 380 to 780 nm [75, 80, 81]. This leaky nature allows a relatively easy extravasation of large molecules or colloid particles to the cancer tissues. In addition, cancer cells also have fewer lymphatic capillaries than healthy tissues, resulting in impaired lymphatic drainage of macromolecules from cancer tissues. The previous phenomenon is known as *Enhanced Permeability and Retention* (EPR) effect [77, 82]. In contrast, healthy tissues are much less permeable to macromolecules and large colloid particles [82, 83]. Therefore, NS have been used to passively control the distribution of anticancer drugs in normal cells and tissues, in order to increase antitumor efficacy as well as reduce the systemic side effects [84], providing physical and chemical protection from rapid plasmatic metabolization at aggressive physiological conditions [85] and solving low water solubility issues [84]. Just for exemplifying purposes, Dox NS having lower cardio and renal toxicity than free drug have been reported [86]; poly(DL-lactide-co-glycolide) (PLGA) NS loaded with 9-nitrocamptothecin have been developed to protect the drug from rapid hydrolysis under physiological pH [84] and; lipophilic derivatives of gemcitabine have been incorporated into poly(alkylcyanoacrylates) (PACA) NS in order to protect them against fast metabolization [85].

Due to their small size, NS may be conveniently administrated through different routes like intravenous or intraarterial, subcutaneous, intraperitoneal, intrathecal, oral, etc. The small size and the spherical geometry (which implies optimal surface to volume ratio), favor solubilization and drug re-

lease compared to other geometric arranges and larger carriers. In addition, polymeric NS have the ability to pass through the gastrointestinal tract barrier by means of active cellular uptake [87].

The clearance kinetics and *in vivo* biodistribution of nanosystems depend on physicochemical and biochemical factors like particle size, nature of the polymer, surface charge and surface hydrophobicity, all of which can be manipulated to enable targeting [88, 89]. Following intravenous administration, drug delivery nanosystems tend to be rapidly cleared by the reticulo-endothelial system (RES), mainly by fixed macrophages in the liver and spleen after opsonization by proteins present in the blood stream [90]. This natural tendency of nanosystems to localize in the RES represents an excellent opportunity for passive targeting of drugs to the macrophages present in the liver and the spleen [91, 92], and has been employed for chemotherapy of the RES localized tumors. For example, recent studies have shown that after intravenous injection, anticancer drug loaded NP are mainly concentrated in Kupffer cells in the liver. These cells act as reservoirs and allow prolonged diffusion of the anticancer molecules into the neighboring tumor cells; therefore, this phenomenon is useful for the treatment of hepatic metastasis [93]. In contrast, this characteristic biodistribution becomes a major obstacle for drugs whose site of action is located in tissues other than the RES.

A variety of methods have been explored to overcome the rapid opsonization and the subsequent RES uptake of nanosystems in order to enhance the circulating time [94-96]. Steric stabilization of NP has been achieved by adsorbing hydrophilic surfactants on the NP surface or by using block/branched copolymers (stealth coating). PEO and PEG are the most successful nonionic hydrophilic polymers used for this purpose [88, 97, 98].

On the other hand, the drug release kinetics from nanosystems should always be tailored so that a minimum amount of the entrapped drug is released prematurely, off-target. One general problem of NP is the premature burst release of drugs in the bloodstream which redounds in low efficiency and toxicity to healthy tissues [99]. After the initial burst release, the drug release from the NP may become very slow. Cancer cells have many drug resistance mechanisms; therefore, if the drug influx into the cancer cell is lower than the capacity of drug removal by ABC transporters and other detoxication mechanisms, the drug cannot build up an effective concentration [3-5, 72, 100-102]. The initial burst release is determined by poorly entrapped drugs, or drugs weakly adsorbed onto the particles surface. In this sense, the interactions between the drug molecules and the NS matrix should be strong enough to provide good encapsulation and not too fast nor too slow release. Appropriate cross-linking can be used to modify the drug release kinetics [103-105].

3.1. Using Polymeric NS to Reach Challenging Active Sites

Other issues to take into account when analyzing the performance of drug delivery by NP are those situations that demand nuclear targeting. Though the transport of nucleic acids, proteins, peptides and drug molecules across the cell membrane using NS/nanotubes has been demonstrated [106], their inefficiency to breach the nuclear membrane limits their use in many applications where the drug molecules should be delivered inside the nucleus. For example, treatment of diseases based on the reversible modifications of histones by HAT (histone acetyltransferase) and HDAC (histone deacetylases) have been proposed as new generation treatments [107]. Therefore, it is highly important to develop new nanomaterials that can deliver molecules which modulate chromatin modifying enzymes specifically to the nuclei [108].

