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Sself-assembling polymerELF-ASSEMBLING POLYMER SYSTEMS FOR ADVANCED TREATMENT OF CANCER AND INFLAMMATIONR.Palao-Suay systems for advanced treatment of cancer and inflammation

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Abstract

Self-assembled nanoparticles have reached a growing interest for the improvement of cancer diseases and associated inflammation processes. This article describes the most representative types of self-assembling nanosystems, including a detailed review of different methodologies for their preparation. Nanoparticles are commonly formed by self-assembling of amphiphilic polymers in aqueous environment. For that reason, the main strategies for the design of amphiphilic polymeric systems are also reviewed, with an emphasis on the different polymerization techniques of synthetic monomers and several strategies of chemical modification of polysaccharides and proteins. Additionally, most advanced applications of self-assembled nanocarriers for the improvement of treatment of cancer and inflammation diseases are also discussed, focusing on the description of drug-loaded and drug-conjugated systems, active targeted strategies and most recently possibilities for the multimodality treatment of cancer diseases.

Keywords: Self-assembling polymers; Nanoparticles; Micelles; Amphiphilic polymers; PEG; Polysaccharides; Cancer; Anti-inflammatory agents

Nomenclature

α-TOS

α-tocopheryl succinate

5-FU

5-fluorouracil

A549

human lung adenocarcinoma epithelial cells

AA

amino acids

ADR

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Adriamycin

ALG

alginates

ATRA

all *trans* retinoic acid

ATRP

atom transfer radical polymerization

CD44

cluster determinant 44

CDDP

hydrophilic derivative of cisplatin

CL

ε-caprolactone

CPT

camptothecin

CSO

chitosan oligosaccharide

DCC

N,*N*′-dicyclohexylcarbodiimide

DCT

docetaxel

DD

deacetylation degree

DEX

dextran

DMAP

N,*N*-dimethyl amino pyridine

DMT

dexamethasone

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DOCA

deoxycholic acid

DOX

doxorubicin

DS

degree of substitution

DSPE

distearoylphosphatidylethanolamine

EDC

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

EGCG

epigallocatechin-3-gallate

EPR

~~Enhanced Permeability and R~~enhanced permeability and retention

FA

folic acid

FDA

Food and Drug Administration

Fol

folate

GRA

glycyrrhetinic acid

HA

hyaluronic acid

HARE

hyaluronan receptor for endocytosis

Hases

hyaluronidases

HCPT

hydroxycamptothecin

HeLa

human cervical cancer cells

HER2

human epidermal growth factor receptor 2

HES

Hydroxyethyl starch

His

histidine

HPMA

poly(*N*-(2-hydroxypropyl)methacrylamide)

IMC

indomethacin

IR

inhibition rate

KB

human nasopharyngeal epidermoid cancer cells

LA

lactic acid

LCST

lower critical solution temperature

LMWC

low molecular weight chitosan

MAA

methacrylic acid

MDR

multidrug resistance

mPEG

methoxypoly(ethylene glycol)

MTO

mitoxantrone

MTX

methotrexate

MW

molecular weight

NC

Nnanocapsules

NCA

N-carboxyanhydride

NHS

N-hydroxysuccinimide

NMP

nitroxide-mediated polymerization

NP

Nnanoparticles

NSCL

non-small cell lung cancer

o/w

oil-in-water emulsion

OX

2-oxazoline monomer

PA

peptide amphiphiles

PAA

poly(amino acids)

PAsp

poly(aspartic acid)

Pba

pheophorbide

PBS

phosphate buffered saline

PCL

poly(ε-caprolactone)

pDNA

plasmid DNA

PDT

photodynamic therapy

PE

phosphatidylethanolamine

PEG

poly(ethylene glycol)

PEGMA

poly(ethylene glycol methacrylate)

PEI

polyethyleneimine

PEO

polyethylenoxide

PEtOx

poly(2-ethyl-2-oxazoline)

PGlu

poly(glutamic acid)

PLA

poly(lactic acid)

PLGA

poly(lactic-*co*-glycolic acid)

PLL

poly(L-lysine)

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PM

polymeric micelles

PMAA

poly(methacrylic acid)

PMDETA

N,*N*,*N*′,*N*″,*N*″′,*N*‴,*N*‴-pentamethyldiethylenetriamine

PNIPAm

poly(*N*-isopropylacrylamide)

Poly(L-HIS)POLY(L-His)

poly(L-histidine)

POX

poly(2-oxazolines)

PPhe

polyphenylalanine

PPO

poly(propylene oxide)

PS80

polysorbate 80

PTX

paclitaxel

RAFT

reversible addition and fragmentation transfer chain polymerization

RES

reticuloendothelial system

RGD

Arg-Gly-Asp

ROP

ring opening polymerization

SCF

supercritical fluid technology

SF

silk fibroin

SPIONs

superparamagnetic iron oxide nanoparticles

TEA

triethylamine

THP

tumor-homing peptides

TPGS

tocopheryl polyethylene glycol 1000 succinate

VEGF

vascular endothelial growth factor

w/o/w

water-in-oil-in-water emulsion

1 ~~INTRODUCTION~~ntroduction

Nanomedicine is growing in directions of diagnostic, treatment and theranostics (diagnostic, drug delivery and therapeutics), and the participation of systems based on a macromolecular concept is well recognized [1–3]. In fact, we have to learn from nature that most of the activities in the living organism, including the human body, are based on the design and application of bioactive systems and drugs developed in a macromolecular or polymeric concept. Nature shows a lot of examples that we have the opportunity to apply; ~~i.e.,~~*i.e.* bioactive systems and macromolecular drugs. Systems such as heparin, chondroitin sulfate, heparan sulfate (polysaccharides), or insulin, growth hormone, fibroblast growth factor, morphogenetic proteins, fibronectin, albumin, fibrinogen (proteins) and other well-known bioactive compounds are designed and fabricated in our organism within a macromolecular architecture.

High molecular weight (MW) polymers with specific molecular architectures present the ability of association and distribution in specific nanodomains, with morphologies and properties depending on the nature of the molecules [4]. The design, composition and morphological assembly of the cells and the extracellular matrix in the human organism are developed on the basis of the interactions and arrangements of long molecules with specific properties. On this way, the cytoplasmic membrane is composed of a bilayer assembled organization of lipid molecules containing a hydrophilic head. These individual and isolated structures develop their activity in a medium of controlled viscosity constituted by a macromolecular hydrogel of unique characteristics (collagen), which allows the development of the cellular activity and at the end, the proliferation of cells and fabrication of tissues and organs.

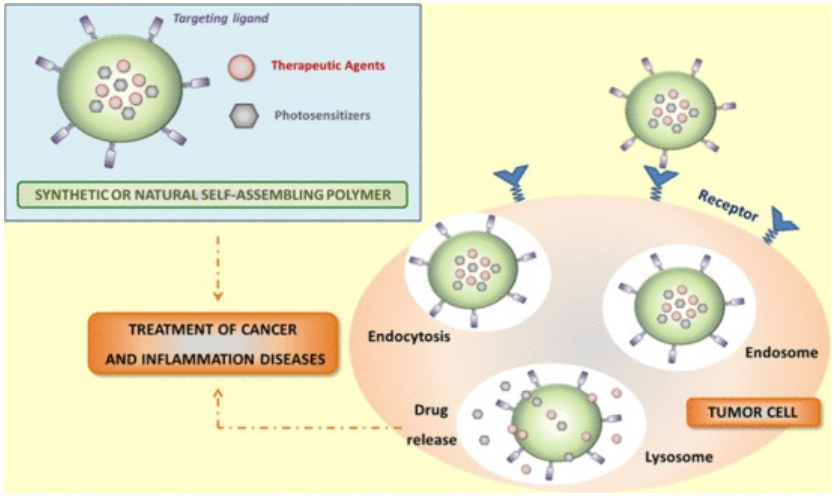
Macromolecular self-assembly is a spontaneous process based on the ensemble of molecules into 3D supramolecular structures with different morphologies such as polymeric micelles (PM), nanoparticles (NP), polymerosomes, ~~*etc.*~~ ~~[5,6]~~*etc.* [5,6]. This process is possible due to the amphiphilic nature of these structures, containing both hydrophobic and hydrophilic domains. Particularly, in the core~~–~~–shell organization, the inner core is composed of the hydrophobic part of the amphiphilic polymer and serves as a nanocontainer of poor soluble drugs. This core is surrounded by an outer shell based on hydrophilic polymers [7–9]. The characteristics of self-assembling systems depend on several important factors:

- Design, molecular composition and structure, considering macromolecular size and size distribution.
- Monomeric or co-monomeric sequences arrangement and distribution along the macromolecular chains.
- Functionality and its distribution in the structure of the macromolecular systems.
- Structural and morphological distribution in nanodomains.

- Macromolecular associations of natural and synthetic polymers, and stability in physiological conditions.

The structural characteristics of self-assembling systems have several advantages to improve the effectiveness and safety of cancer and anti-inflammation therapies for clinical use [10–13]. For example, the encapsulation of chemotherapeutic and anti-inflammatory drugs in the core of these assemblies improves their aqueous diffusion and transport, as well as bioavailability, decreasing their toxic side effects [14,15]. Moreover, their hydrophilic surfaces decrease clearance by the reticuloendothelial system (RES), presumably preventing opsonization by reducing the protein interaction. This strategy also allows the protection of drugs from degradation and produces their controlled release to the tumor site due to the ~~Enhanced Permeability and R~~enhanced permeability and retention (EPR) effect [16–18]. On the other hand, advances in engineering of block copolymers offer a wide range of possibilities to control the most influential properties of the polymeric assemblies, such as the particle size, stability or loading capacity of drugs, and to achieve an optimal active targeting to tumor site by modification of the shell of the macromolecular structures with specific ligands to promote cell targeting [7,19]. In fact the structure and morphology of self-assembled systems in a biomimetic scenario, including the decoration of the NP by ligands with high affinity to receptors overexpressed in cancer cells, are the most important parameters to control and to enhance the most frequent mechanism of the incorporation of the bioactive NP systems that is the endocytosis or pinocytosis. The application of NP systems brings novel and advanced possibilities including the lowering of the toxicity of the applied systems, without a noticeable decrease of the drug activity. The specific decoration of NP offers an additional contribution to new and advanced perspectives for the treatment of cancer and the application of anti-inflammatory therapies.

“Polymer ~~T~~therapeutics” can be considered the first generation nanomedicine. It includes polymers with inherent biological activity, polymer~~-proteins and polymer-~~–proteins and polymer–drug hybrid conjugates, PM, supramolecular assemblies that form multicomponent polyplexes designed for intracellular delivery of genes and proteins [20,21]. According to the properties of the macromolecular systems, they can be designed in different ways and morphologies. Fig~~ure.~~ 1 shows schematically the design and configuration of a sensitive system that can be considered a good nanocarrier for drugs with anti-tumor activity and anti-inflammatory effects. The self-assembled NP contains the drug (normally a non-polar compound with a limited solubility in water or in physiological fluid) in the core and a sensitizer (in this case a photosensitizer), and in the shell layer a targeting ligand for interaction and selective linking to the cytoplasmatic membrane [22]. The design of this micellar system protects the drug from the external medium (the physiological medium) until the cytoplasmatic membrane is reached, where the adequate interaction between the targeting ligand and the receptor of the membrane favors the endocytosis process. The release of the active compounds and the direct activity of the anti-tumor or anti-inflammatory drug, with high efficacy and low toxicity, are produced in the cytoplasm of the cells. If the carrier or NP is designed with a biodegradable or resorbable polymer component, the system is cleared from the cell and from the body without accumulation [23,24].



Fig~~ure.~~ 1 Active targeting of advanced self-assembled nanoparticles. Design of components and mechanism of bioactivity.

This review describes the possibilities of design and formulation of advanced pharmacologically active systems and polymer drugs to obtain self-assembled NP for anti-cancer and/or anti-inflammatory applications, considering the origin and nature of the polymeric materials that can be used as support matrices of nanocarriers.

2 M~~ETHODOLOGIES FOR SELF-ASSEMBLING SYSTEMS~~ethodologies for self-assembling systems

2.1 Types of self-assembling nanosystems

Synthetic or natural-derived amphiphilic polymers can self-associate in aqueous media due to intra- and/or intermolecular hydrophobic interactions [25] giving rise to a variety of nanosized structures with potential application in advanced cancer therapies.

These delivery systems can be classified according to their different supramolecular organization into micelles and diverse types of NP.

PM are among the first polymeric self-aggregates which were described in the literature as nanoscale drug delivery systems [26,27]. It is generally accepted that micelles are built from individual polymeric molecules [28] which are thermodynamically driven to self-assemble due to their amphiphilic nature. PM can be obtained from block or random copolymers, or either from hydrophobically modified hydrophilic polymers where the hydrophobic residues can be attached to one end of the polymer or randomly distributed within the polymeric structure, giving rise to different supramolecular structures (see Figure 2).

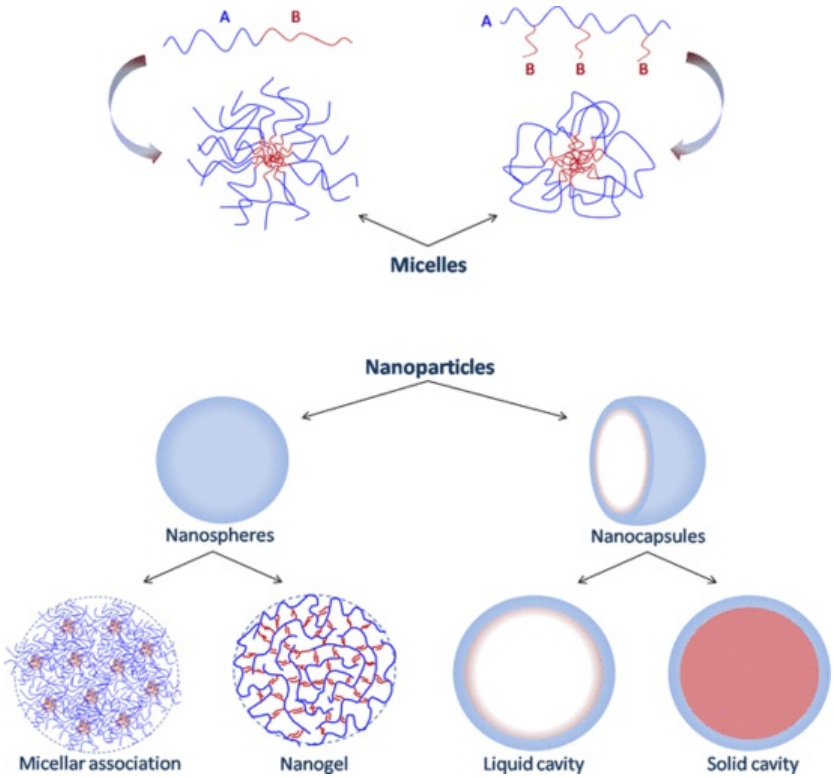


Figure 2 Morphology of self-assembling polymeric nanocarriers.

The cores of the micelles can act as reservoirs for anticancer agents, which are usually hydrophobic, and their hydrophilic shells facilitate their solubility in aqueous media [29]. The small size of PM, commonly between 10 and 100 nm, has several advantages such as the possibility of extravasation of the nanocarriers along with the minimization of the risks of embolism in capillaries [30]. Furthermore, their unique small size facilitates longer blood circulation times and thus leads to enhanced accumulation at tissue sites with vascular abnormalities such as tumor tissues [7].

NP can be prepared by spontaneous self-organization due to the same thermodynamic principles previously described for micelles, which are generally based on the amphiphilic nature of the polymers or the association of oppositely charged polyions. Two different types of self-assembling NP can be obtained depending on the preparation procedure: nanospheres or nanocapsules (NC) (see Figure 2). Nanospheres consist of packed polymeric matrices while NC are vesicular systems with an inner cavity surrounded by a polymeric membrane [31]. When nanospheres are loaded with anticancer agents, these are dispersed throughout the NP. However, these agents are confined in the cavity when loaded into NC, which acts as a reservoir systems.

Nanospheres can in turn be divided into micellar associations, formed by aggregation of individual micelles, and nanogels, consisting of nanosized polymeric matrices which are physically cross-linked by the interaction between the hydrophobic chains or residues of amphiphilic polymers. In addition, hydrogen bond or electrostatic interactions can also support the cross-linking of nanogels to form water-swollen NP [32].

NC can be classified according to the nature of their cavities, which can contain active substances in liquid or solid form, or as molecular dispersions, being this reservoir either lipophilic or hydrophobic [33]. A particular nanocapsular structure are polymersomes, which are similar to liposomes but are composed of amphiphilic polymers instead of amphiphilic lipids [5].

Conventional dendrimers lack self-assembling behavior. However, amphiphilic dendrimers are able to form micelle-like NP by self-assembly, usually exhibiting hydrophilic groups on their surface [5,34]. Moreover, dendronized polymers-based NP, which can combine both the characteristics of self-assembled nanostructures and dendrimers have been prepared by covalently linking hydrophobic dendrons to hydrophilic polymer backbones so that the resulting conjugates have an amphiphilic nature capable of self-organizing into core–shell structures [35]. Alternatively, monomers already carrying dendrons can be subjected to polymerization giving rise to dendronized polymers [36,37].

2.2 Methodologies for the obtaining of self-assembling nanosystems

Self-assembling polymer nanosystems (i.e. micelles, nanospheres or NC) are normally obtained from preformed polymers with an appropriate hydrophobic/hydrophilic balance using the following techniques:

Solvent evaporation: this is a widely used method for the preparation of NP for drug delivery formulations. The polymer is dissolved in a volatile solvent and an emulsion is formulated with or without surfactant using high-speed homogeneization or ultrasonication. The solvent diffuses through the continuous phase and evaporates by continuous magnetic stirring or under reduced pressure, giving rise to a nanoparticle suspension. The process can be carried out in single emulsions, e.g. oil-in-water (o/w) or double emulsions, e.g. water-in-oil-in-water emulsion (w/o/w).

Saeed et al. [38] prepared well-defined smart NP by (w/o)/w double emulsion to encapsulate DNA with high efficiency. NP were based on poly(lactic-*co*-glycolic acid)-S-S-poly(lactic-*co*-glycolic acid) (PLGA-S-S-PLGA) and PLGA-S-S-Poly(ethylene glycol methacrylate)-Folate (PLGA-S-S-PEGMA-Fol) block polymers synthesized using a combination of ring opening polymerization (ROP) and atom transfer radical polymerization (ATRP). The terminal functionality of the ATRP-obtained polymer was modified in order to incorporate an azide moiety to be reactive with propargyl folate via Huisgen [1,3] dipolar cycloaddition. Block polymers self-assembled using the following conditions: PLGA-S-S-PLGA and PLGA-S-S-PEGMA-Fol were dissolved in dichloromethane and mixed with an aqueous solution with or without plasmid DNA using vortex agitation. The emulsion was poured in ethanol. The organic solvent was evaporated under reduced pressure yielding the final NP.

Nanoprecipitation (or solvent displacement method): the polymer is dissolved in a semipolar solvent (miscible in water) and is added dropwise in water (non-solvent of the polymer). A rapid diffusion of the semipolar solvent takes place and a decrease of interfacial tension between the two phases occurs, giving rise to the formation of small droplets of precipitated polymer. This method has been widely used in the preparation of self-assembled NP for the synthesis of anti-cancer drug delivery systems. For example, Sanna et al. [39] prepared resveratrol loaded NP by dissolving poly(ε-caprolactone) (PCL) and PLGA poly(ethylene glycol) COOH (PLGA PEG poly(ethylene glycol)–COOH (PLGA–PEG–COOH) (1.5:1), and resveratrol (2, 3, and 4% w/w) in acetonitrile and added into water under magnetic stirring. The colloidal suspension was stirred at room temperature to remove the organic solvent and NP were isolated by centrifugation and washed with water to eliminate the non-encapsulated drug.

Li et al. [40] used a modified nanoprecipitation method to prepared doxorubicin (DOX) loaded NP based on dextran-*b*-PCL (DEX-*b*-PCL). Briefly, DEX-*b*-PCL was dissolved in DMSO and DOX·HCl was neutralized with triethylamine (TEA) in DMSO. Both solutions were mixed and stirred at 60 °C and added dropwise to water. The resulting suspension was dialyzed against water (MW cutoff 12,000) to remove unloaded DOX and the product was filtered through a 0.45 μm syringe filter before used.

Salting-out: In this method the polymer is dissolved in a solvent that is miscible with water (i.e. acetone, ethanol, ethyl acetate..., etc.) and an emulsion is obtained dissolving high concentrations of salt (e.g. magnesium chloride, calcium chloride or magnesium acetate) or sucrose in the aqueous medium. A strong salting-out effect is produced changing the miscibility of the used solvent. However, dilution of the emulsion by the addition of water produces the precipitation of the polymer dissolved in the droplets of the emulsion by a reverse salting-out effect. For example Cirstoiu-Hapca et al. [41] prepared paclitaxel (PTX) loaded NP using acetone, poly(lactic acid) (PLA) and PTX as organic phase and a solution of 15% poly(vinyl alcohol) (PVA) and 60% w/w of magnesium chloride in water. The surface of these NP was decorated with monoclonal human epidermal growth factor receptor 2 (HER2) antibody (trastuzumab, Herceptin®) in order to target HER2 membrane receptors of certain primary and metastatic tumors.

Dialysis: the polymer is dissolved in an organic solvent and placed in a dialysis membrane with a cut-off low enough to avoid the polymer diffusion. The dialysis is performed against a non-solvent miscible with the organic solvent and the displacement of the solvent in the membrane produces the formation of narrow-distribution of small NP. This simple method has been widely used in the preparation of hydrotropic oligomer-conjugated glycol chitosan NP for tumor-targeted PTX delivery [42].

Supercritical fluid technology (SCF): is an environmentally friendly technology as it avoids the use of organic solvents in the synthetic process providing a residuum-free and effective alternative to traditional methods used in the synthesis of NP. Carbon dioxide (CO_2) in supercritical conditions (72.9 atm or 7.39 MPa and 304.25 K) behaves midway between a gas and a liquid, i.e. expanding to fill the container like a gas, but with a density proper of liquid state. SCF has been used to obtain NP and more specifically to prepare self-assembled NP of amphiphilic polymers. Zhang et al. [43] prepared 5-fluorouracil-loaded-poly(L-lactic)-polyethylene glycol/polyethylene glycol (5-FU-loaded-PLLA–PEG/PEG) NP using a novel supercritical CO_2 technique.

Spray-drying: This method is commonly used in the pharmaceutical industry to obtain a dry powder from a liquid or slurry by the fast drying of a hot gas (air or nitrogen depending on the sensitivity of the compounds). It is not commonly used to obtain NP from amphiphilic copolymers but Meenach et al. [44] have recently used this technique in the preparation of an aerosol of advanced spray-dried chemotherapeutic PEGylated phospholipid particles for dry powder inhalation delivery in lung cancer.

Dispersion polymerization: Shalviri et al. [45,46] designed a novel one-pot method to simultaneously obtain grafting onto starch and NP formation. Briefly, methacrylic acid (MAA) and polysorbate 80 (PS80) were grafted onto starch (PMAA-PS80-*g*-starch) being the obtained amphiphilic polymer sensitive to acidic pH in order to overcome multidrug resistance (MDR).

2.3 Preparation of amphiphilic polymers

Amphiphilic polymers present at least a combination of hydrophilic and hydrophobic segments. They form self-assembled structures when placed in aqueous environments with a hydrophobic inner core and a hydrophilic shell. Chemical composition of the repeating units and the monomer distribution in the macromolecular chains will define the physico-chemical properties of the polymers that will drive the self-assembled process, stability and drug encapsulation efficiency and release profiles. Therefore, the choice of

appropriate monomers, macromolecular architecture and hydrophobic/hydrophilic balance will be crucial in the design of drug delivery systems for anti-cancer and anti-inflammatory applications.

2.3.1 Synthetic polymers

Synthetic polymers have been broadly explored for the design of nanocarriers in the field of biomedical applications and, particularly, for their application in cancer diseases and anti-inflammatory therapies due to their high versatility and optimal balance of physico-chemical and biological properties. The most widely used components for the preparation of amphiphilic polymers are typically PEG, Pluronics®, poly(*N*-(2-hydroxypropyl)methacrylamide) (HPMA), poly(glutamic acid) (PGlu) and different poly(amino acids) (PAA) and poly(esters). Additionally, peptide amphiphiles (PA) and poly(2-oxazolines) (POX) have also been used because of their advanced properties and interesting potentials in this field (see Figure 3).

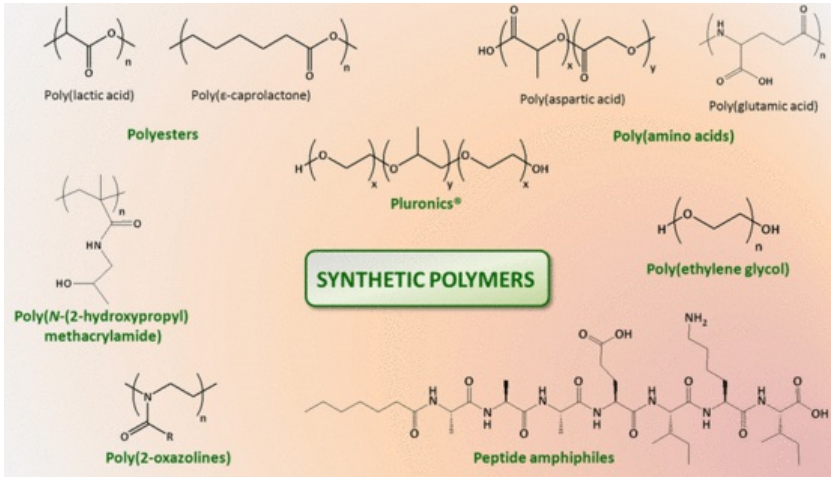


Figure 3 Chemical structures of the most widely used synthetic polymers.

Amphiphilic polymers have been obtained by different synthetic methods including ROP, free radical copolymerization, controlled/living radical polymerization, or click chemistry reactions. ROP is the most straightforward and practical approach for large-scale production of high MW polyethers, polyesters, polypeptides and POX. Bae et al. [47] reviewed the designed efforts to obtain smart PM from functional PEG-*b*-PAA block copolymers. PAA have been obtained by traditional solid-phase peptide synthesis methods, such as the condensation of monomers protected at one carboxyl and activated for polymerization at the other, followed by protecting group removal (protection/conjugation/deprotection). Activated amino acid monomers, e.g. activated esters or *N*-carboxyanhydride (NCA), have been used to obtain sequences longer than 10 repeating units. Fuchs–Farthing method has been used to obtain pure NCA with high yields, and good control of microstructure and MW [48].

Ding et al. [49,50] combined ROP with ATRP to obtain a cationic PAA vesicle for drug and gene-codelivery. ROP was used in the synthesis of poly(γ-2-chloroethyl-L-glutamate) (PCELG) using γ-2-chloroethyl-L-glutamate NCA as starting material and n-hexylamine as initiator. A "grafting from" approach was used to attach oligo(2-aminoethyl methacrylate hydrochloride) (OAMA) to the PCELG yielding amphiphilic poly(L-glutamate)-*g*-OAMA that formed vesicles in phosphate buffered saline (PBS). Authors not only encapsulated and released DOX·HCl but also, the cationic nature of the vesicles allowed the incorporation of DNA, which makes these vesicles promising carriers for codelivery of anti-cancer drugs and genes.

Free radical polymerization is a well-known and cheap synthetic route that can be used to obtain amphiphilic pseudo-block copolymers with an appropriate monomer distribution to allow self-assembling in suitable conditions. This occurs by the copolymerization of a hydrophilic monomer and a hydrophobic monomer of very different reactivity ratios. Lopez-Donaire et al. [51] synthesized copolymers based on oleyl 2-acetamido-2-deoxy-α-D-glucopyranoside (OAG) due to the potent anti-mitotic activity that OAG presents against glioma cells (U-373). For this purpose a methacrylic group was covalently linked through a carboxylic ester function at position C-6 on the pyranosidic ring, and the obtained methacrylic derivative was copolymerized with *N*-vinyl pyrrolidone. The big difference between the monomers reactivity ratios (r*OAGMA = 5.94 and r*$_{OAGMA}$ = 5.94 and r_{VP} = 0.01) gave rise to pseudo-blocks, with long sequences of the hydrophilic or the hydrophobic monomer. The monomer distribution and the hydrophilic/hydrophobic balance allowed the formation of NP in aqueous media [52]. Cell culture assays demonstrated a selective effect of the obtained NP that reduced significantly cell viability of human glioblastoma cell line (A-172) compared to normal human fibroblasts.

García-Fernández et al. also prepared antiangiogenic copolymers by free radical copolymerization of a methacrylic derivative of 5-amino-2-naphthalene sulfonic acid (MANSA) [53] and butyl acrylate (BA) by free radical polymerization. The copolymers poly(BA-*co*-MANSA) formed spontaneously stable NP in aqueous media. The antiangiogenic activity of the heparin-like system was related to the direct interaction of the sulfonic acid groups of MANSA with specific growth factors involved in endothelial cell migration and proliferation, and depended not only on the chemical composition but also on their supramolecular organization [54,55].

Nitroxide-mediated polymerization (NMP) [56], ATRP [57–60] and reversible addition and fragmentation transfer chain polymerization (RAFT) [61,62] are the main approaches used in the development of amphiphilic polymers, however due to its versatility and

the lack of metal catalyzers, RAFT has gained importance in the development of polymeric NP for cancer and anti-inflammatory applications [63,64].

Blunden et al. [65] prepared block copolymers by ring-opening polymerization of D,LD,L-lactide using a RAFT agent with an additional hydroxyl group, followed by the RAFT copolymerization of 2-hydroxyethyl methacrylate and 2-chloroethyl methacrylate. The well-structured polymers were modified to incorporate the ruthenium metallodrug RAPTA-C [$RuCl_2$(*p*-cymene)-(PTA)] in their structure using the Filkestein reaction. RAPTA-C inhibits cell growth by triggering G2/M phase arrest and apoptosis in cancer cells, and slowing cell division resulting selective and efficient on metastases *in vivo*. Zhao et al. have recently developed a pH sensitive acid degradable amphiphilic graft copolymer using two sequential controlled/living radical polymerization processes: RAFT and ATRP. Firstly, 2,4,6-trimethoxybenzylidene-1,1,1-tris(hydroxymethyl) ethane methacrylate (TTMA) was polymerized by RAFT and then 2-hydroxyethyl methacrylate was polymerized by ATRP. The well-structured copolymer formed NP by solvent exchange methods at pH 7.4 that presented low critical micelle concentration (CMC), high loading efficiency, excellent biocompatibility and rapid disassembly in acidic conditions [66].

Click chemistry offers a powerful toolbox for material scientists to design amphiphilic block copolymers for their application in biomedicine as click reactions present high efficiency under mild conditions, minimal and limited side reactions.

Xu et al. [67] reported the synthesis of dendron-like poly(ε-benzyloxycarbonyl-LL-lysine)/linear polyethylenoxide (PEO) block copolymers by the combination of ROP of ε-benzyloxycarbonyl-LL-lysine NCA and click chemistry. The encapsulation and controlled release of anticancer drug DOX highlight the potential of these systems in cancer therapy.

Click chemistry has been used not only in the synthesis of amphiphilic block copolymers of defined structure and architecture, but also in the functionalization of systems to provide them with complex functionalities [68]. In this sense Schoichet et al. [69] performed a Diels–Alder [4 + 2] cycloaddition and a Huisgen 1,3-dipolar cycloaddition azide-alkyne click reactions for surface modification of polymeric NP. The authors prepared poly(lactide-*co*-2-methyl-2-carboxytrimethylene carbonate)-*g*-polyethylene glycol-furan/azide NP that exhibit furan and azide dual functionality in the PEG corona. These moieties enabled orthogonal click reactions to sequentially conjugate trastuzumab-maleimide antibodies and alkyne-functionalized antisense oligonucleotide for gene silencing to the PEG corona. The modified NP were tested using the human ovary cancer line SKOV-3luc that express HER2 and firefly luciferase. The results obtained when cells were treated with dual functionalized NP (having both small interfering RNA (siRNA), and trastuzumab) in terms of gene knockdown were as good as lipofectamine LTX® transfection.

The thiol–ene reaction has also been used in the surface modification of NP. Van der Ende et al. [70] linked targeting peptides to polyester-based 3D-nano-networks. The particles (53 nm) were formulated based on poly(valerolactone-epoxyvalerolactone-allylvalerolactone-oxepanedione) containing 11% epoxy and crosslinked with 2 equivalents of diamines per epoxide. Targeting peptides (HVGGSSV and Arg-Gly-Asp (RGD)) were conjugated to the free allyl groups on the surface of the particles by a thiol–ene reaction carried out in DMSO.

Table 1 summarizes the main methods used in the preparation of amphiphilic synthetic polymers for application in cancer therapy.

Table 1 Representative methods used in the preparation of amphyphilic synthetic copolymers.

Method		Polymeric formulation	Synthesis of NP	Type of drug	Reference
Free radical polymerization		Amphiphilic poly(OAG-*co*-VP)	Nanoprecipitation in water using dioxane as solvent	OAG (antimitotic activity)	[51]
		Amphiphilic poly(BA-*co*-MANSA)	Spontaneous self-assembling in aqueous media	ANSA (antiangiogenic agent)	[53]
Controlled radical polymerization	RAFT	PNIPAm-*b*-PDMAm	Nanoprecipitation in THF	Dipyridamole (DIP)	[61]
		PHPMA-*b*-PPDSM	Crosslinking *via* hydrazone bonds and reducible disulfide bonds in methanol	DOX conjugation *via* acid-sensitive bonds	[223]
		Thermo-sensitive PNAS-*b*-PNIPAm-*b*-PCL	Spontaneous self-assembling in aqueous media into micellar aggregates	DOX encapsulation	[64]
		Amphiphilic triblock HPMA copolymer with enzyme-sensitive peptide GFLGKGLFG in its main chain	Self-assembling in aqueous media mediated by polymer–DOX block	DOX conjugation *via* enzyme-sensitive peptide	[218]
	ATRP	PEO-*b*-PG2MA	Micellization induced by slow addition of water over a solution of polymer in THF.	Indomethacin (anti-inflammatory drug) encapsulation	[59]
		(alkynyl-POEGMA-*b*-PDMA-*b*-PDEA)	Micellization by self-assembling in aqueous media induced by adjusting the solution pH to 9 with 0.1 M NaOH solution	----[60]Ring Opening Polymerization–	[60]
Ring opening polymerization		mPEG-*b*-PGlu	self-assembled NP in aqueous media *via* electrostatic interactions	DOX·HCl encapsulation	[133]

		PEG-*b*-PLA	Oil/water (o/w) emulsion	PTX encapsulation	[144]
		PLGA-*b*-PEG-*b*-PLGA	Dialysis	Curcumin encapsulation	[170]
		PCL-modified Pluronic P105	Dialysis	PTX encapsulation	[164]
Post-modification reactions	Thiol–ene	HVGGSSV and RGD conjugated on the surface of NP based on poly(valerolactone-epoxyvalerolactone-allylvalerolactone-oxepanedione)	Emulsification process	PTX encapsulation	[70]
	Click chemistry	poly(lactide-*co*-2-methyl-2-carboxytrimethylene carbonate)-*g*-polyethylene glycol-furan/azide NP	Co-self-assembly by membrane dialysis	Trastuzumab–maleimide antibodies and oligonucleotide conjugation	[69]
		KGRGDS conjugated on NP based onpoly(TMCC-*co*-LA)	Dialysis	–	[68]

2.3.2 Natural polymers

A variety of amphiphilic derivatives of polysaccharides and proteins for application in chemotherapy and anti-inflammatory therapies have been reported in recent years [71]. Among polysaccharides, it can be said that hyaluronic acid (HA) and chitosan have been the most extensively studied but other polymers such as heparine, chondroitin sulfate, DEX, starch and its derivatives, pullulans, and alginates (ALG) have also been investigated (see Figure 4). Some proteins are amphiphilic by nature or can be modified easily with amphiphiles to form micelles under different conditions, and can be applied as nanocarriers.

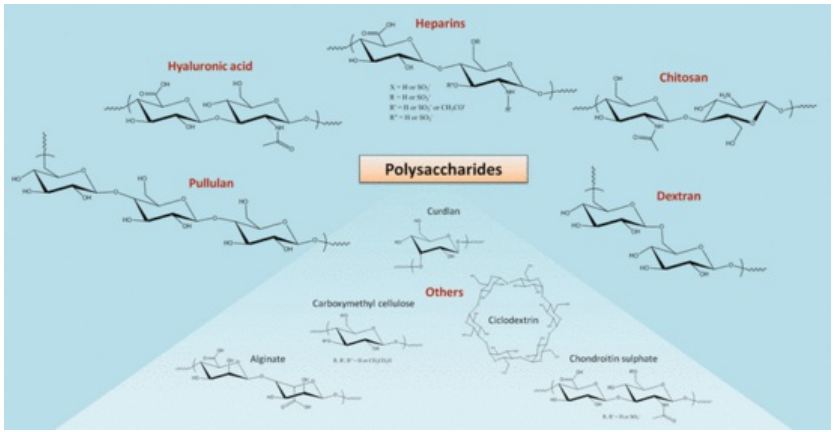


Figure 4 Chemical structures of polysaccharides applied in targeting and drug delivery.

Given the significant importance of a homogeneous and anhydrous environment to achieve high yields in coupling reactions [72], and avoid the limitations of conjugation in mixed water/organic solvents (such as the necessity of multiple activation steps) [73], it is critical to find an organic solvent in which both the natural polymer and the hydrophobic moiety are soluble. Some polysaccharides such as pullulans [72,74,75] or heparins [76,77] can be readily dissolved in organic solvents upon mild heating. For the solubilisation of other polysaccharides in organic solvents, inventive strategies have been designed. For instance, HA was solubilized in anhydrous DMSO by complexing with dimethoxy poly(ethyleneglycol) to generate HA–PTX bioconjugates with high yield without using any salts and blocking agents [73]. HA and heparins can be chemically modified mainly through their carboxylic acid or hydroxyl groups. It is noteworthy that carbonyl compounds preferably bind to their hydroxyl groups at C_6 [75]. Since polysaccharides have one reductive end, they can also be modified through this functional site. Functional amino groups may also be recovered from HA by deacetylation of the *N*-acetyl group [78], and from heparins by *N*-desulfonation [79]. Analysis of the chemical modifications on HA and heparin have been reviewed by Schanté et al. [80] and Fernández et al. [81], respectively. Concerning self-assembling systems for the treatment of cancer HA and heparins have been conjugated to a variety of hydrophobic moieties, from molecules as simple as acetyl groups linked by a thermally activated reaction (40 °C) between acetic anhydride and the hydroxyl groups on the polysaccharide [82], to biomimetic hydrophobic polymers such as poly(γ-benzyl L-glutamate) coupled using more sophisticated Click chemistry (Huisgen 1,3-dipolar cycloaddition using CuBr as the catalyst and *N,N,N′,N″,N‴,N‴*-pentamethyldiethylenetriamine (PMDETA) as a ligand) [83].