A patent of Kundu *et al*. [109] provides a possible solution to this problem, through the development of fluorescent surface-functionalized carbon NS with nuclear targeting ability, which are non-toxic and have particle sizes ranging from about 100 nm to 500 nm. These NS are derived from a sugar (e.g. glucose) and are surface-functionalized with polymeric alcohols, salicylaldehyde groups and α, β-unsaturated aldehydes to provide a negatively charged, hydrophilic surface. The preparation process of this fluorescent carbon NS involves a step of polymerization of the sugar solution by hydrothermal decomposition at about 180°C followed by a second step where the active molecule(s) are adsorbed onto the NS surface. The obtained NS are made up of a dense hydrophobic core of polyaromatic units covered by layers of highly functionalized carbon chains with a thickness of a few nanometers. The authors state that the hydrophilicity / hydrophobicity balance of the NS helps them penetrating the cell. This penetration process takes about 1 h, and after 3 h they reach the perinuclear region. Active molecule(s) such as 4-chloro-3-trifluoromethylphenyl-2-ethoxybenzamide (CTPB, a p300 histone acetyltransferase activator), DNA, polycations, peptides, antibodies and RNA are adsorbed onto the carbon NS. Remarkably, photo luminescence studies showed a weak emission at 660 nm on excitation at 514 nm, attributed to the radiative relaxation of excited electrons (possibly due to the presence of conjugated aromatic systems). This helps imaging the NS in the confocal microscope over extended periods without the aid of any fluorescent tag, which is an added advantage due to the risks involved in the case of quantum dots or organic dyes.

The authors used the carbon NS to deliver CTPB into HeLa cells, and demostrated that CTPB carriers were capable of crossing the blood-brain barrier (BBB) and inducing hyperacetylation *in vivo*. The NS were distributed preferentially to the brain, followed by spleen and liver.

These are very promising results since the invention reaches not only the cellular nuclei but also are able to cross the BBB, which is another challenging objective when designing a pharmaceutical formulation. Due to the BBB, the central nervous system (CNS) is well protected and its homeostasis is maintained, but also many potential drugs for the treatment of CNS diseases cannot reach the brain in sufficient concentrations. In fact, only highly lipophilic molecules can passively diffuse through BBB. There have been a lot of recent and meaningful advances in the field of nanotechnology for brain delivery of therapeutics and good reviews can be found on the topic [110-112]. One strategy is to conjugate the NP with functional groups promoting translocation through the intact BBB (as in the case of primary

brain tumors). Another option is to use nanotechnological approaches as tools to cross the BBB by both prolonging the plasma half-life of the drugs and crossing fenestrations of BBB damaged by brain metastases [113]. Moreover, polymeric NP may not only successfully cross the BBB but they may also reach specific objectives or action sites within the brain as well. In recent studies, Wolfhart *et al.* determined the amount of surfactant-coated Dox-loaded NP in the whole brain and the fraction of drug in the brain parenchyma after crossing the BBB [114, 115]. This evidence suggests that this technology holds great promise for non-invasive therapy of the CNS diseases.

3.2. Biodegradable Polymeric NS

Biodegradable synthetic polymers as drug carriers have many advantages including good biocompatibility, nontoxicity, and adjustable controlled-release properties [116]. Among the different polymers used for polymeric NS, Polylactide-co-glycolide (PLGA) is a commonly used compound that presents lower toxicity than other polymers. Natural polysaccharides such as albumin, chitosan and alginate have also attracted increasing attention in drug delivery due to their favorable properties including pH-sensitivity and mucoadhesive ability, as well as their biocompatibility and biodegradability [117, 118].

A patent by D'Souza [119] describes a method for the preparation of NS containing bioactive material. The method comprises dissolving a polymer matrix, such as albumin or β-cyclodextrin (a non-antigenic and biodegradable material) in an aqueous medium and contacting the dissolved polymer with a cross-linking agent (e.g. glutaraldehyde), followed by the neutralization of the cross-linking agent excess and the dissolution of the bioactive material in the resulting aqueous solution of the polymer matrix. Finally, the mixture is spray-dried to produce the NS. By controlling the extent of cross-linking of the polymer matrix, the release of the drug can be very effectively controlled and designed. It should be highlighted that organic solvents are not involved in the process, therefore this green process avoids the risk of biomolecules denaturation caused by organic solvents [120].