Amphiphilic HA has been obtained via esterification of its carboxylic acids by conjugation of with hydrophobic molecules such as monostearin [84] or octadecyl moieties [85] through 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/*N*-hydroxysuccinimide (EDC/NHS)-mediated reactions. EDC/NHS activation was also used for the amidation of HA via its carboxylic acids using histidine (His) [86], a number of different fatty amines and polyamines [87], as well as amine-functionalized 5β-cholanic acid [88] and deoxycholic acid (DOCA) [89], Amine-terminated hydrotropic oligomers have also been reacted with the carboxylic acid of HA in the presence of EDC and HOBt [90]. Heparin derivatives have also been obtained via amidation of its carboxylic acids. Heparin–DOCA

conjugates were prepared by linker-mediated coupling of *N*-(2-aminoethyl)-deoxycholylamide with the carboxylic groups of heparin in the presence of EDC [77]. Similar approaches were used to conjugate heparin with amine-terminated poly(β-benzyl-~~L~~-aspartate) using EDC [76] and aminated folate using EDC [76,91,92] or *N,N'*-dicyclohexylcarbodiimide (DCC)/NHS [93]. Aminoacids have been used as spacers for the conjugation of HA with PTX, where HA tetrabutylammonium salt was conjugated with 2'-aminoacyl-PTX using EDC/NHS activation [94]. Similar approaches consisting of previous amination of PTX or (docetaxel) DCT for subsequent reaction with the carboxylic acids of heparin in the presence of EDC/NHS were exploited to obtain heparin-PTX [95] and heparin-DCT conjugates [96], respectively. Curcumin and DOX have also been conjugated to HA, either by direct conjugation ~~via~~*via* DDC/*N,N*-dimethyl amino pyridine (DMAP)-mediated esterification [97], or by linker-mediated coupling using spacers such as adipic dihydrazide [98,99]. Retinoic acid has been conjugated with heparins in a number of works, either by amidation of their carboxylic groups with previously aminated retinoic acid using EDC [91,92,100,101] or ~~via~~*via* reaction of DCC/NHS-activated all-~~trans~~*trans* retinoic acid (ATRA) with hydroxyl groups of heparin [93]. Several strategies for the preparation of amphiphilic ALG with potential application in cancer therapy as nanocarriers exist in the literature. A library of amphiphilic ALG esters with different degree of substitution (DS) and hydrophobic alkyl length were synthesized by the reaction between partially protonated sodium ALG and aliphatic alcohols (octanol, dodecanol or hexadecanol) [102,103]. NP of alginic acid (ALG-H) and poly[(2-(diethylamino)ethyl methacrylate)] (PDEMA) were successfully prepared in aqueous medium using a polymer–monomer pair reaction system consisting of the anionic ALG and the cationic monomer DEA in presence of potassium peroxydisulfate ($K_2S_2O_8$) as a radical initiator. Ca^{2+} was introduced to gel the ALG moieties at the outer shell of NP that encapsulated hydroxycamptothecin (HCPT) [104]. Chitosan has been chemically modified by linking hydrophobic groups on hydroxyl [105] or amino groups. Due to the higher reactivity of amino groups, the majority of the modifications have used this group mainly through ~~N~~*N*-acylation reactions [106~~]~~,107].

N-octyl-*N,O*-carboxymethyl–low molecular weight chitosan (LMWC) was synthesized by two-step modification of LMWC to prepare self-assembled PM for encapsulation of PTX. Alkyl chains were conjugated to the firstly prepared carboxymethylchitosan using octanal and sodium borohydride ($NaBH_4$) as a reducing agent [108]. Conjugates of chitosan oligosaccharide with linoleic acid have been synthesized using EDC to obtain micelles as a carrier for DOX [109]. Stearic acid grafted chitosan oligosaccharide with different DS was also synthesized by EDC mediated coupling reaction. PTX was incorporated into the micelles and the surfaces of the micelles were further cross-linked with glutaraldehyde to form drug loaded and shell cross-linked NP with improved ~~in vivo~~*in vivo* stability [110]. Micelles of this type presented excellent internalization into cancer cells and accumulation in cytoplasm, either for PTX-loaded [111] or DOX-loaded [112]. Self-assembled amphiphilic *N*-(2,3-dihydroxypropyl)-~~chitosan-~~chitosan–cholic acid was synthesized by linking small molecules of cholic acid and glycidol onto primary amine group of chitosan, respectively [113]. Hydrophobically modified glycol chitosan (HGC) NP conjugated with interleukin-4 receptor binding peptides were prepared to enhance cellular uptake of NP in tumor tissues [114]. Some polysaccharides can only be modified through their hydroxyl groups or reductive ends, because of these are the only available functional groups. This is the case of pullulans, starch and its derivatives, DEX, cellulose or curdlan.

Various amphiphilic pullulans have been prepared for cancer treatment applications. Cholesteryl pullulan has been prepared by reacting pullulan with cholesterol succinate as a carrier for mitoxantrone (MTO) [115]. Further modification of cholesteryl pullulan with biotin was achieved by direct esterification of its hydroxyl groups with EDC/DMAP [116]. Folated pullulan was likewise prepared as a carrier for DOX by direct conjugation using DDC/DMAP in a simple, one-step reaction obtaining very high substitution yields [75]. Following the same method, folated pullulan was further coupled with the photosensitizer pheophorbide (Pba) [72]. Alternatively, DOX was chemically linked to pullulan by first functionalizating the polysaccharide with carboxylic acid groups ~~via~~*via* reaction with maleic anhydride, using TEA as a catalyst, and subsequent amidation with DOX in the presence of EDC/NHS and TEA [117]. This conjugate was further folated using the same catalytic system [117]. Folated pullulan–DOX conjugates were also obtained using a different strategy: oxidized pullulan was first conjugated to cysteamine and diamino-PEG, followed by conjugation of DOX to the cysteamine thiol groups and coupling of folic acid (FA) to the pending amino groups of diamino-PEG [118].

Akiyoshi et al. [119] have prepared very interesting amphiphilic systems through the conjugation of cholesterol to pululans of different MW. The addition of only 5~~%mol~~ mol% of cholesterol to the polysaccharide chains was enough to give self-assembled NP which offer excellent opportunities to be applied as nanocarriers for specific drugs with good stability and very low toxicity for healthy cells.

Amphiphilic starch derivatives have been prepared for cancer therapy using different routes. Hydroxyethyl starch (HES) was esterified with lauric, palmitic, and stearic acids under mild reaction conditions using DCC/DMAP. The amphiphilic derivatives gave rise to the formation micelles and polymeric vesicles [120]. Other researchers synthetized amphiphilic PLA grafted HES for the preparation of DCT-loaded micelles by graft polymerization of ~~D,L~~D,L-lactide with HES under partial protection of the hydroxyl groups with trimethyl chlorosilane (TMSCl) [121]. The preparation of starch–drug conjugates with 5-FU-1-acetic acid (FUAC) through ester bonds has been recently reported [122] ~~via~~*via* the coupling reaction with DCC/NHS.

Carboxymethylated curdlan was substituted with *N*-[4-[2-[(4-carboxyphenyl (amino) ethyl)] phenyl] sulfonyl]-N9-cyclohexylurea as a hydrophobic moiety by DCC/DMAP-mediated ester formation for the encapsulation of ATRA. This derivative was further conjugated to lactobionic acid using ethylenediamine linkers in the presence of DCC [123].

Amphiphilic protein derivatives with self-assembling properties to entrap anti-tumor drugs have been prepared by reaction of the amino groups of the protein. Li et al. [124] reported the reaction of gelatine with different amounts of hexanoyl anhydride in alkaline conditions. Conjugates of gelatine and oleic acid were synthesized by a novel aqueous solvent-based method that overcame the opposite solubility between gelatin and oleic acid [125]. Gelatin was conjugated with high contents of cholesterol using *N,N'*-disuccinimidyl carbonate. The gelatine–cholesterol conjugates formed micelles that entrapped curcumin for cancer research [126]. Hydrophobically modified casein, casein-*g*-poly(*N*-isopropylacrylamide) (PNIPAm), was prepared as a novel dual stimuli responsive. The amphiphilic casein was synthesized by free radical graft copolymerization of NIPAm in presence of the protein, using *t*-butyl hydroperoxide (TBHP) as an initiator [127]. A different strategy was reported for SF that basically consisted of silk based ionomers obtained ~~via~~*via* carbodiimide coupling of fibroin with poly(~~L~~-L-lysine) (PLL) hydrobromide and PGlu sodium salts, respectively. The assembly of the ionomers ~~via~~*via* electrostatic interactions led to particles where the PAA chains formed the core and the protein backbone the surface. DOX was encapsulated in the core of the ionomeric NP and it was released in a pH-dependent manner [128].

3 ROLE OF SYNTHETIC BIOSTABLE AND BIODEGRADABLE SYSTEMSole of synthetic biostable and biodegradable systems

A wide range of synthetic polymers have been extensively studied for the preparation of self-nanoassemblies. Focusing on the hydrophilic part of amphiphilic polymers that typically form the shell of nanocarries, commonly used synthetic polymers are: PEG, Pluronics®, HPMA and PGlu. Furthermore, hydrophobic polyesters such as PLA and PCL have been successfully used to prepare self-assembled nanocarriers. Other advanced polymers based on PA and POX are new promising platforms to create self-assembling nanostructures [4,129–131]. Advanced applications of these synthetic polymers for cancer therapies and anti-inflammatory therapies are described below.

3.1 Self--assemblies based on PEG

Among all hydrophilic polymers, PEG hydrophilic block is the most used for the preparation of self-assembling polymers due to several reasons. Mainly, PEG is a water soluble polymer, nonionic, nonvolatile, biocompatible and a poorly immunogenic polymer approved by the Food and Drug Administration (FDA) for its use in drugs products and pharmaceutical applications. PEG with a MW less than 30 kDa can be cleared from the body through renal filtration.

Additionally, the PEGylation is an essential strategy to improve the stealth properties of polymeric NP, and therefore, to reduce the adhesion of opsonins that are present in the blood serum. This methodology avoids the NP recognition by the RES, being camouflaged or invisible to phagocytic cells. Several theories have been proposed to explain this behavior. However, the most widely accepted is based on the extended conformation PEG chains on the NP surface which can create repulsive forces that effectively block the interactions of opsonins. The MW, the surface chain density and the conformation of PEG are critical factors to improve the blood circulation half-life of polymeric NP and therefore to favor their preferential accumulation in tumor tissues.

Table 2 relates the main nanocarriers developed using PEG for delivery of anticancer drugs in chemotherapy.

Table 2 A selection of main nanocarriers prepared with PEG and applied in cancer therapy.

Type of copolymer	Nanocarrier type	Drug/Bonding type	Cancer cell type	Biological/Clinical status	Reference	
PEG-*b*-PAA	PASA	PEG-*b*-PASA micelles protected by benzyl esters	DOX encapsulation	Human prostate and lung cancer	*In vitro*	[141]
		PEG-*b*-PASA NP	PTX encapsulation	12 human tumor cell lines	Phase III clinical trials (NK105)	[135]
		PEG-*b*-PASA NP (pH-sensitive)	DOX Conjugation (hydrazide linker)	----------	-	[192]
	PGlu	Hybrid micelles (PPhe hydrophobic core, cross-linked PGlu shell layer and PEG external shell)	CDDP, PTX encapsulation	Human ovarian cancer cells	*In vitro* and *in vivo*	[142]
		PEG-*b*-PGlu NP with FA conjugated on the surface	DOX, SPIONs encapsulation	Human hepatic carcinoma cells	*In vitro*	[134]
		Polymer--metal complex formation between PEG-*b*-PGlu block copolymers	CDDP conjugation	12 human tumor cell lines	Phase II (NC6004)	[195]
PEG-*b*-polyester	PLA	mPEG-*b*-PDLLA micelles	PTX encapsulation	Lung, ovarian, breast and gastric cancer	Commercial market Genexol®-PM	[147]
		PEG-*b*-PLA polymersomes	DOX encapsulation	Breast adenocarcinoma cells	*In vitro*	[148]
		c(RGDyK)-PEG-*b*-PLA micelles	PTX encapsulation	Glioblastoma cells	*In vitro* and *in vivo*	[159]
		PEG-*b*-PLA NP	Cisplatin, PTX conjugation	SKOV-3 human ovarian carcinoma cells, U14 xenograft model of cervical carcinoma	*In vitro* and *in vivo*	[207]
	PCL	mPEG-*b*-PCL micelles	Luteolin encapsulation	4T1 breast and C26 colon adenocarcinoma cells	*In vitro* and *in vivo*	[163]
		PEG-*b*-PCL micelles	PTX, cyclopamine, gossypol encapsulation	ES-2 and SKOV3 human ovarian cancer cells	*In vitro* and *in vivo*	[164]
		CGKRK-PEG-*b*-PCL micelles	PTX encapsulation	HUVEC and Human U87MG cells	*In vitro* and *in vivo*	[166]
PEG-b-PLGAPEG-*b*-PLGA		PEG-*b*-PLGA micelles	THPP encapsulation	HN5 head and neck and H2009 lung cancer cells	*In vitro*	[169]

		Folate-targeted PLGA-*b*-PEG NP	17-AAG encapsulation	MCF7 human breast adenocarcinoma cells	*In vitro*	[175]
		APRPG peptide-modified PEG-*b*-PLGA NP	TNP-470 encapsulation	HUVECs and SKOV3 ovarian cancer cells	*In vitro* and *in vivo*	[176]
~~Others~~Others		PEG-*b*-poly(methacrylic acid) cross-linked micelles	Cisplatin, encapsulation and metal complex formation	A2780 human ovarian carcinoma cells	*In vivo*	[183]
		PEG-*b*-DSPE and TPGS NP	PTX, parthenolide, encapsulation	A549 and A549-T24 human lung adenocarcinoma cell lines	*In vitro*	[185]

3.1.1 Drug-loaded self-assembled systems based on PEG

Self-assembled PM with hydrophilic shell of PEG can be composed of a wide range of hydrophobic polymers to form the inner core of different self-assembling structures that have been successfully used to encapsulate several drugs. The most frequently investigated block copolymers to design these drug-loaded self-assembled systems are PEG-*b*-PAA, PEG-*b*-polyesters and PEG-*b*- PLGA, as are described below in detail.

3.1.1.1 PEG-b-PAA block copolymers Self-assembling block copolymers of PEG-*b*-PAA are probably one of the most promising vehicles to improve anti-tumor drug delivery. Self-assembled NP are formed by a hydrophilic PEG shell and a PAA core that can encapsulate different anti-tumor and anti-inflammatory drugs with significantly low toxicity compared with free drugs. PAAs provide interesting properties such as an excellent biocompatibility, biodegradability and nontoxicity. Moreover, PAAs have high versatility with a wide range of functional groups such as hydroxyl, carboxyl, amino and thiol groups that can be used for the modification of the chemical structure of the nanoassembled systems in order to improve the drug loading and other physico-chemical properties. Several self-assembling polymer systems based on PEG-*b*-PAA have been widely studied for advanced cancer and inflammation therapies. The most typical PAA used for these systems are poly(aspartic acid) (PAsp) and PGlu [47,132–134].

Micelles based on PEG-*b*-PAsp block copolymers have been extensively investigated to encapsulate other hydrophobic drugs. Hamaguchi et al. incorporated PTX by physical entrapment into the inner core of micelles based on PEG-*b*-PAsp copolymers modified with 4-phenyl-1-butanol by esterification reaction (designated as NK105). After freeze drying, the drug loading was about 25%. These micelles were stable and significantly small in size (approximately 85 nm) that allowed a uniform distribution and great accumulation in tumor tissues. Particularly, NK105 was evaluated *in vitro* on 12 human tumor cell lines, obtaining a similar cytotoxicity to PTX. *In vivo* results showed substantial tumor suppression in a dose dependent manner [135–137]. Currently, Phase III clinical trials are ongoing with promising results. In fact, NK105 showed a reduced toxicity with few adverse reactions in patients at the early stage (Phase I/II clinical trials) of clinical development [7,138–140].

Eckman et al. [141] investigated the importance of interactions between drugs and polymers by the preparation of DOX-loaded micelles based on PEG-*b*-PAsp block copolymers in three different core environments. Basically, the carboxyl groups of PEG-*b*-PAsp were protected by benzyl esters (hydrophobic interactions) and ionized by sodium salt or remained as free acids, in order to encapsulate DOX through hydrophobic or ionic interactions, respectively. The ionic interactions between DOX and inner core of micelles favored the stability, the prolonged release of the drug in a pH-dependent manner and the effective cytotoxicity against prostate and lung cancer cell lines. The increase of the hydrophobic character of NP could be an interesting route to improve their anti-cancer activity. In this way, Shixian et al. [132] investigated the incorporation of polyphenylalanine (PPhe) in order to increase the stability of these micelles through hydrophobic and aromatic interactions. Particularly, they synthesized methoxypoly(ethylene glycol) mPEG-*b*-poly(glutamic acid-*co*-~~L~~-PPhe) triblock copolymers that self-assembled into NP where DOX·HCl was successfully loaded by simple mixing in the aqueous phase. The appropriate combination of electrostatic interactions between PGlu domains and the cationic drug and the stabilizing effect of PPhe domains allowed to optimize the therapeutic efficacy of these micelles that exhibited higher cell proliferation inhibition and toxicity compared with free DOX·HCl against human pulmonary carcinoma cells (see Fig~~ure~~. 5).

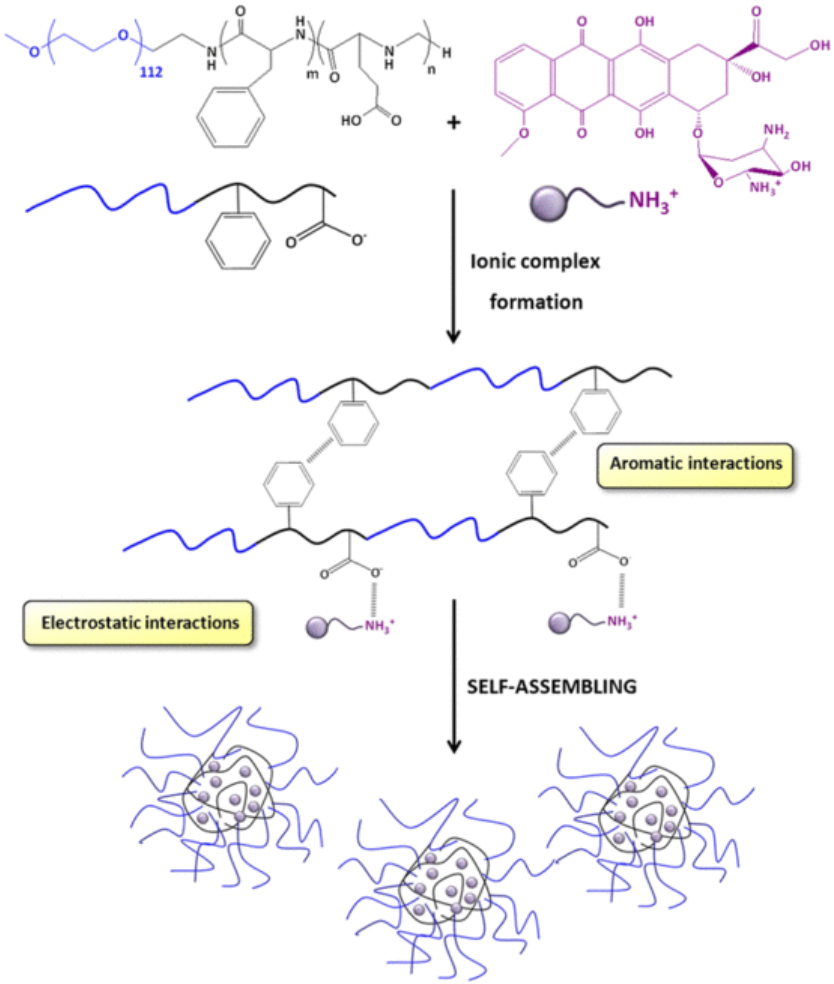


Figure. 5 Aromatic and electrostatic interactions involved in the formation of DOX-loaded NP.

Desale et al. [142] reported the synthesis of triblock copolymers formed by PEG, PGlu and PPhe (PEG-b-PGlu90-b-*b*-PGlu$_{90}$-*b*-PPhe$_{25}$) with the aim to prepare hybrid micelles for the loading of drugs with different physico-chemical properties. In particular, a hydrophilic derivative of cisplatin (CDDP) and hydrophobic PTX. Specifically, hybrid micelles were designed with multi-compartment morphology, characterized by PPhe hydrophobic core, cross-linked PGlu intermediate shell layer and PEG external shell. In addition, micelles exhibited higher anti-cancer activity compared to individual drug-loaded micelles. These results suggested the promising potential of these carriers for chemotherapeutic drug combination delivery with attractive advantages such as the synergistic therapeutic effects of the drugs and spatial-–temporal synchronization of drug exposure. Other route to enhance the intracellular delivery of low MW drugs is the use of disulfide bearing carriers. In this way, Thambi et al. [143] prepared amphiphilic PEG-*b*-poly(γ-benzyl L-glutamate) (PEG-*b*-PBLG) block copolymers that were synthesized by ring-opening polymerization of benzyl glutamate in the presence of a PEG macro-initiator bearing disulfide bond (PEG-SS-NH$_2$). These amphiphilic copolymers self-assembled in aqueous medium, encapsulating effectively camptothecin (CPT) with a drug loading of about 12%. These biodegradable micelles released the drug in the presence of glutathione (GSH) that is an abundant cytoplasmic peptide capable to reduce disulfide bonds.