The authors provide examples of adjuvant-free vaccines formulations to induce immunity after administration: one of them involves an oral tumor vaccine that induces mucosal immunity to oral melanoma. A mouse tumor model was used to evaluate a nanoencapsulated vaccine preparation of the extracellular antigen MECA. The antigens used in the vaccine were derived from a B16 murine melanoma cells culture. The C57BL/6 mouse, syngeneic to the B16 murine melanoma cells, was used. The mice were vaccinated to induce an anti-tumor response. The animals were then challenged to determine if such response was induced by assessing their capacity to reject the establishment of the murine melanoma. They were subsequently monitored for the development of tumors. The MECA group in this study remained 80% tumor free at day 60. The studies suggest that encapsulating tumor antigens could have an adjuvant effect in inducing tumor immunity by targeting professional antigen presenting cells. Based on evidence of M-cells targeting by lectins [121] the authors tested several lectins to promote targeting M-cells in the Peyer's patches, among them wheat germ agglutinin, which showed an excellent targeting ability.

Another situation that demands biocompatible materials is when pharmaceutical formulations are intended to be administered by injection, in order to reduce irritation at the injection site. Currently one of the main drug therapies for the treatment of estrogen-receptor (ER) positive breast cancer involves inhibiting or down-regulating ER [122]. Fulvestrant is an antiestrogen which competitively binds to ER and blocks receptor dimerization. The unstable fulvestrant-ER complex then undergoes accelerated degradation, resulting in down-regulation of the ER with subsequent inhibition of ER-DNA binding [123, 124]. Regrettably, the drug is insoluble in water, causing poor oral bioavailability [122, 125]. Thus, conventional oral preparations and common water solutions for injection are not applicable to this drug. Although there are a number of sustained release injectable formulations which have been commercialized, they commonly use a solvent system that includes ethanol, benzyl alcohol, benzyl benzoate, and castor oil. All these chemical ingredients have strong irritation properties and often cause damage to the injection site. Furthermore, after injection of fulvestrant, the non-aqueous chemical solvent is absorbed into the muscle tissue and then the drug is precipitated, followed by slow absorption [126-128]. A recent patent by Hu *et al.* [129] describes the development of a non-irritating injectable formulation with fulvestrant NS. The carrier material of the fulvestrant NS is a methoxy ended PEG-polylactic acid block copolymer, which has an adequate hydrophilicity-lipophilicity balance, good thermoplasticity and thermosetting, and it is also biodegradable and biocompatible, causing no irritation to the injection site or blood vessels. Using such material as the drug carrier the organic solvents that cause pain and inflammation to the injection site may be avoided, and moreover, the NS could be administered by subcutaneous or intravenous injection. *In vitro* studies on fulvestrant release from the NS and pharmacokinetics studies in rats demonstrated the good performance of this invention. After injection, the drug carrier material begins to degrade and fulvestrant is slowly released from the NS. The release rate can be regulated by adjusting the molecular weight of the hydrophilic PEG fragments and the lipophilic polylactic acid fragments.

3.3. Multifunctional NS

Recently, the concept of multifunctional nanocarriers has emerged and attracted increasing attention [130-132].

A recent patent from Yu *et al.* [133] describes the preparation of NS containing antioxidant derivatives of camptothecin (CPT). CPT is a plant alkaloid that exhibits its antineoplastic effect by the inhibition of DNA relaxation by DNA topoisomerase I. However, CPT is essentially insoluble in water, and therefore numerous derivatives have been developed to increase its water solubility [134, 135]. CPT consists of a pentacyclic structure having a lactone in the E-ring, which is essential for the antitumor effect of the molecule. It has been demonstrated that the main transformation and elimination pathways of the drug are lactone hydrolysis and urinary excretion. In fact, around 50% of the lactone is hydrolyzed 30 minutes after administration [84]. On the other

hand, the (1,2)-dithiolane containing lipoic acid/dihydrolipoic acid redox system has been regarded as a universal antioxidant. Lipoic acid and dihydrolipoic acid are capable of trapping a number of radicals both in lipid and aqueous environments. The two thiol groups present in the (1,2)-dithiolane ring system confer it a unique antioxidant potential. A probable role of nitric oxide (NO), reactive oxygen species (ROS) and the metabolism of glutathione has been demonstrated in a number of conditions, among them cancer [136-140]. Lipoic acid inhibits the activity of NO-synthase enzymes and regenerate endogenous antioxidants which trap ROS [141, 142].