3.1.1.2 PEG-b-b-polyester block copolymers PEG-*b*-polyester copolymers have received considerable attention for the preparation of self-assembled nanostructures. Particularly, nanoassemblies composed of PLA inner core have demonstrated a great potential for the delivery of different agents [144]. In fact, Genexol®-PM is a polymeric micelle-based formulation of PTX encapsulated in mPEG-*b*-poly(D,L-D,L-lactide) (mPEG-*b*-PDLLA) micelles that is commercially available for treatment of non-small cell lung cancer (NSCL), ovarian cancer, breast cancer and gastric cancer. This polymeric formulation was synthesized by a ring-opening polymerization and PTX was loaded using a solid dispersion technique with a drug loading of about 17%. *In vitro* and *in vivo* studies demonstrated that mPEG-*b*-PDLLA micelles were biocompatible with remarkable anti-tumor efficacy against various human cancer cell lines. Moreover, a rapid release of PTX from micelles was observed, probably due to their decomposition in the presence of α- and β-globulins in the blood [7,137]. Particularly, Genexol®-PM showed superior efficacy and less adverse reactions compared with free PTX. Due to the

encouraging anti-tumor activity and the safety profile, different Phases of clinical trials have been developed in patients with excellent results, reaching the commercial market [145–147].

In a study by Garkhal et al. [148], DOX-loaded polymersomes were prepared by nanoprecipitation method using amphiphilic PEG-*b*-PLA copolymers. These polymersomes showed a favoured profile release at acidic pH, exhibited enhanced cellular uptake of DOX and improved cytotoxicity compared to free DOX using breast adenocarcinoma cancer cells. Currently, polymersomes represent an excellent alternative platform to NP or micelles for the encapsulation of different drugs in the treatment of cancer and inflammation diseases [149,150].

Blanco et al. encapsulated β-lapachone (β-lap) that is a novel poor water soluble anti-cancer drug. Particularly, PEG-*b*-PLA micelles were prepared using a film sonication technique, obtaining particles with core–shell architecture and optimal size (approximately 30 nm). In two types of subcutaneous lung carcinoma models, these micelles showed prolonged blood circulation times, significant accumulation in tumor tissues ~~via~~ via EPR effect and interesting anti-cancer efficacy [151,152]. On the other hand, Siddiqui et al. [153] demonstrated that the load of an extract of green tea, epigallocatechin-3-gallate (EGCG), into biodegradable micelles based on PLA-*b*-PEG block copolymers allowed retaining its biological effectiveness and enhancing its tumour growth suppressive properties. Moreover, the use of over 10-fold dose advantage of EGCG was possible to exert its proapoptotic and angiogenesis inhibitory effects in both *in vitro* and *in vivo* models for the treatment of prostate cancer [153,154].

Mu et al. [155] prepared mixed micelles of PEG-*b*-PLA and Pluronic® copolymers in order to encapsulate DCT and improve significantly its therapeutic effectiveness compared to Taxotere®. The appropriate ratio of PEG-*b*-PLA and Pluronic® copolymers enabled to obtain stable micelles with more potent anti-tumour activity than the commercial DCT formulation, probable due to the inhibitory activity of Pluronic® copolymer against ~~P-glycoprotein (P~~-glycoprotein (*P*-gp) [152,155].

On the other hand, different authors have explored the preparation of Fol targeting NP with pH-sensitivity [152,156]. In this direction, Gao et al. synthesized different pH-sensitive mixed micelles formed by two types of copolymers: mixture of FA-conjugated to PEG-*b*-PLA and PEG-*b*-poly(~~L-HISTIDINE) (POLY(L~~-histidine) (poly(L-His)) copolymers as well as PEG-*b*-poly(~~L~~L-His-*co*-PPhe) (PEG-poly(~~L~~L-His-*co*-PPhe)) copolymers (designed as first and second generation of micelles, respectively). In both cases, these micelles were successfully used to encapsulate DOX. The incorporation of poly(~~L~~L-His) allowed to endowing of pH sensitivity to the particles. The combination of Fol targeting and pH sensitivity considerably improved the anti-cancer activity of DOX [157,158].

Zhang et al. studied other class of tumour targeting ligands based on the use of different sequences of peptides that recognize integrins, overexpressed in a wide range of solid tumours [152]. Specifically, they prepared PTX-loaded PEG-*b*-PLA micelles. Moreover, cyclic RGD peptide, cyclic Arg–Gly–Asp-d-Tyr-Lys, c(RGDyK), was effectively conjugated in order to enhance the treatment of integrin αvβ3 overexpressed glioblastoma. Biological experiments confirmed the efficacy and great potential of the incorporation of RGD-containing peptides into these micelles, improving the anti-glioblastoma cell cytotoxic efficacy by 2.5-folds and exhibiting a potent tumour growth inhibition [159].

PCL is another biodegradable polyester that has been extensively investigated as hydrophobic block to prepare micelles based on PEG copolymers for advanced treatments of cancer and inflammation diseases. The optimal combination of hydrophilicity, biodegradability and mechanical properties of these assemblies has enabled their use as delivery systems for a wide range of poor soluble drugs [160]. For the treatment of inflammation, Wang et al. [161] synthesized tacrolimus-loaded micelles by self-assembly of PCL-*b*-PEG-*b*-PCL triblock copolymers. Tacrolimus-loaded assemblies were prepared by a solid disperse method with a high drug loading and encapsulation efficiency (maximum values of 22.5 and 95.5%, respectively). *In vitro* results of these micelles showed a sustained drug release and suitable cytotoxicity for their use for immunosuppressive therapy.

Combination of two complementary drugs is an attractive approach. Gong et al. developed curcumin-loaded biodegradable self-assembled polymeric PEG-*b*-PCL micelles with a size lower than 30 nm. These micelles were also used to encapsulate luteolin. *In vitro* and *in vivo* studies for curcumin and luteolin loaded micelles confirmed their potential for the treatment of breast and colon cancers [162,163]. Additionally, PEG-*b*-PCL micelles have been investigated as vehicles for combined cytotoxic agents delivery in the treatment of tumours with a high rate of chemoresistance. Lai et al. [164] loaded 3 types of drugs in the core of PEG-*b*-PCL micelles: PTX (cytotoxic agent), cyclopamine (hedgehog inhibitor) and gossypol (Bcl-2 inhibitor). They investigated exhaustively the most optimal combination of these drugs to achieve their solubilization, obtaining a maximum load efficiency of each drug of about 2%. After intravenous injection of these multidrug loaded micelles, a significant tumour growth inhibition and prolonged survival was demonstrated against metastatic ovarian cancer. Furthermore, Peng et al. [165] studied the growing interest of drug and photodynamic combination therapies in order to improve cancer treatments. Particularly, a derivative of campthothecin (7-ethyl-10-HCPT (SN-38)) was loaded into chlorine-core star-shaped micelles that were prepared by conjugation of chlorine derivative to mPEG-*b*-PCL block copolymer.

Different authors have also decorated the surface of PEG-*b*-PCL micelles with small molecules with the aim to enhance the delivery of poor soluble drugs ~~via~~ via EPR effect and achieve active targeting. Antibodies, herceptin, FA and peptides are representative examples of molecules that target specific receptors, overexpressed in inflammation processes and involved in the most aggressive cancers. Hu et al. [166] decorated the surfaced of PEG-*b*-PCL micelles with a specific peptide sequence (CGKRK) to obtain angiogenic blood vessels and tumour cells dual targeting effect. Moreover, PTX was successfully loaded in order to evaluate the enhancement of its cytotoxicity using human umbilical vein endothelial cells (HUVEC) and tumour cancer (U87MG) cells. In fact, peptide decoration improved the apoptosis induction and anti-proliferative activity of PTX.

Finally, PLGA is one of the most successfully biodegradable and biocompatible polyesters used for the development of nanomedicines, because its hydrolysis leads to lactic acid (LA) and glycolic acid that are endogenous and easily metabolized by the body. In fact, PLGA is approved by the FDA and European Medicine Agency (EMA) for its use as a drug delivery system in humans. PLGA NP have been used as carriers for chemotherapeutic and anti-inflammatory drugs, photosensitizers and imaging agents for the diagnosis and treatment of cancer and inflammation diseases. The PEG conjugation to PLGA enables the preparation of self-assembling micelles with a hydrophilic shell based on PEG and a hydrophobic core of PLGA [23,167–169]. Song et al. [170] loaded curcumin into the inner core of NP based on PLGA-*b*-PEG-*b*-PLGA copolymers. A dialysis method was used to form self-assembled micelles with an average size of 26 nm and entrapment efficiency of 70%. *In vivo* studies demonstrated an improved biodistribution of curcumin. The conjugation of different target moieties is an effective method for improving the targeted ability of these NP by the ligand-receptor recognition. Different peptides, antibodies and small molecules as FA have been successfully linked to PLGA-*b*-PEG NP [171–174]. Saxena et al. [175] conjugated FA to PLGA-*b*-PEG NP and their inner core was used to load of 17-allylamino-17-demethoxy geldanamycin (17-AAG), that is a hydrophobic inhibitor of heat shock protein 90 (HSP90). Fol-targeted NP were selectively uptaken by breast cancer cells. Moreover, the anti-cancer activity of these micelles was 2-fold higher than that of non-targeted NP. Additionally, Wang et al. [176] modified PLGA-*b*-PEG NP with the aim to incorporate Ala-Pro-Arg-Pro-Gly (APRPG) peptide.

3.1.1.3 Other drug-loaded self-assembled systems based on PEG One of the most important drawbacks of the use of PEG nanocarriers for cancer treatment is their poor stability. This disadvantage has an appreciable impact for the appropriate control of circulation times of assemblies in blood and the drug delivery efficiency. Different strategies have been proposed in order to improve the stability of polymeric systems that include PEG in their structure.

Lai et al. reported the use of new lipophilic moieties based on cinnamic acid and 7-carboxyl methoxycoumarin that are conjugated using the terminal groups of mPEG. These small molecules have π–π conjugated structures that enhance the load of poor water soluble drugs into the nanoassemblies as well as their stability. Particularly, micelles were used to encapsulate different anti-cancer drugs, such as DOX or an active derivative of CPT (9-nitro-20(S)-CPT). In all cases, the results showed that both hydrophobic and π–π conjugated interactions contributed to the self-assembly. Furthermore, these assemblies had promising anti-cancer activities and could represent a promising strategy to produce stable nanocarriers for cancer diseases [177–179].

Other attractive alternative to improve the stability of nanoassemblies is the use of cross-linked micelles. The characteristic morphology and structural properties of these nanoassemblies allow achieving an exceptional stability, a prolonged circulation time in blood and an appropriate regulation of the drug release. Cross-linked micelles have been investigated to load anti-cancer drugs, like DOX and cisplatin [180–182]. Particularly, Oberoi et al. [183] prepared core cross-linked micelles composed of PEG-*b*-poly(methacrylic acid) (PEG-*b*-PMAA) where cisplatin was successfully loaded. The average size of these micelles was 110 nm with a loading capacity of about 30%. *In vitro* and *in vivo* studies demonstrated the efficacy of these assemblies for the treatment of ovarian cancer with an improved safety profile, tumour accumulation and anti-cancer activity relative to the use of free cisplatin. On the other hand, Talelli et al. [184] synthesized core cross-linked micelles based on block copolymers of PEG-*b*-poly[*N*-(2-hydroxypropyl) methacrylamide-lactate]. After the formation of micelles by rapid heating, DOX was encapsulated and then copolymerized through the methacryltate groups. For this purpose, DOX was previously functionalized with a methacrylamide group using hydrazone bond in order to obtain core-crosslinked micelles with pH-sensitivity. In these specific conditions, the cellular uptake by endocytosis and intracellular release of DOX were significantly favoured due to the slightly acidic pH of the intratumoral environment. Moreover, these assemblies had excellent anti-tumoral behaviour against human ovarian carcinoma cells.

Effective strategies to improve the solubility of poor water soluble drugs have been proposed combined with advanced clinical trials and final commercialization. In this sense, PEG-*b*-lipid micelles formed by hydrophilic PEG blocks and hydrophobic distearoyl phosphatidylethanolamine (DSPE) segments have been extensively investigated. These vehicles have interesting properties for delivery of hydrophobic drugs with appropriate control of their release and high stability due to the presence of long fatty acyl chains [185]. Tong et al. [186] prepared docetaxel-loaded micelles based on mPEG-*b*-DSPE. DCT-loaded micelles showed anti-tumour efficacy and retarded tumour growth in mice bearing breast cancer.

Finally, the introduction of an “intelligent” stimulus to improve the drug release from micelles has been extensively applied in last years. Zhu et al. [187] evaluated the anti-tumour activity of acid-sensitive micelles that were obtained by directly conjugating PEG to a hydrophobic derivative of stearic acid through hydrazone bond. These assemblies served as vehicles of an analogue of gemcitabine (4-(*N*)-stearoyl gemcitabine). *In vivo* assays demonstrated a higher anti-cancer activity of pH-sensitive micelles than that of non-sensitive assemblies using murine B16–F10 tumors.

3.1.2 Drug-conjugated self-assembled systems based on PEG

All above reported nanoassemblies incorporated different drugs into their hydrophobic segments through physical entrapment. However, a wide range of active molecules has been covalently attached to amphiphilic copolymers that include PEG in their structure. The preparation of polymer–drug conjugates was proposed by Ringsdorf in the mid-1970s. Since then, the covalently attaching of chemotherapeutic and active agents to polymer chains has greatly developed due to improvement of their physico-chemical, biopharmaceutical and pharmacokinetic properties [188–191]. These systems now are called ‘polymeric drugs’ and its application ‘polymer therapeutics’.

Liu et al. used PEG with the aim to conjugate DCT through an ester linkage and to form amphiphilic micelles in aqueous medium. The efficiency of these micelles was improved by encapsulation of free DCT into their inner core. The *in vitro* anti-cancer activity of this formulation was demonstrated using three different human cancer cell lines [192].

Conjugates based on PEG-*b*-PAA copolymers, including PAsp and PGlu as hydrophobic blocks, have reached various stages of clinical trials for the treatment of cancer diseases [137,139,189,191,193]. NC-6004 is a hydrophilic derivative of cisplatin (CDDP) formulation based on polymer–metal complex forming chain. Particularly, the coordinate complex of PGlu and CDDP forms the inner core of micelles with outer shell of PEG. These micelles were exhaustively characterized by Uchino et al. [137,194]. This formulation was evaluated on 12 human tumor cell lines, preserving the anti-tumor activity of CDDP and reducing its nephrotoxicity. For those reasons, Phase I/II clinical trials with NC6004 have been recently developed with advanced pancreatic cancer [139,195].

Additionally, Cabral et al. prepared analogous micelles (designed as NC-4016), containing dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt) that is an oxalilplatin parent complex [196,197]. The safety and anti-tumor activity of NC-4016 was evaluated by Nanocarrier ® in Japan. In fact, Phase I clinical trials started in 2009 [137].

Koizumi et al. developed PEG-*b*-PGlu PM (named as NK012) to encapsulate an active metabolite derivative of CPT (SN38). In particular, this drug was conjugated to PGlu segment of block copolymer [198,199]. Micelles exhibited a controlled size of 20 nm and approximately 20% of drug loading. Furthermore, these PM showed excellent *in vitro* and *in vivo* anti-tumor activities, especially in highly vascular endothelial growth factor (VEGF) secreting tumors [139,200]. Different clinical trials are currently undergoing with the aim to evaluate the real efficiency of this formulation in patients [201,202]. Up to date, the anti-tumor activity of this conjugate has been evaluated in several orthotopic tumor models including glioma, renal cancer, stomach cancer, and pancreatic cancer with promising results. They demonstrated enhanced distribution and prolonged SN-38 release as well as partial responses and several occurrences of prolonged stable diseases in patients [203].

PEG-*b*-PAsp copolymers have also served to attach poor soluble water drugs. The micelle carrier NK911 was formed by the block copolymer of PEG (MW of about 5000) and PAsp (about 30 units) was chemical conjugated with DOX. Moreover, free DOX was encapsulated in the inner core of these micelles. This combination allowed the formation of self-assembling micelles with particle size of about 40 nm that could be accumulated in tumor tissues [137,204,205] and possessed higher anti-cancer activity than the free DOX. Phase I/II clinical trials were performed at National Cancer Center Hospital in Japan with promising results. Among the 23 patients, a partial response was obtained in one patient with metastatic pancreatic cancer and 8 had stable disease [7,206].

Recently, Ponta and Bae [192] have applied PEG-*b*-PAsp block copolymers to conjugate DOX through different hydrazide bonds as spacers. NP were prepared by a dialysis method with hydrodynamic sizes lower than 50 nm. *In vitro* studies demonstrated that the drug release from micelles was pH-dependent and it was tuned as a function of the chain length of the blocks and the type of spacer used.

Other researchers have also prepared conjugates based on PEG and polyesters, such as PEG-*b*-PLA block copolymers. Xiao and Song [207] prepared micelles by co-assembling the 2 different conjugates containing cisplatin and PTX. Both hydrophobic drugs were conjugated to PEG-*b*-PLA copolymers that self-assembled into micelles with an inner core composed of PLA block and PEG hydrophilic shell (see Figure 6). These nanoassemblies showed an effective anti-tumor activity and inhibiting the tumor growth using a U14 xenograft model of cervical cancer with superior efficacy compared to free drugs solutions.

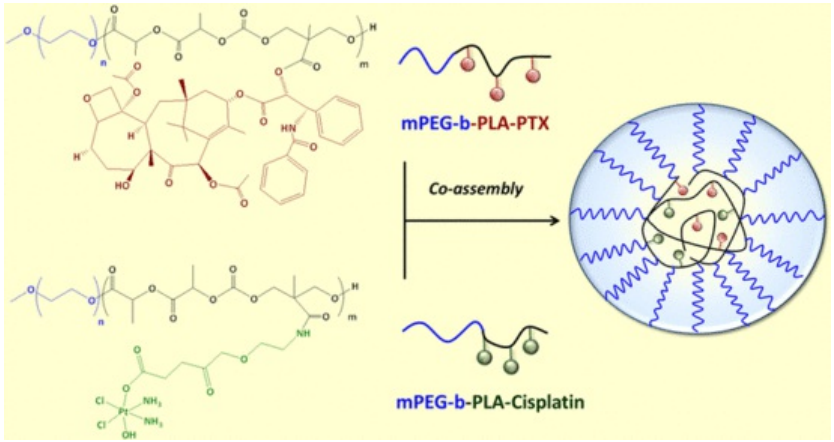


Figure 6 Chemical structures of cisplatin and paclitaxel conjugated to PEG-*b*-PLA copolymers and representative mechanism of their co-assembly in aqueous enviroment.

3.2 Self-assemblies based on Pluronics®

Pluronic® block copolymers have been exhaustively investigated as an excellent alternative for the preparation of self-assembling systems and the solubilization of hydrophobic drugs. These amphiphilic polymers are composed of hydrophilic PEO and hydrophobic poly(propylene oxide) (PPO) segments, organized in triblock structures (PEOx-*b*-PPOy-*b*-PEOx) with a wide range of molecular weights and PEO/PPO ratios. Pluronic® copolymers have important advantages for their use in nanoassemblies for cancer treatments because they can be modified by reactions through the terminal hydroxyl groups in order to achieve the optimal targeting in tumor site [208].

Different binary mixing Pluronic® micelles have been developed to form stable self-assembling carriers for anti-cancer drugs. Particularly, Fang et al. [209] prepared Pluronic® P105 and F127 block copolymers that self-assembled into micelles in order to encapsulate methotrexate (MTX) as anti-tumor compound. MTX-loaded mixed micelles exhibited *in vitro* and *in vivo* anti-tumor activities and optimal pharmacokinetic parameters. Wang et al. [210] prepared a PTX-loaded Pluronic® P105 micellar system by a thin-film method, obtaining micelles with small particle size, spherical shape and excellent stability. *In vitro* and *in vivo* activities, pharmacokinetics and biodistribution parameters were evaluated with promising results that suggested that P105 PM could be a useful drug carrier for intravenous administration of PTX.

Other mixed Pluronics® micelles have been studied to encapsulate DOX. In fact, Pluronics® can interact with MDR cancer cells obtaining a significant sensitization of resistant tumors with respect to DOX. For instance, DOX-loaded micelles composed of Pluronic® L61 and F127 (formulation designed as SP1049C) have already achieved Phase III of clinical trials. In this formulation, the most effective regulator of DOX activity against a wide range of tumor cell lines was Pluronic® L61. Promising results indicated that out of 19 patients evaluated, 9 patients had a partial response without a complete response and 8 patients had a stable disease. Moreover, the median overall survival was longer than for formulation based on free DOX [211–213].

Currently, active targeted delivery therapy using Pluronic® block copolymer micelles is being investigated. Song et al. [214] developed PTX-loaded Pluronic® P123 micelles that were used to chemically conjugate anti-HIF-1a antibody with the aim to selectively target cancer cells with overexpression of HIF-1a. In the same way, FA-functionalized Pluronic® P123/F127 mixed micelles were evaluated *in vitro* and *in vivo* by Zhang et al. [208]. The biological assays demonstrated the selective and active targeting of these self-assembled micelles.

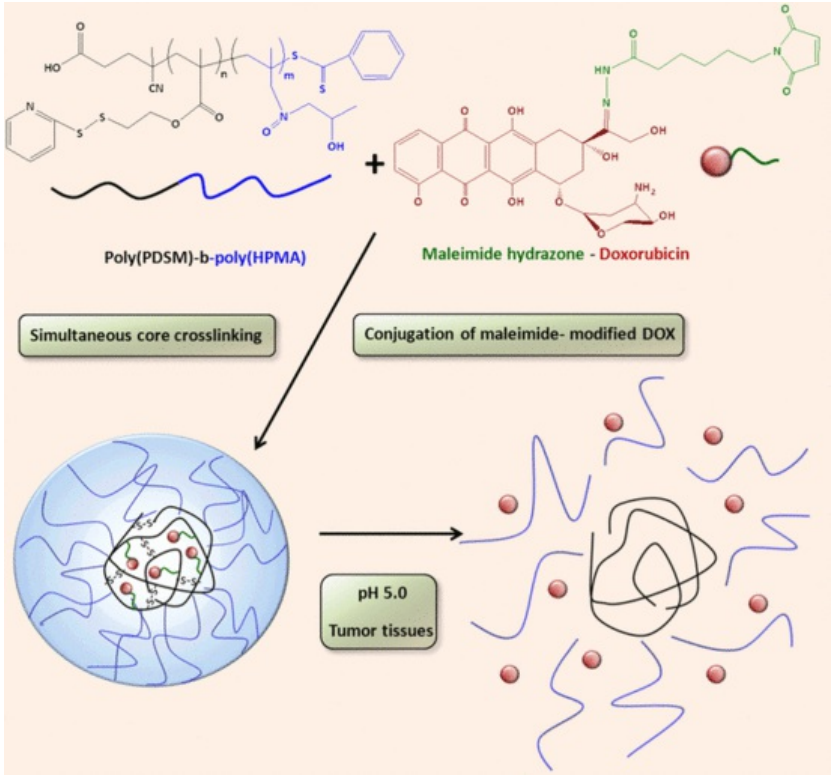
3.3 Self-assemblies based on 2-hydroxypropyl methacrylamide (HPMA)

HPMA is a hydrophilic, non-immunogenic and biocompatible polymer that is very attractive to form the shell of polymeric nanoassemblies. Moreover, HPMA has an interesting multifunctionality based on the presence of secondary groups in its structure that facilitates the conjugation with different poor water soluble drugs and targeted moieties. In fact, the conjugation of hydrophobic drugs to hydrophilic HPMA polymer facilitates the formation of nanoassemblies that could be attractive drug delivery systems for the treatment of inflammation and cancer diseases [215–218]. Chytil et al. [219] conjugated DOX to a HPMA copolymer using different contents of various hydrophobic substituents, particularly, dodecyl, oleoyl and cholesterol moieties. These conjugates self-assembled in aqueous solutions forming supramolecular structures with an optimal stability and a range of hydrodynamic sizes between 13 and 37 nm as a function of the type and content of hydrophobic substituents. Moreover, these assemblies released DOX at acidic pH

(approximately 70% DOX released after 24 h at pH 5.0), that is, the conditions in endosomes and lysosomes of tumor cells. *In vivo* experiments showed that theses micelles exhibited significant anti-tumor activity against EL-4 T lymphoma cells and enhanced tumor accumulation due to the EPR effect.

In a study by Miller et al., a conjugate of HPMA copolymer with anti-angiogenic and cytotoxic drugs, aminobisphosphonate alendronate (ALN) and PTX, was successfully prepared. Particularly, PTX was conjugated through specific peptide linker that can be cleaved by lysosomal enzymes in order to release both drugs. This conjugate self-assembled in aqueous environment into particles with sizes of approximately 100 nm. These nanoassemblies demonstrated significant anti-angiogenic activity, inhibiting the proliferation of prostate carcinoma cells. These nanoconjugates have potential for the treatment of prostate cancer bone metastases and osteosarcomas [220–222].

Jia et al. [223] synthesized new amphiphilic block copolymers of HPMA with 2-(2-pyridyldisulfide)ethylmethacrylate (PDSM) ~~via~~via RAFT polymerization. The high versatility of PDS groups was used to covalently conjugate maleimide-modified DOX ~~via~~via acid-sensitive bonds with the aim to obtain assemblies with pH-sensitivity (see Fig~~ure.~~ 7). In aqueous environment, this block copolymer self-assembled through acid-cleavable hydrazone and cross-linked ~~via~~via reducible disulfide bonds. *In vitro* studies showed that cross-linked micelles released preferentially DOX at pH 5.0.



Fig~~ure.~~ 7 Preparation of advanced HPMA nanoparticles by simultaneous conjugation of DOX and core-crosslinking ~~via~~via reducible disulfide bonds.

On the other hand, different authors have been investigated the use of nanoassemblies based on polymer conjugates in order to improve the selectivity and tumor-imaging capacity of small molecular photosensitizers that are usually used for photodynamic therapy (PDT) [224,225]. In this way, Nakamura et al. [226] conjugated zinc protoporphyrin (ZnPP) to HPMA polymer for its application for diagnosis and cancer treatment by imaging and light exposure, respectively. NP based on this conjugate exhibited a hydrodynamic diameter of 82 nm with excellent stability in aqueous medium. These assemblies accumulated preferentially in tumors and inhibited tumor growth after light-irradiation. ZnnPP-conjugated NP represent a promising method for cancer detection due to their easily visualization by fluorescence after intravenous injection.

3.4 Self-assemblies based on polyesters

PLA has been used as an excellent matrix to form self-assembling NP to encapsulate different chemotherapeutic drugs. This polymer can be also combined with peptides to create polymer–peptide hybrids with the aim to enhance cell uptake without

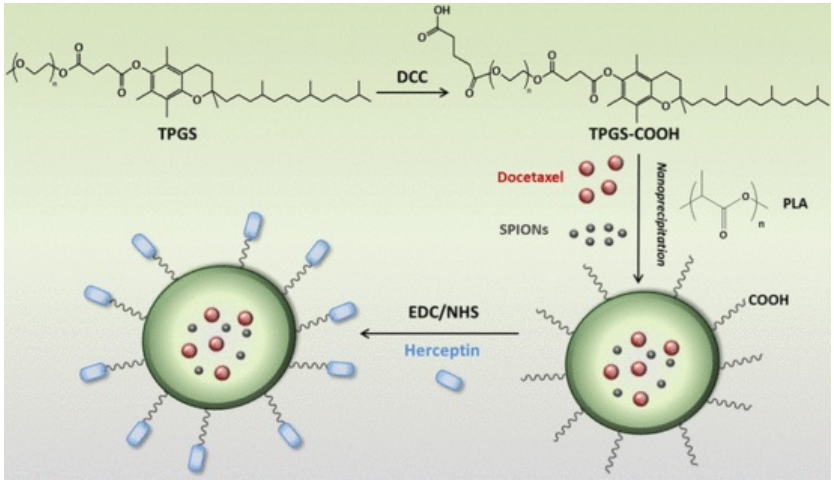
changing their functionality. Jabbari et al. [227] prepared self-assembled polymer–peptide NP formed by a VVVVVVKK peptide (V6K2) conjugated to PLA matrix. These NP with spherical shape and average size of 100 nm were used to encapsulate DOX or PTX and their cell uptake and cytotoxicity was tested against murine breast carcinoma and marrow stromal cells. *In vitro* and *in vivo* studies demonstrated that PLA-V6K2 NP exhibited a high tumor cell uptake due to interactions between the lysine groups of peptide and negatively charged moieties at the cell surface.

Also, peptides can be incorporated at the surface of NP. Xu et al. [228] prepared PLA NP to load oridonin that is an anti-cancer drug successfully used for the treatment of liver cancer and esophageal carcinoma. The surface of these NP was modified by incorporating the peptide RGD ~~via~~via cross-linking. The results of *in vivo* studies showed the anti-cancer efficiency of these NP, a decrease in the tumor growth, improving the survival time of mice bearing H22 tumo~~u~~rs.

PCL is other polyester extensively studied to prepare nanocarriers for medical applications. Ortiz et al. [229] studied the therapeutic efficacy of the combination of chemotherapeutic agents with gene therapy using 5-FU loaded PCL NP, prepared by an interfacial polymer disposition method. These NP exhibited 40 times superior anti-cancer activity than the free drug. Moreover, the utility of gene therapy based of the cytotoxic suicide gene E was demonstrated against colon cancer cells and it was attributed to the synergistic effect with the 5-FU loaded NP.

Huang et al. [230] synthesized complex amphiphilic NP composed of PCL-~~b~~*b*-poly-(propargyl methacrylate-click-mercaptosuccinic acid-~~co~~*co*-PEG methyl ether methacrylate) (PCL-~~b-p(PMA-click-MSA-co~~*b-p*(PMA-click-MSA-*co*-PEGMA)). In this case, superparamagnetic iron oxide NP (SPIONs) were loaded into the inner PCL core. Moreover, cisplatin was coordinated with pendant dicarboxylic groups in the hydrophilic shell. These multifunctional NP revealed great anti-cancer activity against UMUC3 bladder cancer cells. Moreover, the anti-cancer efficacy of these NP by cisplatin delivery can be combined with SPIONs-induced hyperthermia.

On the other hand, PLA-*b*-tocopheryl polyethylene glycol 1000 succinate (TPGS) NP could be successfully applied for the multimodality treatment of cancer [231]. For instance, Mi et al. [232] loaded DCT and SPIONs in the inner core of NP formed by PLA-*b*-TPGS copolymers. Moreover, carboxyl group-terminated TPGS was added to conjugate Herceptin® (see Fig~~ure~~. 8). The combination of these different active molecules could allow the treatment of cancer diseases with chemotherapy, hyperthermia and an appropriate active targeting to HER2-overexpressing cancer cells. In fact, the results demonstrated that the treatment with these NP using an *in vitro* model of the HER2-~~-~~positive breast cancer was 2130-fold more efficient than the corresponding single modality treatments.



Fig~~ure~~. 8 Preparation of herceptin-conjugated NP loaded with DCT and SPIONs by nanoprecipiattion and surface modification.

3.5 Self-assembled systems of peptide-~~-~~amphiphiles

Currently, peptides represent an emerging platform to create self-assembling nanostructures for the cancer treatment and inflammation diseases [233]. These small molecules have attracted much attention due to their inherent biocompatibility, biodegradability, weak immunogenicity and high versatility based on the combination of a wide range of chemical compositions and bioconjugation strategies and their ability to adopt different supramolecular structures [234–236].

Typically, PA are formed by a hyprophobic peptide block, in most cases a long alkyl chain, attached to a short peptide sequence, capable of forming intermolecular hydrogen bonding, usually β-sheet formation. PA include charged amino acids (AA) following the hydrophobic peptide sequence to enhance their solubility in water and to design intelligent materials with, for example, pH sensitivity. Finally, different peptide epitopes as bioactive signals can be incorporated with several purposes, for instance, the improvement of cell adhesion [235].

The appropriate combination of different regions of PA allows the self-assembly into a variety of different structures, such as micelles, vesicles, or nanofibers, through hydrophobic collapse of the aliphatic tails and the formation of intermolecular hydrogen bonding. This spontaneous organization allows to minimizing unfavorable interactions with hydrophobic tails in the core and bioactive residue on the surface of the nanoassemblies. The structural characteristics and physico-chemical properties of these assemblies can be modified by changing the segments of amphiphilic molecules. The hydrophobicity can be tuned with the use of different alkyl chain lengths and the solubility or responsive behaviour of these materials can be controlled with the appropriate number of charged AA [235,237].

Over the last decade, Stupp and co-workers have exhaustively investigated self-assembling peptide-based materials for different applications, including cancer therapy and inflammation treatment [238]. For these applications, peptides provide a wide range of biological specific interactions, environmental responsive phase behaviors that can be properly controlled, different alternatives to direct self-assembly and a unique source of functional units into a single polymer that can target different tissues [239].

Stupp et al. [240] integrated a cationic α-helical (KLAKLAK)2 peptide into a PA that self-assembled into cylindrical and bioactive nanofibers by stabilization of its bioactive α-helical conformation. The biological activity of this PA was evaluated using human breast cancer cells. The results of these studies demonstrated that (KLAKLAK)2 nanostructures selectively reduced the viability of cancer cells by induction of apoptosis. Additionally, PA incorporating specific cancer biomarkers in their structure have also been investigated. One interesting example is the incorporation of a consensus substrate sequence specific to protein kinase A (PKA) to form filamentous nanostructures with enzyme-responsive behaviour. In fact, these nanostructures disassembled as a result of enzymatic phosphorylation, upon treatment with PKA. However, the treatment with an enzyme to cleave the phosphate group allowed to reconstructing the filamentous nanostructures. Furthermore, these nanostructures were used to encapsulate DOX, exhibiting significant cytotoxicity against a cancer cell line that secretes high levels of PKA. This strategy could be considered for the preparation of biodegradable dynamic assemblies with a wide range of biological stimuli-sensing capabilities [241].

Different authors have investigated the use of PA to encapsulate a wide range of poor water-soluble drugs. Stupp et al. [242] encapsulated CPT into self-assembling PA nanofibers. This strategy allowed to improve significantly the solubility of this drug. Moreover, the anti-cancer activity of these nanofibers was superior to that of the free drug using human breast cancer cells. Chilkoti et al. [243] developed biodegradable chimeric polypeptides that were attached to a short cysteine-rich segment and self-assembled into sub-100-nm-sized NP. The modification of polypeptides with cysteine residues allowed their conjugation to DOX. *In vivo* experiments showed that these assemblies inhibited completely the tumour growth using a murine cancer model. This advanced strategy offers the possibility to design diverse multifunctional nanoassemblies due to their capability to incorporate drugs, targeting molecules and imaging agents. On the other hand, Wiradharma et al. [244] prepared an oligopeptide amphiphile as a delivery system of DOX. Cationic core–shell nanostructures of approximately 100 nm were obtained with load efficiency up to 22%. The biological behaviour of these nanostructures was tested using HepG2 cells. The results showed the significant anti-proliferative activity of DOX loaded assemblies.

The control of drug release in PA can be achieved covalently attaching a drug to a hydrolytically labile bond. Particularly, the incorporation of hydrazone bonds represents an excellent alternative to control the drug release as a function of the pH of the surrounding media [245]. Recently, Stupp et al. [246] developed a PA covalently conjugated to dexamethasone (DMT) through a labile hydrazone bond. This conjugate self-assembled spontaneously in water into long supramolecular nanofibers. *In vitro* and *in vivo* experiments demonstrated the anti-inflammatory response of these assemblies with an optimal control of drug release and without systemic immune suppression.

3.6 Self-assemblies based on poly(2-oxazolines) (POX)

Recently, the use of POX for biomedical applications has reached growing interest due to their biocompatibility as well as their stealth behaviour. The living cationic ring-opening polymerization of 2-oxazoline monomer (OX) that was discovered in the middle of the 1960s allows the preparation of a wide variety of well-defined polymers. Additionally, the properties of POX can be easily modified by varying the side chain of OX. This strategy enables the easy access to hydrophilic and hydrophobic polymers for the synthesis of a wide range of self-assembled amphiphilic copolymer structures. For all these reasons, drug loaded micellar carriers formed from POX block copolymers have been developed for the treatment of cancer diseases [247–249].

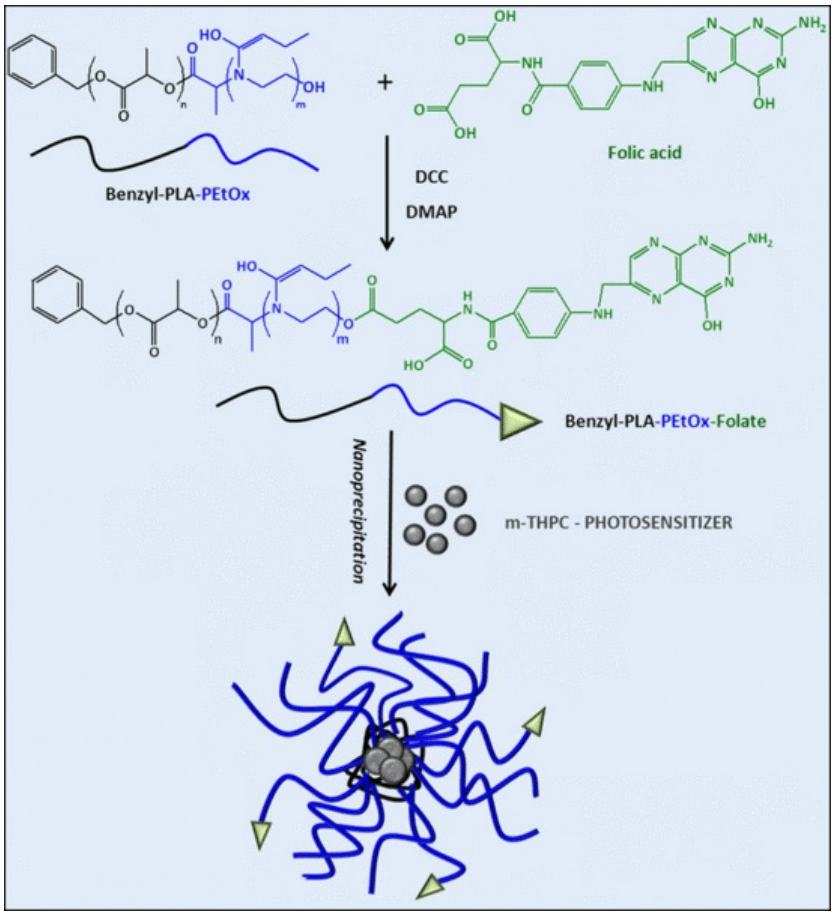
The most commonly POX investigated to prepare nanoassemblies is poly(2-ethyl-2-oxazoline) (PEtOx). This polymer exhibits a lower critical solution temperature (LCST) in water that facilitates the development of thermoresponsive micelles. Cheon et al. [250] prepared micelles composed of PEtOx-*b*-PCL amphiphilic block copolymers for the loading of PTX. PTX-loaded micelles exhibited an *in vitro* anti-proliferation activity against human nasopharyngeal epidermoid cancer cells (KB) comparable to that observed with the current clinical formulations. However, this micellar formulation reduced side effects such as hypersensitivity and neurotoxicity [250].

Wang et al. [251] encapsulated DOX in the inner core of micelles formed by PEtOx-*b*-PLA block copolymers. These micelles exhibited pH-sensitivity in aqueous solution. This behaviour allowed the targeted release of DOX in endosomes and lysosomes of cancer cells. In addition, DOX-loaded NP showed significant cytotoxicity against non-small-cell lung carcinoma CL3 cells and preferentially accumulated in the acidic compartments of the cells.