The invention provides a NS that includes compounds produced by conjugation of an alpha lipoic acid and CPT or CPT analogs (Fig. (**3**)). These antioxidant CPT analogs NS can deliver the active ingredient for a prolonged period of time.

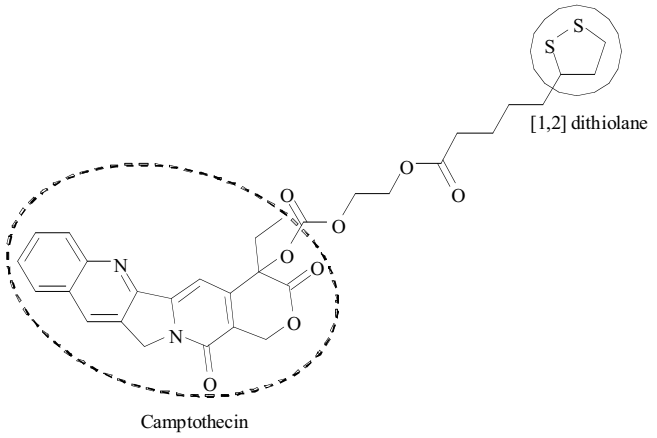

Fig. (3). Schematic representation of the compounds prepared by Yu *et al.* [133]. Camptothecin (CPT) or a derivative of CPT is conjugated with lipoic acid analogs containing the antioxidant (1,2) dithiolane ring system.

The authors present the results of *in vitro* intracellular uptake of the antioxidant-antineoplastic NS (AA-NS) labeled with a hydrophobic dye Coumarin 6 by U87 glioma cells, as well as the distribution and intra-tumoral accumulation of AA-NS in an animal model. Improved accumulation in U87 glioma cells and enhanced, selective distribution to tumor tissues in mice were observed.

4. NANOCAPSULES

Nanocapsules (NC) have been defined as vesicular systems in which the drug is commonly confined to an oily or aqueous cavity surrounded by a polymeric shell [72, 73]. A more general definition that we will embrace here considers the NC as nano-vesicular systems that exhibit a core-shell structure in which the drug is generally confined to a reservoir surrounded by a polymeric membrane or coating [16].

Active ingredients may be adsorbed onto the surface or included in the central core. The NC core may be of hydrophilic or oily nature. Some of the advantages of NC over NS are their low polymer content and a high loading capacity for lipophilic drugs [143].

4.1. NC for Delivering of Biomolecules

One of the main advantages of NC drug delivery systems is the possibility to be used for the *in vivo* transportation and delivery of biomolecules, which would otherwise be rapidly degraded. Biomolecule-based therapeutics that functions intracellularly has enormous potential for the treatment of human diseases; however the development of intracellular biomolecules therapeutics has been hampered by the limitations arising from the nature of these molecules: structural fragility, low serum stability and poor membrane permeability, among others. All these obstacles require suitable delivery vehicles that can protect the molecule from degradation and denaturation during circulation and endocytosis, and release the biomolecule in native form when the desired destination (i.e. the cytosol) is reached [144].

A 2011 patent by Yungfeng *et al.* [145] describes NC that comprise a single-protein core covalently anchored to an artificial thin shell. The invention has been conceived to protect the core-protein from proteolysis and denaturation, and to improve cellular uptake. The authors indicate that the surface features (e.g. charge), solubility and other properties (e.g. temperature-sensitivity) may be adjusted by varying the weight ratio of the different monomers and/or cross-linkers used to form the nanocapsule shell. Specific embodiments provided as examples and tested by the authors include carriers for Horseradish peroxidase (HRP) in combination with indole-3-acetic acid as enzyme-mediated prodrug cancer therapy [146], and caspases, a family of cystein proteases that induce apoptosis [147]. The authors warn that the activity of the encapsulated protein can be reduced up to 30% compared to the native protein. Studies in HeLa cells with encapsulated enhanced green fluorescent protein (EGFP) confirmed the improved stability of encapsulated EGFP versus free EGFP, observing a fluorescence signal from the encapsulated protein even after 144 hours. A positive correlation between the surface charge of the NC and the internalization of EGFP was also observed. Incubation of the EGFP NC at 4ºC suggested that the NP were uptaken into the cell by endocytosis. Intraperitoneal delivery of EGFP NC and control EGFP to nude mice and subsequent fluorescent confocal microscopy analysis of organ sections revealed that, after 8 hours, strong fluorescence signals were detected in all tissues (meaning that at least some of the fluorescent protein was intact) while only background signal was detected for control EGFP. The signal from the injected NC remained high up to 50 hours. The discussed evidence indicates that both the stability in the biological media and the delivery of proteins within the cells are improved through the single protein NC. Remarkably, HRP NC retained 92% of the native HRP activity.

4.2. Improving the Cellular Uptake of NC

A relevant issue in the particular case of anticancer drugs delivery is the slow cellular uptake by cancer cells. The previously discussed coatings that are used to evade the clearance by RES can substantially slow down the cancer cellular uptake of the NP [148-151]. As a result, PEG-coated NP may passively accumulate in cancer interstitial space and release drugs there. Surface engineering (grafting ligand moieties onto the nanoparticle surface) has been used to en-

hance the cellular uptake, e.g. transferrin receptor-transferrin system [152, 153] and folate receptor-folic acid system [154-158]. In particular, folate receptors (FR) are over-expressed on various types of cancer cells, and mediate endocytosis (FR-Endo) of folic acid-conjugated carriers [156]. Nevertheless, coated NP functionalized with folic acid only may not be sufficiently effective to grant adequate uptake. The steric repulsion of the coating may prevent the folic acid on the NP from finding and binding the FR. Hence, FR-Endo needs to be optimized. Electrostatic-enhanced adsorptive endocytosis (AD-Endo) has been used to facilitate the cellular uptake by using a polycation complex that is effectively taken up through adsorptive endocytosis mediated by the electrostatic interaction with the negatively charged cell membrane [159]. So, carriers with cationic charges are easily adsorbed onto negatively charged cell membranes and enter the cell via AD-Endo, which may explain, at least partially, the correlation between the surface charge and the internalization of EGFP described in the previously reviewed patent from Yungfeng *et al.* [145]. Intending to improve cellular uptake, a recent patent by Radosz *et al.* [160] introduces multi-layered long-circulating NP for the delivery of high levels of anticancer drugs preferentially to cancer cells. The patented NP are NC with diameters between 10 and 500 nm, comprising an outer shell, an inner solid core, and an intermediate layer. The core consists in the active ingredients (anticancer drug or drugs) and polymer chains that are soluble at lysosomal pH (similar to 5). The intermediate layer involves polymer chains that are insoluble at the pH of healthy tissue, but soluble at the pH of the cancer interstitium. The outside layer is made of hydrophilic water-soluble polymer chains (like PEG, PEO, among others) to avoid RES recognition, linked to the intermediate layer (the shell) by acid-labile bonds. The outside layer also includes folic acid and cationic-charges that enable the NC to be efficiently internalized via combined endocytosis mechanisms (FR-Endo, AD-Endo). Consequently, three endocytosis processes may occur: 1) The folic acid moieties on the NC surface may bind FR on cell membranes and trigger FR-Endo; 2) In the acidic tumor interstitium where the pH is approximately 6 [161], the acid-labile bonds begin to break and the PEG chains are shed off from the NC surface to expose the shell layer. At such pH the shell layer is positively charged and thus adsorbed on the negatively-charged cell membrane, leading to electrostatically AD-Endo; and 3) the adsorption of the NC on the cell surface also makes it easy for folic acid to find and bind FR, resulting in adsorption-promoted FR-mediated endocytosis (AD-FR-Endo). Once the nanocapsule is transferred to a lysosome the core of the nanoparticle is protonated. Due to severe osmotic imbalance, the lysosome swells and its membrane is disrupted: amine-containing polymers act as proton sponges buffering acidic lysosomes and disrupting membranes by increasing the internal osmotic pressure within the vesicle [162-167]. The membrane disruption quickly dumps all the carried drugs into the cytoplasm. An illustration of the previous processes can be found in Fig. (**4**). The patent authors show, through *in vitro* studies, that cisplatin encapsulated in fast-releasing NC made of pH-responsive poly[2-(N,N-diethylamino)ethyl methacrylate) (PDEA), synthesized from PDEA-PEG copolymer has a significantly higher cytotoxicity than free cisplatin and cisplatin encapsulated in slow-release NC of pH-resistant poly(ecaprolactone) (PCL) cores, synthesized from PCL-blockPEG (PCL-PEG) NC. *In vivo* characterization of the antitumor effects of cisplatin loaded NC in immunocompromised mice showed that the group treated with cisplatin/PDEA-PEG NC exhibited a significant decrease in the number of tumors compared to free cisplatin control treatment.