Recently, Luxenhofer et al. [252] synthesized well-defined amphiphilic block copolymers composed of PEtOx, poly(2-methyl-2-oxazoline) (MeOx) and poly(2-Bbutyl-2-oxazoline) (BuOx) with different compositions (MeOx$_a$-*b*-BuOx$_b$-*b*-MeOx$_a$ and EtOx$_a$-*b*-BuOx$_b$-*b*-MeOx$_a$ and EtOx$_a$-*b*-BuOx$_b$). These copolymers self-assembled in aqueous environment because BuOx exhibited limited water solubility. For this reason, BuOx formed the inner core of self-assembled micelles that were an excellent platform for the solubilization of diferent hydrophobic drugs such as PTX, cyclosporine A, etopiside and amphotericin B. In fact, loading capacities up to 45% were obtained using these novel formulations. *In vitro* studies demonstrated that PTX-loaded micelles exhibited more efficient anti-cancer activity in comparison with the commercial formulation of this chemotherapeutic drug.

On the other hand, micelles based on POX have also been used to incorporate other different agents such as photosensitizers and to be conjugated with small molecules in order to achieve an optimal targeted drug release mechanism and an improvement of cellular uptake [253,254]. Syu et al. [255] conjugated FA to micelles based on PEtOx-*b*-PLA block copolymers for the loading of meta tetra(hydroxyphenyl)chlorin (THPC) (see Figure. 9). TPHC-loaded NP were selectively internalized into cancer cells with more

potent anti-cancer activity in KB s.c. xenograft-bearing mice compared to free THPC and non-targeted micelles.



Fig~~ure~~. 9 Preparation of PEtOx-*b*-PLA block copolymers and schematic illustration of TPHC-loaded NP for PDT.

4 A~~PPLICATION OF NATURAL FUNCTIONALIZED POLYMERS~~pplication of natural functionalized polymers

4.1 Hyaluronic acid derivatives

Among all naturally occurring polysaccharides, HA is of particular interest in the development of advanced cancer therapies. HA is an anionic, linear glycosaminoglycan made of alternating disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine with β(1,4) and β(1,3) glycosidic linkages [256]. As a biocompatible, non-immunogenic and biodegradable polysaccharide with a number of biological roles and attractive physicochemical characteristics [257] it has been extensively investigated for a wide range of biomedical applications [80], including drug delivery for cancer therapies. In fact, increased levels of HA in tissues and body fluids have been observed in many cancers [258], suggesting its use as prognostic marker of various types of tumors [259,260]. This accumulation has been related to the overexpression of HA-binding receptors such as the cluster determinant 44 (CD44), which is implicated in the regulation of tumor growth and metastasis [261], the receptor for HA-mediated motility (RAHMM) or the lymphatic vessel endothelial receptor-1 (LYVE-1) in cancer cells [82]. Indeed, Choi et al. [262] demonstrated that NP obtained by self-assembling of HA–5β-cholanic acid conjugates were efficiently uptake by SCC7 cancer cells overexpressing CD44 while no significant uptake was observed by normal fibroblasts. Hence, the preparation of HA-based drug delivery systems can simultaneously exploit active targeting of HA-binding receptors on cancer cells along with the passive targeting of tumors based on the EPR effect, without the need of further modification with targeting ligands [188]. Furthermore, HA can be readily degraded by hyaluronidases (Hases), which are present in abundance in the cytosol of tumo~~u~~r cells, facilitating a rapid release of loaded anti-cancer drugs [263]. Besides, HA also acts as a signaling molecule in inflammation processes [264–266] which are greatly related with cancer progression [267,268]. Table 3 shows a list of the main amphiphilic nanocarriers based on hyaluronic acid for cancer treatment developed in recent years.

Table 3 A summary of amphiphilic nanodelivery systems of cancer drugs based on hyaluronic acid.

Polymer backbone	Type of modification	Nanocarrier type	Drug/Bonding type	Cancer cell type	Biological/Clinical status	Reference
HA	Acetylation	Nanogels	DOX encapsulation	HeLa cells	*In vitro*	[82]
	Conjugation of 2-(4-(vinylbenzyloxy)-*N,N*-diethylnicotinamide)	NP	PTX encapsulation	SCC7, squamous cells carcinoma	*In vitro*	[90]
	Esterification with monostearin	Noisomes	α-tocopherol encapsulation	4T1 mouse breast tumour cells	*In vitro* and *in vivo*	[84]
	~~Conjugation of 6-O~~Conjugation of 6-*O*-(3-hexadecyloxy-2-hydroxypropyl)	NP	DOX encapsulation	EAC, Ehrlich's ascites carcinoma	*In vitro*	[271]
	Conjugation of histidine residues	NP	DOX encapsulation	MCF-7 human breast adenocarcinoma cells	*In vitro*	[86]
	Conjugation to drug (PTX)	Micelles	PTX conjugation	MCF-7 breast and HCT-116 colon adenocarcinoma cells	*In vitro*	[73]
	Conjugation to drug (PTX)	-	PTX conjugation	Human bladder cancer	*In vitro* and *in vivo*	[279]
	Conjugation to drug (PTX)	NP	PTX amino acids Linkers	MCF-7	*In vitro*	[94]
Cystamine-modified HA	Conjugation of DOCA	Micelles	PTX encapsulation	MDA-MB-231 human breast adenocarcinoma cells	*In vitro*	[89]
Adipic dihydrazide-modified HA	Conjugation to drug (PTX)	-	PTX linker mediated coupling	Ovarian carcinoma xenografts	*In vitro*	[279]
PEGylated HA	Conjugation of 5β-cholanic acid plus black hole quencher3	NP	Chlorin e6 (photosensitizer) encapsulation	HT29 human colorectal adenocarcinoma cells	*In vitro* and *in vivo*	[280]

4.1.1 Drug-loaded self-assembled systems based on HA

Park et al. [269] reported the self-assembling of DOX-loaded nanogels based on HA with cancer cell selectivity by simple acetylation of HA with acetic anhydride. They confirmed that an increase in the DS of acetyl groups produced a reduction of the critical aggregation concentration values and the size of the nanogels due to the increase in hydrophobicity, which in turn increased the drug-loading efficiency and capacity, and prolonged the half-maximal DOX release time [82]. Similar tendencies were observed for other systems described below [84,90].

Several other hydrophobic moieties have been used to modify HA in order to give rise to drug-loaded, self-assembled nanostructures for cancer therapies. Saravanakumar et al. [90] conjugated an amine-terminated 2-(4-(vinylbenzyloxy)-*N,N*-diethylnicotinamide) (VBODENA) oligomer to HA for the encapsulation of PTX upon self-assembling into NP, achieving drug loadings up to 20.7% for an optimal DS of 3.17. The proposed system exhibited selective cytotoxicity to cancer cells overexpressing CD44. Interestingly, the PTX release rate was lower for NP containing the larger drug loadings, as observed also in other core–shell structured systems [270]. Kong et al. [84,270] esterified HA with monostearin to develop self-assembled HA-based noisomes as transdermal drug delivery systems for cancer therapy and loaded them with α-tocopherol. The noisomes underwent transdermal permeation both *in vitro* (in a stratum corneum model) and *in vivo* (in male Kunming mouse), and exhibited higher endocytosis by mouse breast tumour cell (4T1) than the control (Ch NP). Ray et al. [271] developed self-assembled 6-~~O~~*O*-(3-hexadecyloxy-2-hydroxypropyl)–HA (HDHA) NP and loaded them with DOX (4%) for intravenous infusion with simultaneous oral administration of EGCG, a green tea polyphenol. This system significantly enhanced the toxicity against Ehrlich's ascites carcinoma (EAC) cells as compared to free DOX. One of the drawbacks of some self-assembled polymeric systems is their usual sustained drug release over long time periods, which may decrease the efficacy of anti-cancer drugs. Recently, Li et al. [89] designed HA-based redox-sensitive micelles for targeted, rapid intracellular release of encapsulated PTX. The nano-sized micelles self-assembled from amphiphiles prepared by conjugation of DOCA to a cystamine-modified HA, efficiently encapsulating PTX (up to 34.1% wt load). Under reducing conditions, which mimic the reducing environment of tumor cells, the micelles disassembled due to the cleavage of the disulfide linkage triggering a fast drug release. As a consequence, the redox-sensitive micelles exhibited an enhanced cytotoxicity for human breast adenocarcinoma cells (MDA-MB-231) as compared with an insensitive control prepared by using adipic dihydrazide instead of cystamine as a linker for the conjugation of HA–DOCA. Wu et al. [86] also exploited stimuli responsiveness of HA derivatives for the targeted delivery of anti-cancer drugs to tumor cells. In their work they used His as a hydrophobic residue to induce the self-assembling of His-modified HA and encapsulate DOX. Under weakly acidic pH, such as the slightly acidic extracellular pH of solid tumors, the His residues in the His-HA NP became hydrophilic as a consequence of the protonation of their imidazole group, resulting in a higher DOX release at acidic pH than at neutral pH. The prepared DOX-loaded His-HA NP showed similar dose and time-dependent cytotoxicity against MCF-7 cells than free DOX and could exploit both pH-sensitivity and selective receptor-mediated endocytosis to target solid tumors.

Regardless of the selective intracellular uptake of HA-based systems by receptor-mediated endocytosis in tumor cells, successful efforts have been made in order to further improve tumor targetability of self-assembled HA derivatives by reducing their accumulation in the liver after systemic administration, which had been observed in other works [262,272,273] and is attributed to the presence of another HA receptor in the liver sinusoidal endothelial cells, namely the hyaluronan receptor for endocytosis (HARE) [274]. Because of the relevant role of HA in inflammation processes, this polysaccharide is also especially interesting for the development of polymer-based anti-inflammatory therapies. For instance, DMT was recently loaded in self-assembled NP of Flt1 peptide–HA conjugates showing efficient internalization into lung epithelial cells by HA-receptor mediated endocytosis, exhibiting long-term retention in deep lung tissues attributed to the mucoadhesive property of HA, and decreasing cytokine levels of lipopolysaccharide-stimulated cells more efficiently than free DMT [275].

4.1.2 Anti-cancer agent-conjugated self-assembled systems based on HA

Instead of physically loading the anti-cancer agent into the nanocarriers, some authors suggest its chemical conjugation with HA, serving both as active anti-cancer agent and as the hydrophobic residue required to obtain an amphiphilic self-assembling construct. Lee et al. prepared round-shaped self-assembled micelles (~200 nm) by direct conjugation of PTX to HA, achieving up to 12% PTX loadings. The generated ester linkage was cleavable in acidic conditions, potentially favoring the differential PTX delivery to acidic tumor tissues as shown in the drug release assays performed *in vitro* at different pHs. In addition, these micelles showed enhanced cytotoxicity in CD44-overexpressing MCF-7 and HCT-116 cells as compared to Taxol®, but lower cytotoxic effects than the commercial formulation for NIH-3T3 (no CD44-overexpression), suggesting a HA-receptor mediated internalization process for PTX uptake [73]. Previous works had already reported the development of HA–PTX conjugates by linker mediated coupling [276–278], their selective uptake by malignant cells ~~via~~via receptor-mediated internalization [276,277] and the anti-tumor activity of HA–PTX derivatives against human ovarian carcinoma xenografts [279] or superficial bladder cancer.

Despite the predominant role of self-organizing HA-based systems as vehicles for the delivery of anti-cancer drugs, they have also been exploited for other strategies to combat cancer, such as photodynamic imaging and therapy. Yoon et al. [280] used PEGylated HA–5β-cholanic acid conjugates and further coupled them to black hole quencher3 (BHQ3), obtaining self-assembled NP in which they incorporated chlorin e6 (Ce6), a hydrophobic photosensitizer capable of generating fluorescence and singlet oxygen upon irradiation for combined cancer diagnosis and therapy (see Figure 10). Being smaller than 300 nm, these NP were selectively uptaken by HT29 human colon cancer cells ~~via~~via CD44-binding interactions, as compared to normal NIH3T3 mouse embryo fibroblasts, and they effectively accumulated in the tumor tissue of HT29 tumor-bearing miceCovalent attachment of steroidal and nonsteroidal anti-inflammatory drugs to functionalized HA has also been accomplished [281]. However, scarce literature can be found regarding self-assembling properties of these conjugates into nanoparticulate systems for cancer or inflammation therapies.

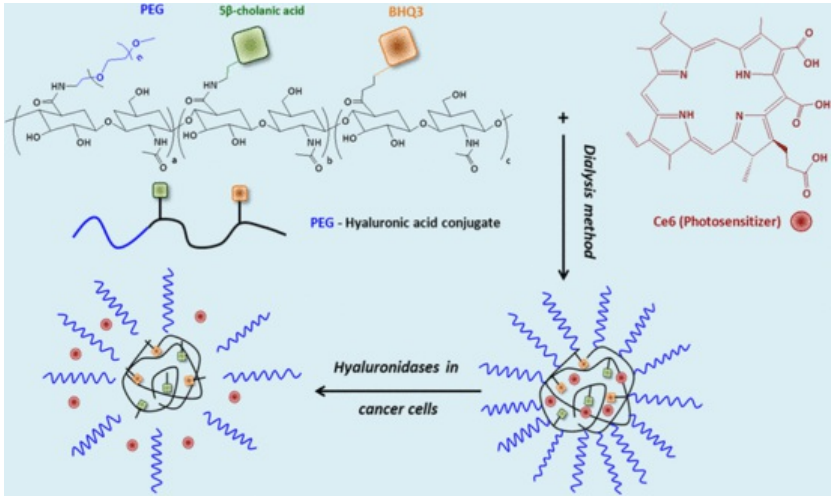


Figure 10 Representative preparation and enzimatic release mechanism of a HA based system for photodynamic imaging and cancer therapy.

4.2 Heparin derivatives

As negatively charged polysaccharides, heparins have the ability to bind to a variety of proteins and other biomolecules altering their activity. Hence, apart from their known anticoagulant effects, heparins can play a role in a number of biological activities including interference with malignant processes [282]. Although the exact mechanism of action is still unknown, several works suggest that heparins can efficiently inhibit the proliferation and induce apoptosis on different cancer cell lines [101,283], thus constraining tumor growth and metastasis. In recent studies has been proposed that the mechanism is related to the ability of heparin to interact with tumor related factors such as selectins, heparanases and growth factors [35]. Furthermore, specific binding properties to certain receptors in metastatic cells, such as peptides in B16F10 cells, have been attributed to heparin [101,284], potentially facilitating binding and internalization into those cells ~~via~~via targeted endocytosis. For this reasons, heparin-derived self-assembled systems have also

been extensively investigated for their use in advanced cancer therapies.

4.2.1 Drug-loaded self-assembled systems based on heparin

Over the last decade, a number of anti-cancer drug-loaded self-assembled systems based on heparin have been developed. Park et al. [77] prepared self-organizing heparin–DOCA conjugates and efficiently loaded them with DOX up to 12%. They observed that bare heparin-DOCA NP inhibited tumour and endothelial cell proliferation in tumour tissues by their own, and were more active than free heparin *in vitro*. When loaded with DOX, these NP where more effective reducing tumour growth and had better anti-tumour activity than free DOX, reducing its side effects. Li et al. [76] did attempt the design of targeted delivery systems for antineoplastic drugs by investigating the role of chemically linked Fol residues in the selectivity of self-assembled heparin-*b*-poly(benzyl-L-aspartate) (PBLA) block copolymers encapsulating PTX towards cancer cells. They found that Fol-conjugated NP were more efficiently uptaken by KB cells (positive for Fol-receptor) than the ones which did not incorporate targeting ligands. Further, they showed that when a PEG fragment was inserted as spacer between Fol and heparin the cellular uptake was improved to a greater extent. In addition, Fol-conjugated PTX-loaded NP exhibited enhanced selective cytotoxicity towards KB cells as compared with Fol-receptor-negative human lung adenocarcinoma epithelial (A549) cells, especially when PEG was used as spacer.

4.2.2 Drug-conjugated self-assembled systems based on heparin

Heparin based anti-cancer prodrugs have also been developed by chemical conjugation of the free drug with heparin. Park et al. [95] conjugated PTX with heparin obtaining spherical NP with diameters in the range of 200–400 nm which exhibited enhanced cytotoxicity against KB cancer cells compared to free PTX. These NP also showed inferior anticoagulant activity than free heparin, another clue feature to take into account in order to reduce hemorrhagic side effects when using heparin-based prodrugs. By chemically modifying heparins, critical functional groups or units on their antithrombin-binding sequence may be inactivated, thus reducing their anticoagulant effect [283]. Park et al. [93] also modified heparin by chemical conjugation of retinoic acid and FA to the polysaccharide backbone and obtained self-assembled NP (150–300 nm). The presence of FA showed to improve the cellular uptake of the NP in Fol receptor-positive cells, and the cytotoxicity of retinoic acid against these cells was also enhanced using this system compared to the free drug. Again, the modification of heparin reduced its anticoagulant activity. Tran et al. [91] used folated heparin-retinoic acid self-assembling prodrugs to encapsulate the photosensitizer Pba for combined PDT and chemotherapy. Apart from the improved cytotoxicity of retinoic acid and the enhanced and selective cellular uptake in Fol receptor-positive human cervical cancer (HeLa) cells compared to Fol receptor-negative HT-29 cells, these complex NP selectively enhanced the phototoxicity of Pba in HeLa cells upon irradiation.

Hou et al. [285] also designed a dual cancer therapy based on heparin-retinoic acid conjugates. Their strategy consisted of combining the chemical conjugation of ATRA and the physical encapsulation of PTX in a single heparin-based delivery system for simultaneous delivery of both anti-cancer drugs. The obtained self-assemblies, with particle sizes ranging from 228 to 108 nm and PTX contents of up to 33%, extended the plasma circulation periods for PTX and ATRA and showed improved cytotoxicity to human hepatocellular carcinoma HepG2 cells compared to free PTX. Most Recently, an innovative approach which combines both the features of self-assembled nanostructures and the benefits of dendrimers for chemotherapeutic drug delivery was proposed by She et al. [35] In their work, they prepared “dendronized” heparin by covalently linking polypeptide dendrons to heparin, and subsequently conjugated DOX to these dendrons ~~via~~via acid-liable hydrazone bonds, obtaining 9% DOX contents. The dendronized heparin-DOX conjugates self-assembled in aqueous media to form compact NP displaying average hydrodynamic diameters around 90 nm, a suitable size to accumulate in tumor tissues by the EPR effect. Due to the liability of the hydrazone bonds, these NP exhibited pH sensitivity, with greater DOX release rates at acidic pH. The *in vitro* and *in vivo* assays showed considerable anti-tumour activity by anti-angiogenic and apoptotic effects against 4T1 breast cancer cells and in 4T1 tumour bearing-mice.

As oral drug delivery is the most convenient route of administration for the patients, some authors have focused on improving the oral absorption and targeting of chemotherapeutics. Khatun et al. [96] designed a strategy based on the conjugation of taurocholic acid (TCA), capable of interacting with the bile acid transporter of the small intestine, with heparin as a therapeutic polymer, and further modification of this conjugate by chemical linkage of DCT. These ternary bioconjugates self-assembled in water giving rise to NP (115–124 nm) which showed positive results in MDA-MB231 and KB tumour bearing mice, indeed enhancing oral absorption compared to bare heparin, efficiently accumulating in tumo~~urs and improving tumour~~rs and improving tumor growth inhibition compared to free DCT.

4.3 Chitosan derivatives

Chitosan, the *N*-deacetylated derivative of chitin (poly-β-(1→4)-*N*-acetyl-D-glucosamine), has drawn rising interest in the development of nanocarriers due to its unique and versatile physico-chemical properties and biodegradability. In particular, development of colloidal amphiphilically modified chitosan nanocarriers has increasing attention for chemotherapy applications [286] due to the fact that chitosan and its oligosaccharides were reported to be potent angioinhibitory and anti-tumour compounds, as confirmed by inhibition of angiogenesis and inducing apoptosis as a function of DNA fragmentation [287]. Thus, together with HA, chitosan has a relevant role in the development of nanocarriers for cancer therapy [288].