A recent patent of Lozano López *et al.* [168] introduces an easy-to-obtain nanocapsule system wherein an oily core comprising an oil and a negative phospholipid component is surrounded by a polyarginine (PArg) shell. PArg belongs to the family known as protein transduction domains (PTD, or cell penetrating peptides) [169-171] which are small cationic peptides able to carry other peptides, nucleic acids, viral particles and NP across the cellular membrane, into cells. PTD engage the cellular surface through electrostatic interactions and induce their own internalization through endocytosis. Besides their proven utility in the promotion of cellular uptake of different molecules such as siRNA, fluorescent markers and hormones [172-174], pArg has immunostimulant properties [175] and induces changes in paracellular transport at epithelia through tight junction disruption [176]: all these properties have enormous potential for drug delivery in general and anti-cancer treatment in particular. Therefore, the development of pArg-shell NC seems as a very attractive idea for the delivery of anticancer drugs.

What is more, as the authors state, the highly positive surface charge of the NC provides greater interaction with mucosae and, especially, tumor cells, which are often more negatively charged than healthy tissue (e.g. due to cell surface sialylation) [177]. Additionally, PEG stearate can be used as auxiliary component to modulate surface charge and to improve stability in biological fluids. Regarding surface charge modulation, it is worth mentioning that a number of studies suggest that charge may be optimized to control intra-tumor distribution of drug carriers [178, 179]. Preferred embodiments of the invention include using docetaxel or adsorbed genetic material as active ingredients. The authors obtained an encapsulation efficiency of 74% for docetaxel. It should be noted that the strong basic character of pArg guanidine groups determines that the positive charge is maintained through a very wide pH range.

4.3. Enhancing the Selectivity of Drug Delivery

Two original applications towards targeted-release of NC content have been recently patented by Wheatley *et al.* [180, 181]. The novelty of the work involves the preparation of echogenic NC whose distribution may be monitored through ultrasound imaging, while degradation of the nanoparticle (and thus, drug release) can also be enhanced by application of ultrasound pulses. The idea of merging an ultrasound contrast agent with ultrasound-sensitive drug release is particularly attractive to deliver anticancer agents to tumors, especially if one takes into account that drug delivery usually works well in the highly perfused regions of the tumor (generally the periphery) while thermal treatment through high intensity focused ultrasound can ablate poorly perfused tumor cores [182]. The first invention aims to selectively delivering the therapeutic agent in the liver, in order to treat liver cancer, by localized rupture of the delivery system and,

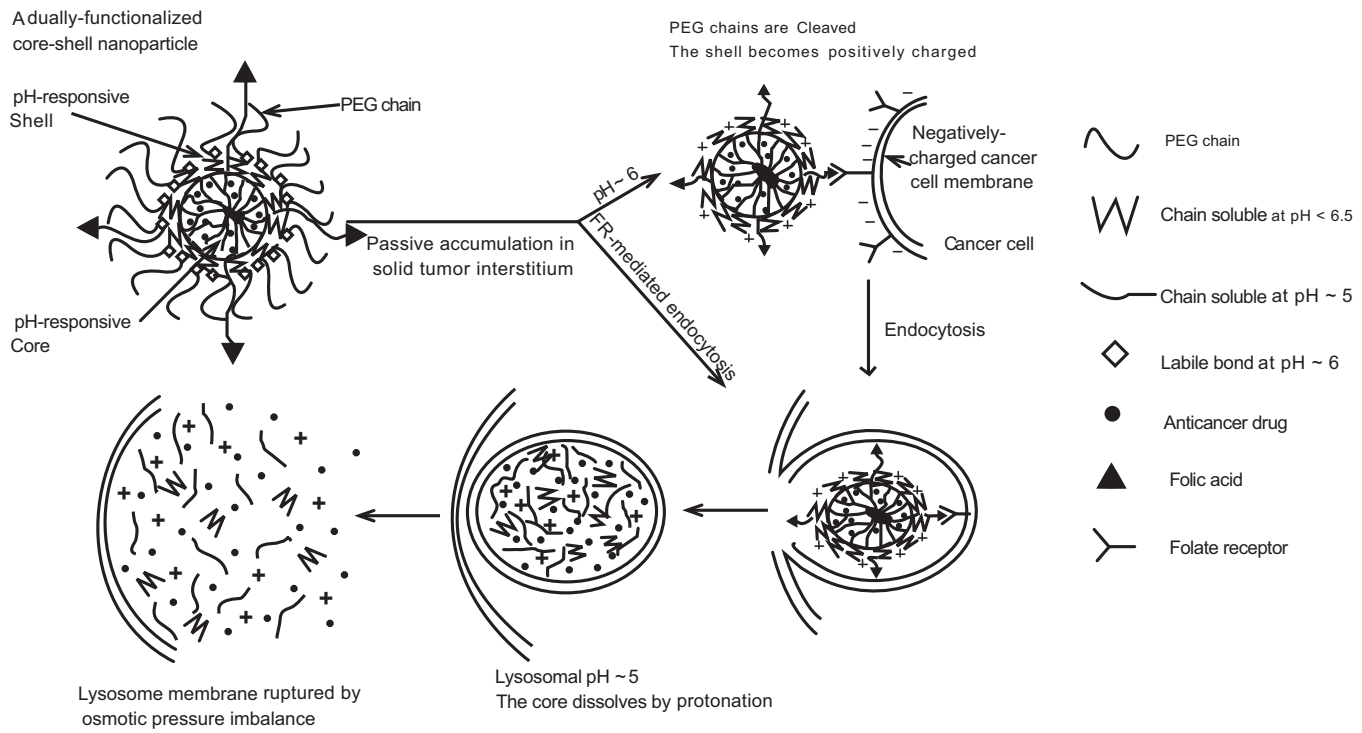

Fig. (4). Schematic representation of the different stages involved in the drug release from the NC by Radosz *et al.* [160].

eventually, by including a targeting moiety (e.g. an antibody) onto the particle surface.

In order to present an echogenic behavior (i.e. to produce a detectable echo signal when isonated with ultrasound waves) the nanocapsule should be hollow or porous. Such feature is achieved by inclusion of sublimable compounds during the preparation of the NC, which are later evaporated leaving gas-filled holes or pores. Two preparation methods are described: single and double emulsion methods, both involving a initial step of mixing a biodegradable polymer with a substance that can easily sublime in the lyophilizer (e.g. camphor, naphthalene), and a final step of freeze-drying to remove the sublimable agent. More details on the procedure to obtain the NC can be found in the work of El-Sherif and Wheatley [183].

Dox, irinotecan, paclitaxel, oxaliplatin and 5-fluorouracil loaded NC were produced. The NC give up to 25 dB enhancement when isonated in the medical imaging range, although the echogenicity can be partially lost when loading chemotherapeutic agents. Although, Wheatley claims that isonation of the NC releases the drug to a significantly greater extent than in the absence of ultrasound, it should be mentioned that this statement is not reflected in the correspondent graph disclosed in US20080247957 [180]. Modifications of the NC surface for targeting purposes has also been reported by the Wheatley group [184]. Patent US20090196827 [181] discloses an optimized method for the obtention of the previously discussed drug-loaded echogenic NC. The improved method contemplates loading the drug in pre-hardening or hardening steps. In other words, prior to hardening, the NC are put into contact with a solution of the drug in a non-solvent for the polymer that constitutes the NC shell (pre-hardening loading), or, alternatively, the NC are contacted with a solution of the drug in the hardening solvent itself (e.g. hexane). The loading efficiency is enhanced by this optimized preparation method, as shown in Table **1**.

In 2012, Singh *et al.* disclosed an ingenious method to avoid hair loss during paclitaxel treatment [185]. The authors propose a passive targeting approach by taking advantage of EPR effect. To this purpose, paclitaxel-loaded human serum albumin NC are prepared by means of the double emulsion method and the size distribution of the resulting NP is carefully assessed. The general idea is to achieve NC of such a size distribution that 90% of the particles have a particle size of less than 450 nm, 10% have a particle size equal or lower than 80 nm and around 50% of the particles are about 200 nm. This size distribution responds to two purposes: limiting removal by the RES and preventing permeation from normal blood capillaries to skin (and hence hair roots) while allowing permeation through tumor leaky vessels. It should be mentioned that vesiculo-vacuolar organelles, which provide the major route of extravasation of macromolecules at sites of augmented permeability induced by vascular endothelial growth factor (e.g. tumor vessels), measure on average 108 $\pm$ 32 nm (mean $\pm$ SD) in internal diameter, while capillary caveolae present an internal diameter of 58 $\pm$ 9nm [186]. When assessing tumor retentiveness and leakiness behavior of the NP of the present invention administered through intratumor route to ICRC mice carrying spontaneous mammary tumors, the authors found that two particular embodiments of the invention presented a paclitaxel tumor plasma