4.3.1 Drug-loaded self-assembled systems based on chitosan

Zhang et al. [289] prepared micellar systems of amphiphilic chitosan derivatives based on alkyl chains (~~n~~*n* = 8, 10, 12) and sulfated groups of which the *N*-octyl-~~O~~*O*-sulfate chitosan system was selected to encapsulate PTX in amounts up to 25%. The PTX-loaded micelles showed slow *in vitro* released of PTX (up to 220 h) from micellar solution [290]. This chitosan amphiphile showed no intravenous stimulation, injection anaphylaxis, hemolysis and cytotoxicity [291]. Chitosan derivatives with hydrophobic moieties of *N*-octyl and hydrophilic moieties of sulfate and mPEG groups were reported by Qu et al. [292]. These amphiphiles formed micelles that were charged with PTX. The tissue distribution studies in mice indicated that PEG conjugated micelles were phagocytized less than

unconjugated micelles by RES. Furthermore, the higher targeting efficiency of PEGylated micelles to uterus (including ovary) suggested that this carrier could be promising for the chemotherapy of ovarian cancer.

Dufes et al. [293] prepared targeted carrier systems for DOX by covalent linking of transferrin (TfR) to DOX-loaded palmitoylated glycol chitosan vesicles using dimethylsuberimidate (DMSI). For comparison purposes, glucose targeted niosomes were prepared using *N*-palmitoyl glucosamine. *In vivo* experiments with a mouse xenograft model showed that all vesicle formulations had a superior *in vivo* safety profile compared to that of the free drug. And also, all vesicles reduced tumour size on day 2 but were overall less active than the free drug.

Micelles with high *in vivo* stability based on stearic acid grafted chitosan oligosaccharide amphiphiles (CSO-SA) were developed by Hu et al. [110]. To obtain an active-targeting carrier to cancer cells, Fol-conjugated CSO-SA amphiphiles were synthesized. The targeting ability of these micelles was investigated against two kinds of cell lines, A549 and HeLa, which have different amounts of Fol receptors on their surfaces. The results revealed good internalization of the micelles into both types of cells. Then, PTX was encapsulated into the micelles, and the anti-tumour efficacy was investigated *in vitro*. The cytotoxicity of PTX-loaded micelles was improved sharply for both strains of cells compared with that of Taxol®, what was attributed to the increased intracellular delivery of the drug [294]. Curcumin-loaded CSO-SA micelles were effective for inhibiting subpopulations of CD44+/CD24+ cells (putative colorectal cancer stem cell markers) both *in vitro* and *in vivo* [295]. Amphiphilic micelles of stearyl chitosan (SC) and sulfated stearyl chitosan (S-SC) were developed for the controlled delivery of atorvastatin (ATV) in cancer cells. Micelles encapsulating ATV exhibited a sustained release and more cytotoxic activity against MCF 7 and HCT 116 cell lines than ATV alone [296].

An effective and safe vehicle for systemic administration of hydrophobic drugs based on novel chitosan derivatives carrying linoleic acid and poly(β-malic acid) was synthesized by Zhao et al. [297]. This double grafted chitosan self-assembled into NP which could encapsulate PTX. The PTX-loaded NP demonstrated to have a potent tumor inhibition efficacy relative to that of Taxol® in sarcoma-180 bearing mice. Self-assembled NP of 5β-cholanic acid–glycol chitosan conjugates were efficiently loaded with PTX up to 10% content using a dialysis method (400 nm, average diameter). Injection of PTX-loaded NP into the tail vein of tumor-bearing mice prevented increases in tumor volume for 8 days. PTX was less toxic to the tumor-bearing mice when formulated in NP than when formulated with Cremophor® EL [108,298]. pH-sensitive self-aggregated NP (ranging from 87 to 174 nm) based on DOCA modified carboxymethyl chitosan (DCMC), were developed by Jin et al. [299] for delivery of DOX. The unloaded NP showed an acidic pH-induced aggregation and deformation behavior. The DOX-loaded NP exhibited a sustained drug release profile, dependent on pH and DS of the hydrophobic chitosan. DOX-loaded NP effectively suppressed both sensitive and resistant MCF-7 cells in a dose- and time-dependent manner.

Yang et al. [300] proposed a novel thermal sensitive amphiphilic chitosan containing hydroxybutyl groups and DOCA moieties as a drug carrier in combined hyperthermia and chemotherapy. By tuning the hydrophobic/hydrophilic balance of DOCA decorated hydroxybutyl chitosan (DAHBC), LCST of this novel polymer was adjusted to 38.2 °C for hyperthermia therapy. These NP delivered the encapsulated DOX at a temperature above the LCST. DOX-loaded nanocarriers exhibited an improved drug uptake by mouse embryo fibroblasts MCF-7 cells with the incubation temperature rising from 37 °C to 43 °C.

N-acetyl histidine-conjugated glycol chitosan (NAcHis-GC) self-assembled NP are a promising system for intracytoplasmic delivery of PTX. At neutral pH, the conjugates formed self-assembled NP with mean diameters ranging between 150 and 250 nm. In slightly acidic environments, such as those in endosomes, the NP were disassembled due to breakdown of the hydrophilic/hydrophobic balance by the protonation of the imidazole group of NAcHis and could release the encapsulated PTX into the cytosol (see Figure 11) [301].

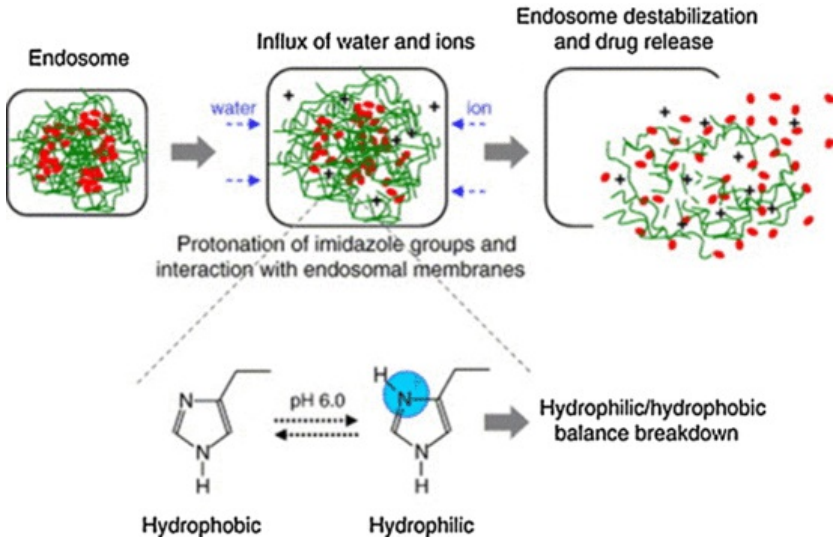


Figure 11 Schematic representation of endosomal escape and drug release of histidylated polymer nanoparticles. The pH-dependent endosomal-membrane destabilization by histidine correlated with the protonation of its imidazole groups. The p*Ka* of the imidazole group is around 6.5. In a slightly acidic milieu, such as in endosomes, the imidazole group is protonated, interacts with negatively charged lipid bilayers and induces the influx of water and ions into endosomes, thus causing endosome destabilization and drug release into the cytosol. [301], Copyright 2006. Reproduced with permission from Elsevier Ltd.

A CSO–arachidic acid (CSOAA) amphiphilic derivative of chitosan was successfully synthesized as a self-assembled nanocarrier of DOX by Termsarasab et al. [302]. *In vitro* release of DOX was sustained and pH-dependent. Cellular uptake of DOX in FaDu cells was higher in the NP-treated group compared to the free DOX group. The anti-tumor efficacy of DOX-loaded NP was also verified in FaDu tumor xenografted mouse model. The amphiphilic grafted copolymer *N*-phthaloylchitosan-*g*-mPEG was proposed as a carrier for ATRA by Bouterfa et al. [303]. It was found that deacetylation degree (DD) of chitosan, which corresponded to the *N*-phthaloyl groups in the inner core of the micelles, was a key factor in controlling the loading efficiency, stability of the drug-loaded micelles and drug release behavior. As the % DD increased, the loading efficiency and ATRA-loaded micelles stability increased. The sustained release profiles were also obtained at high % DD (90 and 95%) [99]. Similar results were reported for the encapsulation of CPT in this carrier [304].

NP based on graft copolymers of PNIPAm onto chitosan were developed as a pH-sensitive carrier of CPT for targeting tumors. The cumulative release rate of CPT was optimal at pH 6.8 and decreased rapidly either below pH 6.5 or above pH 6.9 in 37 °C. Based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) tests performed with SW480 cells at pH values of 6.8 and 7.4, CPT-loaded NP showed enhanced cytotoxicity at pH 6.8 but minimal cytotoxicity at pH 7.4 what was attributed to pH-sensitivity release of the drug. Particularly, for a PNIPAm:chitosan mass ratio of 4:1 the CPT-loaded NP were more sensitive to tumor pH [305].

Non-steroidal anti-inflammatory drugs (NSAIDS) are emerging as a particularly valuable class of drugs due to their recently recognized anti-tumor activity in colorectal cancer. Micellar nanocarriers (108–252 nm) composed of amphiphilic chitosan with encapsulated ibuprofen were readily internalized by tumor cells and deliver the drug in the intracellular compartment provoking a remarkable reduction in cancer cell viability (<13%), at a relatively low drug dosage, what illustrated the anti-tumor activity of ibuprofen when delivered to breast cancer cells [306].

4.3.2 Conjugated self-assembled systems based on chitosan

A variety of polymer drug conjugates of chitosan have been reported in recent years. Adriamycin (ADR) glycol chitosan conjugates *via* an acid-labile cis-aconityl linkage [307] were capable of forming spherical nano-sized self-aggregates in an aqueous medium, when the ADR content in the conjugate was in the range of 2–5%. The release of ADR was significantly dependent on the pH of the medium due to the cis-aconityl linkage. The cell viability results demonstrated that free ADR showed more potent cytotoxicity than the conjugates, primarily attributed to the sustained release of ADR from the self-aggregates.

PTX-loaded tocopheryl succinate (α-TOS) conjugated CSO (CSO–α-TOS) NP were proposed for synergistic chemotherapy by Tao et al. [308]. PTX-loaded CSO–α-TOS NP had excellent cellular uptake ability by human glioma U87 cells. *In vitro* cytotoxicity studies revealed that the PTX-loaded NP system was more potent than free PTX, and a synergistic effect between α-TOS and PTX was observed. *In vivo* pharmacokinetic results indicated that the PTX-loaded CSO–α-TOS NP had a longer systemic circulation time and slower plasma elimination rate than those of Taxol®.

Selective targeting of drugs to kidneys may improve renal effectiveness and reduce extrarenal toxicity. For that purpose Yuan et al. [309] developed and evaluated a novel renal drug carrier randomly based on 50% *N*-acetylated LMWC and prednisolone covalently coupled with chitosan *via* a succinic acid spacer. The mean residence time in plasma of prednisolone conjugates increased as the MW of the chitosan increased. The conjugate with MW of 19 kDa displayed the highest accumulation rate in the kidneys. The total amount of this conjugate in the kidneys was 13-fold higher than that of prednisolone.

pH Sensitive NP based on polyethylenglycol thetethered carboxylated chitosan modified with FA/DNA nanocomplexes containing a high mobility group box1 (HMGB1) have been developed recently as an efficient non-viral gene delivery system (see Figure 12). This kind of complex bioactive NP gives very good transfection and expression efficiency in most folate receptor (FR-α)-positive cancer cells [310].

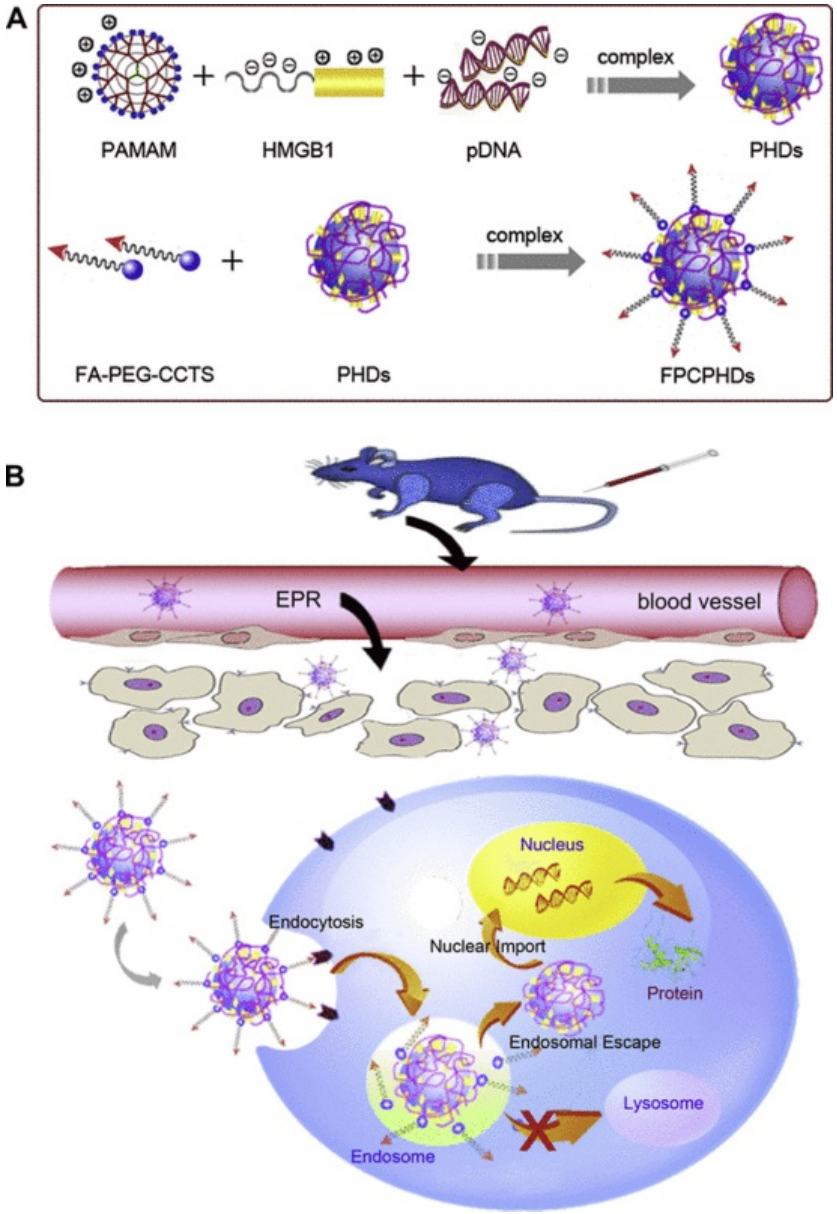


Figure 12 A schematic diagram showing (A) the formation of FPCPHDs nanocomplexes and (B) the extracellular and intracellular trafficking for the systemic delivery of plasmid DNA to a tumor. FPCPHDs accumulates in the tumor via the EPR effect and is associated with the tumor cell surface, followed by cellular uptake by folate receptor-mediated endocytosis. FAPEG-CCTS dissociates from PHDs ternary complexes in the acidic endosomes and then PHDs escapes from endosomes through the proton sponge effect. Finally, the plasmid DNA is delivered to the nucleus with the help of HMGB1 due to its nuclear locating ability. [310], Copyright 2013. Reproduced with permission from Elsevier Ltd. Abbreviations: FPCPHDs, FAPEG-CCTS/PAMAM/HMGB1/pDNA nanocomplexes; FAPEG-CCTS, folate-modified polyethylene glycol tethered carboxylated chitosan; PAMAM, polyamidoamine dendrimer; EPR, enhanced permeability and retention effect; PHDs, PAMAM/HMGB1/pDNA nanocomplexes.

4.4 Pullulan derivatives

Pullulan is a linear homopolysaccharide of glucose consisting of α-(1→→6) linked maltotriose units, which is secreted mainly by *Aureobasidium pullulans*. Its distinctive and regular linkage pattern gives rise to unique properties such as structural flexibility and superior water solubility as compared to other polysaccharides [311]. This polysaccharide is a good candidate for chemical modification with hydrophobic residues leading to self-organizing systems with potential application in cancer treatment since it can be readily dissolved in organic solvents such as DMSO upon mild heating, [72,74,75] facilitating its chemical derivatization. Besides, it can be easily modified to include active targeting ligands like biotin or folate in its structure to confer specificity to cancer cells.

4.4.1 Drug-loaded self-assembled systems based on pullulan

Yang et al. [116] proposed the use of biotin as a targeting ligand to develop pullulan-based self-aggregated NP for the encapsulation and targeted delivery of MTO to cancer cells, as biotin specific receptors are generally overexpressed on numerous tumor cells [312]. Previously, they had developed cholesterol-modified pullulan NP as carrier for MTO [115], however, these self-aggregates lacked active targeting to tumor tissues. To overcome these limitation, in their latter work they suggested the conjugation of biotin to cholesteryl pullulan and the obtaining of MTO-loaded NP by self-organization in aqueous media. The prepared NP were able to encapsulate MTO with loading capacities up to 14% and showed pH-dependent drug release behavior. However, their efficacy and selectivity towards tumor cells has not been reported [116].

4.4.2 Conjugated self-assembled systems based on pullulan

Zhang et al. [117] preferred conjugation of DOX onto the pullulan backbone instead of physically loading it and they prepared pullulan–DOX conjugates with maleic acid as spacer, followed by further modification of this prodrug to incorporate FoI the residues by direct conjugation. Although the effectiveness of this system against ovarian carcinoma A2780 cells was improved with respect to the parent drug, its self-assembling properties were not reported. Pullulan self-assembling derivatives have also been exploited in the field of PDT. Bae and Na [72] used Fol–pullulan conjugates previously described by Kim et al. and further coupled them to Pba, obtaining self-assembling and self-quenching nanogels with the aim of both reducing the phototoxicity of Pba in normal tissue and improving the efficacy of the PDT using this photosensitizer. Indeed, owing to a self-quenching effect between photosensitizer moieties, the photoactivity of Pba was not detected when the nanogels were suspended in PBS. However, upon digestion by the enzymes present in the lysosome, the photoactivity of Pba could be restored. Moreover, their results suggested that these nanogels could be uptaken by HeLa cancer cells by FoI receptor-mediated endocytosis (see Figure 13).

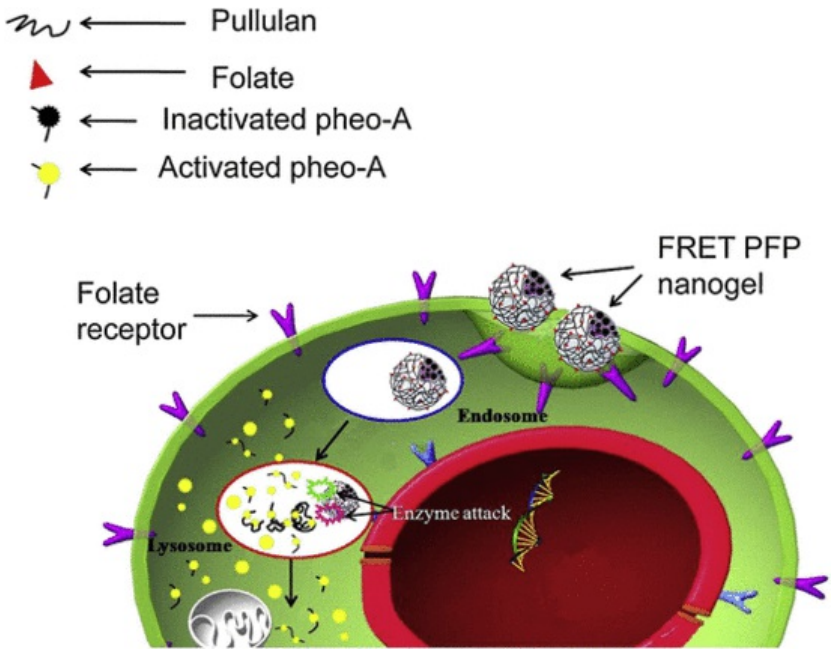


Figure 13 Conceptual image of the change in photoactivity of PFP nanogels in cells. PFP nanogels do not show photoactivity during blood circulation due to self-quenching of photosensitizers (FRET effect). When the nanogels are internalized in cancer cells, photoactivity is restored due to loss of the FRET effect by enzymatic attack within cellular compartments such as lysosomes. [72], Copyright 2010. Reproduced with permission from Elsevier Ltd. Abbreviations: PFP, pullulan/folate-pheophorbide–pheophorbide–conjugates; FRET, fluorescence resonance energy transfer.