Table 1. Comparison of the Loading Efficiency (Expressed as mg Dox/g PLGA) of Different Drug Loading Methods, for PLGA NC. Dry-Absorption Refers to putting into Contact an Aqueous Solution of the Drug with the Lyophilized NC for Loading Purposes. Incorporation Refers to Adding the Drug to the Aqueous or Organic Phase at the Beginning of the Double Emulsion Method. Table Adapted from Reference [181].

% Starting Concentration (mg DOX/mg Polymer)	Dry-Adsorption	Pre-Hardening Adsorption	Hardening Absorption	Incorporation
0.10	0.20	1.00	1.00	1.00
0.26	0.52	-	2.60	2.60
0.55	0.72	-	5.50	5.50
1.44	0.84	13.21	14.40	12.16
3.00	0.84	21.00	21.01	15.20
4.00	0.94	20.00	14.23	17.71

ratio of 71.6 and 355.7, while a commercially available albumin bound paclitaxel achieved only 19.96. The authors also tested the performance of the proposed formulations in a murine model to study chemotherapy-induced alopecia, finding the proposed strategy had been successful to reduce paclitaxel-associated hair loss (Table **2**).

CURRENT & FUTURE DEVELOPMENTS

Drug disposition is a crucial aspect of cancer treatment. Traditional (small molecule) anticancer drug therapies often present solubility issues, fast-metabolization and side-effects related to off-target/systemic distribution. Novel biomolecule-based therapies (e.g. gene or protein therapies) tend to present absorption issues (that hamper oral administration) and lack of stability in physiological conditions (enzymatic cleavage and denaturation).

Nanosystems are providing unprecedented solutions to the previously mentioned problems. First, they can provide a water soluble vehicle to poorly soluble drugs, which due to its small size can be administered even through the intravenous route. Second, their size allows easy extravasation to interstitial fluids and they can be uptaken into cells via active mechanisms, mainly endocytosis. Third, they may prolong the half life of both small and large molecules by providing physical and chemical protection to entrapped/encapsulated entities. Fourth, they may be engineered to target a given cell or tissue through either passive or active targeting. Fifth, the drug release profile may involve different processes (biodegradation or dissolution of the NP constituents, passive diffusion, induced or active release) and may be tailored to achieve controlled or sustained release.

Passive targeting approaches include the use of pH-, termo-, or redox environment-sensitive materials, and exploitation of different size restrictions in different tissues (e.g. the EPR effect). Active targeting includes termo-, ultrasound-, magnetic-sensitive NP and surface modifications (including targeting moieties such as antibodies, lectins and many others).

We have overviewed recent inventions on nanogels, NS and NC oriented to cancer diagnostic, treatment and prevention, among them small molecules, biomolecules and vaccine carriers, and contrast agents. Among the reviewed patents, we might highlight the inventions of Chirwa *et al.* and Sinko *et al.* oriented to the prevention of metastases of different types of cancer; the work of the Wheatley group towards NP than can simultaneously act as targeting, ultrasound-sensitive drug carriers and contrast agents; the double targeting NC by Radosz *et al.*, which exploit over-expressed folate receptors and negative cell surfaces at cancer cells to induce a two-mechanism selective endocytosis and the work of Lozano López *et al.*, which presents positively-charged NC whose shell is entirely made from a PTD (pArg).

Table 2. Comparison of Paclitaxel-Induced Hair Loss in a Murine Model of Chemotherapy Induced Alopecia. Controls Are Injected Saline Solution. Mice are induced, by Depilation, to synchronically enter Anagen Phase. The Hair Growth Score at Day 10 after Treatment is presented. A Score Of 0 means no Hair Growth, while a Score of 3 Represents Good and Uniform Hair Growth.

NC of D50 = 97.9 nM		NC of D50 = 178.5 nM	
Group	**Hair Growth Score (Day 10)**	**Group**	**Hair Growth Score (Day 10)**
Control	3.00	Control	2.25
Test (20 mg/kg iv)	2.66	Test (20 mg/kg iv)	2.00
Reference (20 mg/kg iv)	2.00	Reference (20 mg/kg iv)	1.50

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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