4.5 Dextran derivatives

DEX is a polysaccharide containing glucose units linked by α-(1→→6) glycosidic linkages in the main chains and α-(1→→3) linkages in the branches. DEX has been also widely used in the development of drug delivery systems for anti-cancer therapy.

Several anti-tumor drugs such as DOX, CPT, MTX and mitomycin have been conjugated to DEX either directly or using spacers to form anti-cancer prodrugs [313]. Oxidized DEX-DOX conjugates and carboxymethyl DEX CPT derivative conjugates have entered clinical trials [314]. A review of this was reported by Goodarzi et at. [257] In recent years, DEX nanocarriers for cancer therapy have been developed based on the modification of DEX to obtain amphiphilic derivatives that self-assemble into micellar or nanoparticulate systems.

Varshosaz et al. [315] prepared DEX stearate PM as carriers of etoposide. The composition was tuned by changing MW of DEX and molar ratio of stearate. Both parameters demonstrated to have a dominant role on particle size of etoposide-loaded micelles and also on cytotoxicity and cellular uptake of etoposide-loaded PM using CT-26 colorectal carcinoma cell line.

Jeong et al. [316] reported the preparation of DEX amphiphilic derivatives by linking DOCA to DEX. These amphiphiles showed self-aggregation behavior at aqueous environment and gave rise to DOX-incorporated NP with sizes lower than 200 nm. These NP had higher anti-tumor activity compared to free DOX on DOX-resistant CT26 colon carcinoma cells. In addition, DOX-loaded NP were properly entered into tumor cells and maintained longer compared to DOX by itself.

Stimuli responsive DEX based nanocarriers have also been developed. DEX-*b*-polyHis block copolymers were synthesized by Hwang et al. [317] to prepare pH-responsive NP for DOX targeting. The viability of DOX-loaded NP using HuCC-T1 cholangiocarcinoma cells was decreased at acidic pH in cells treated with active NP, whereas cell viability did not vary according to changes of pH for free DOX. Sun et al. [318] reported the preparation of reduction-responsive biodegradable micelles from block copolymers of disulfide-linked DEX-SS-PCL and used them as a targeting delivery system of DOX. *In vitro* studies revealed that DOX-loaded micelles released DOX quantitatively in 10 h under a reductive environment, mimicking that of the intracellular compartments such as cytosol and the cell nucleus, however, only about 20% DOX released was measured in 20 h under the non-reductive conditions. In cellular experiments using mouse leukemic monocyte macrophage cell line (RAW 264.7), it was observed that DOX was rapidly released to the cytoplasm as well as to the cell nucleus. Cytotoxicity studies revealed an enhanced drug efficacy of DOX-loaded micelles compared to DOX-loaded reduction-insensitive micelles.

Prabu et al. [319]–[320] proposed a self-assembled core–shell micellar vehicle for PTX based on PCL-grafted DEX. PTX-loaded NP were prepared by a modified oil/water emulsion method. PTX-loaded NP so-obtained presented significant drug encapsulation efficiency, cellular uptake, and cancer cell mortality using the human gastric cancer cell line (SNU-638).

Graft copolymers of DEX-*b*-poly(ethyleneimine) (DEX-*b*-PEI) were synthesized by Liu et al. [321] for developing redox-responsive DOX prodrug micelles (100–140 nm average size) to overcome MDR. DOX was conjugated to the graft copolymer through redox-responsive cleavable disulfide linkers. DOX-conjugating micelles enhanced the cellular accumulation of DOX and achieved endosomal escape in human breast carcinoma multidrug resistant (MCF-7/ADR) cells. The therapeutic efficacy of DOX prodrug micelles against MCF-7/ADR cells was remarkably enhanced compared with free DOX.

Thermosensitive NP as carriers of indomethacin (IMC) have been prepared from a graft copolymer of DEX and PNIPAm by Tan et al. [322] In absence of drug, compact NP were formed at a temperature above the LCST, however, in presence of IMC uniform IMC-loaded NP were formed even below the LCST that was attributed to hydrogen bonding between IMC and the PNIPAm side chains. The IMC release rate was accelerated at a higher temperature because of the dissociation of the hydrogen bonds.

4.6 Derivatives of other polysaccharides

Alginates (ALG) are (1→4) linked linear copolysaccharides composed of β-D-mannuronic acid (M) and its C-5 epimer, α-L-guluronic acid (G). ALG also can play a role in cancer therapy [323]. It has been demonstrated that ALG obtained from *Sargassum* sp. (Phaeophyta) showed a considerable anti-tumor activity against various murine tumors, such as Sarcoma 180 (solid and ascitic types), Ehrlich ascites carcinoma and IMC carcinoma [324]. For that reason, self-assembled nanocarriers of amphiphilic ALG were developed recently.

Zhang et al. [325] have proposed DOX-loaded amphiphilic NP consisted of glycyrrhetinic acid-modified alginate (GRA-ALG) for targeting therapy of liver cancer. After intravenous administration of DOX-loaded GRA-ALG NP in Kunming mice, the biodistribution study showed that the concentration of DOX in the liver was higher compared with non-GRA-modified NP and DOX·HCl, respectively. The liver tumor growth inhibition rate (IR) *in situ* was 76.6% and no mice died in the DOX-loaded GRA-ALG NP group. Histological examination showed that the heart and liver cells surrounding the tumor were not affected by administration of DOX loaded GRA-ALG NP, whereas myocardial necrosis and apparent liver cell swelling were observed after DOX·HCl administration.

Recently, Du et al. [326] used a reactive template method to fabricate alginate-based hydrogel microcapsules with a high drug loading capacity for application in cancer therapy. The capsules coated by a Fol-linked lipid mixture on the surface possessed higher cell uptake efficiency due to the molecule recognition between Fol and the Fol-receptor overexpressed by the cancer cells. Moreover, in this bioconjugate, the lipid could also encapsulate the hydrophobic photosensitizer hypocrellin B, giving drug carriers for combined treatment of cancer using chemotherapy and photodynamic. This strategy may be extended to fabricate other multidrug carriers for combined anti-cancer treatment.

Shalviri et al. [46] synthesized new pH-responsive NP to overcome MDR [45] based on graft copolymers of PMA and PS80 onto starch (PMA-PS80-*g*-starch) by using a one-pot method that achieved simultaneous grafting and NP formation in an aqueous medium. The relatively spherical NP exhibited pH-dependent swelling in a physiological pH with magnitude of phase transition dependent on polymer composition and formulation parameters. The NP were able to load up to 50% of DOX maintaining good colloidal stability. DOX-loaded NP released the drug at a higher rate at acidic pH attributable to weaker DOX-polymer molecular interactions. The DOX-loaded NP were taken up by MDR1 cells *in vitro* and significantly enhanced cytotoxicity of these cells with respect to that of free DOX, showing a 20-fold decrease in the IC_{50} values [327]. In further studies the activity of the self-assembled DOX-loaded NP was evaluated *in vivo* using a murine orthotopic breast cancer model and compared with the activity of preformed NP prepared by cross-linking graft polymerization reaction. Blood circulation, tumor uptake, penetration and tumor growth inhibition of self-assembled DOX-loaded NP was superior to those of preformed NP, what was attributed to a denser structure of the self-assemblies, suggesting the

usefulness of these bi-functional NP as nanotheranostics [327].

A number of other polysaccharides have been used for the preparation of self-assembling drug conjugates. CPT was covalently conjugated to a linear cyclodextrin-PEG copolymer, and the obtained conjugates self-assembled into NP. Preclinical studies showed that these self-aggregates exhibited enhanced pharmacokinetics compared to the parent drug, and their anti-tumor activity was superior compared to FDA-approved Irinotecan® in a number of xenograft models (see Figure 14) [328,329]. This novel nanopharmaceutical, named CRLX101®, is currently in Phase 2 clinical studies and evidence has been provided that its behavior in animals is translatable to humans [330].

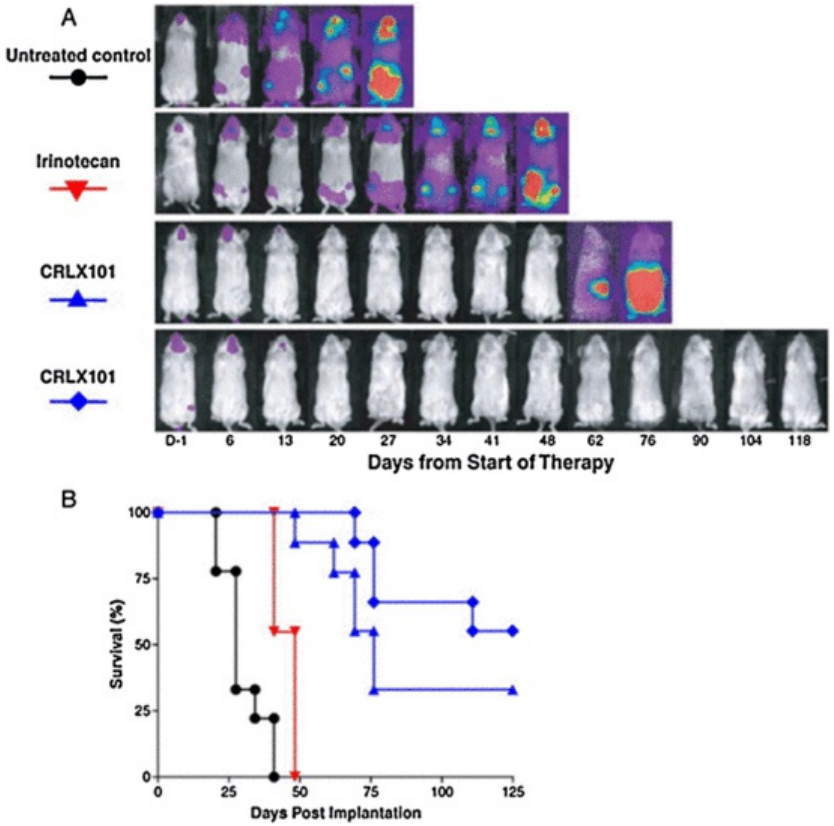


Figure 14 Efficacy study of CRLX101 compared to irinotecan using a disseminated Daudi B-cell lymphoma xenograft in mice. Above: bioluminescence study using Daudi B-cell line expressing luciferase activity. Weekly dosing × 3 at 100 mg/kg (irinotecan), 5 mg/kg (CRLX101, triangles), and 10 mg/kg (CRLX101, diamonds). Below: corresponding survival graphs. CRLX101 achieved 55.6% complete tumor response at Day 125 post-treatment at the 10 mg/kg dose, while no complete tumor responses were observed in irinotecan-treated mice. [328], Copyright 2011. Reproduced with permission from Elsevier Ltd. Abbreviations: CRLX101, Cyclodextrin-poly(ethylene glycol) copolymer (CDP) conjugated to CPT.

4.7 Proteins and proteins derivatives

Proteins also played an important role in the development of nanocarriers for chemotherapy and inflammation diseases. Some protein anti-tumor drug conjugates have been approved by FDA in treatment of cancer, such is the case of PTX albumin conjugate (Abraxane®, ABI-008, nab-PTX) approved for treatment of metastatic breast cancer. This formulation does not use solvents as vehicles but instead, it is based on the natural properties of albumin to reversibly bind PTX and transport it through the endothelial cell to tumor site [331]. This was confirmed in preclinical studies that in fact showed that the concentration of PTX-albumin conjugate in endothelial cells and in the extravascular space was significantly increased (3–10 fold) [332]. These data suggest that albumin may have intrinsic targeting ability to tumors, although the EPR effect may also contribute to their in tumor accumulation. Overall, the albumin-bound PTX formulation allowed higher dosages than the Taxol® one and demonstrated improved efficacy and safety [333]. In addition, albumin was tested as a platform for delivery of other molecules that have anti-proliferative activity, such as rapamycin (~2.5 mg/ml). Albumin-bound rapamycin (ABI-009) has been in a clinical phase trial for the treatment of non-hematologic malignancies since January 2008 [334]. These recent advances as well as multiple clinical trials currently in progress for other types of cancer, opened the development of protein based NP for delivery of therapeutic agents [5].

Taking advantage of the high affinity that PTX showed for strong binding to human serum albumin (HAS), Lee et al. obtained shell crosslinked NC employing HAS and amine reactive multi-arm PEG. The NC were prepared by emulsifying the branched PEG in dichloromethane into aqueous solution of HAS, followed by cross-linking at the organic/aqueous interface [335]. PTX-loaded NC were spherical with an average diameter of about 280 nm. Surface modification was conducted using a flexible PEG linker with a cell-penetrating peptide, Hph1. ~~*In vitro*~~*In vitro* cellular studies using different cell lines, human breast adenocarcinoma (MCF-7), human ovarian carcinoma (OVCAR-3), human nasopharyngeal epidermal carcinoma (KB) and human coronary artery and smooth muscle cells (hCASMCs), it was observed that the peptide facilitated cellular uptake and apoptosis effects of PTX. The targeted anti-tumor activity of the PTX-loaded NC tested in a mouse tumor model indicated minimal clearance of the NC in the liver, and hence targeting to the tumor tissue. Tumor growth was significantly reduced in mice after intravenous administration of the system compared to control group.

With respect to the use of other proteins in the preparation of NP, Lu et al. [336~~H~~,337] prepared PTX loaded gelatin NP which showed good perspectives for intravesical therapy of superficial bladder cancer. PTX-loaded NP were obtained using a desolvation method with sizes from 600 to 1000 nm and they resulted active against human RT4 bladder transitional cancer cells. Results of *in vivo* experiments performed in dogs giving an intravesical dose of PTX-loaded NP, showed PTX concentration in the urothelium and lamina propria tissue layers 2.6 times the concentrations reported for dogs treated with the commercial Cremophor formulation.

For the treatment of lung cancer, one of the most malignant cancers today, gelatin NP were grafted with NeutrAvidin® FITC on the particle's surface, and afterward~~s~~, biotinylated EGF (bEGF) was conjugated with NeutrAvidin® FITC to improve targeting efficiency, forming a core~~–~~shell-like structure (bEGF-Av-NP of 220 nm average size). *In vitro* studies on adenocarcinoma A549 cells showed that entrance efficiency of bEGF-Av-NP and lysosomal entrapment was higher than that on normal lung cells (HFL1), and the uptake of bEGF-Av-NP by A549 cells was time and dose dependent [338]. Specific accumulation of the nanosystem in cancerous lung was confirmed after *in vivo* aerosol administration to cancerous lung of the SCID mice model [339].

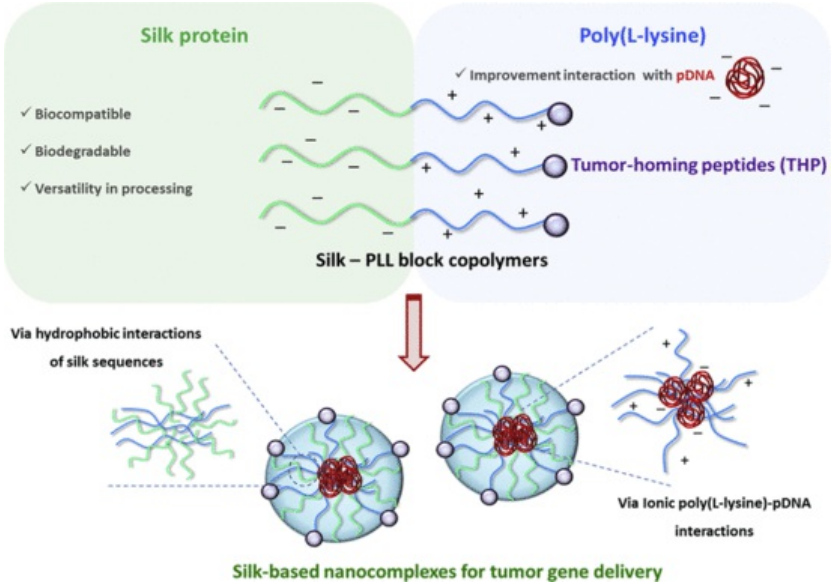
Thiolated gelatin NP were modified with PEG chains giving NP of 300~~–~~350 nm average diameter, to improve circulation and *in vivo* tumor-targeting of breast cancer. The *in vivo* behavior of the system was evaluated by injecting indium-111 (^{111}In)-labeled NP into breast tumor (MDA-MB-435) bearing nude mice. *In vivo* circulation times were found to be longer in PEG modified NP, showing plasma and tumor half-lives of 15.3 and 37.8 h, respectively, and preferential localization of thiolated NO in the tumor mass was detected [340]. These PEG modified thiolated gelatin NP were proposed as nanovectors for systemic delivery of therapeutic genes to human solid tumors. Thus, plasmid DNA (pDNA) encoding for the soluble form of the extracellular domain of VEG factor receptor-1 (VEGF-R1 or sFlt-1) was encapsulated in these NP. pDNA delivery produced the highest levels of sFlt-1 expression in the MDA-MB-435 human breast adenocarcinoma cell line. After intravenous administration in female Nu/Nu mice bearing orthotopic MDA-MB-435 breast adenocarcinoma xenografts, efficient *in vivo* expression of sFlt-1 pDNA was confirmed qualitatively and quantitatively. The expressed sFlt-1 was therapeutically active as shown by suppression of tumor growth and microvessel density measurements [341].

Tran et al. [342] synthetized amphiphilic gelatin with oleoyl moieties that successfully formed NP with versatile potential in drug delivery and tumor targeting. The amphiphilic NP entrapped PTX and they were further conjugated with FA for targeting HeLa cells. All NP were stable in human blood serum and their average size was below 300 nm, suitable for passive targeting. The release of PTX from both plain PTX- and FA conjugated PTX-loaded NP was controlled for a long time. The cytotoxicity results demonstrated great advantages of PTX-loaded NP either conjugated or not with FA, over the conventional dosage form of PTX (Taxol®) [125]. Wareechuensook et al. [126] modified gelatin into an amphiphilic molecule ~~*via*~~*via* conjugation with cholesterol. These amphiphiles aggregated in micelles in water at pH 5, and subsequently fabricated with entrapment of curcumin for a cancer research.

Silk fibroin (SF) is a protein that possesses inherent amphiphilicity because the repetition of the hexapeptide -Gly-Ser-Gly-Ala-Gly-Ala- gives rise to hydrophobic and hydrophilic blocks which self-assemble into micelles. In addition, the fibroin chains adopt the β-sheet conformation that acts as a stabilizing element of the nanostructures [343]. Another advantage of fibroin is that it possesses reactive carboxylic end groups that can be used for preparation of fibroin derivatives. Based on all these features, numerous silk SF micellar and nanoparticulate systems have been developed for cancer and inflammation treatment.

Chiang et al. [344] prepared silk fibroin NP with controllable size by a simple method in which the encapsulation of PTX was successfully achieved, leading to PTX-loaded NP with average size from 270 to 520 nm. PTX release was controlled over 9 days, and the release could be prolonged for 2 weeks varying the drug charge, what improved the potential of this system for chemotherapy in clinical applications. Other SF nanocarriers were prepared by Zhao et al. [345] using a novel solution-enhanced dispersion method using supercritical CO_2. The anti-inflammatory drug IMC was charged. Treatment with ethanol did not affect the biocompatibility of the system. *In vitro* IMC release from the IMC-loaded NP after ethanol treatment was significantly sustained over 2 days. These studies indicated the suitability of the supercritical CO_2 process to achieve the co-precipitation of drug and protein to form active NP with potential in the treatment of inflammatory processes.

SF has been chemically modified for tumor specific gene delivery by Numata et al. [346]. In this work, block copolymers of SF with PLL domains were prepared to interact with pDNA and the tumor-homing peptides (THP), to bind specific tumor cells and achieve pDNA delivery. Globular nanocomplexes of average diameter in the range 150~~–~~250 nm were obtained. After *in vitro* transfection experiments into MDA-MB-435 melanoma cells and MDA-MB-231 metastatic human breast tumor cells, using non-tumorigenic MCF-10A breast epithelial cells as a control, this system was proposed as a new platform for nonviral gene delivery in tumor cells. Further studies focused on the optimization of the content of THP to enhance specificity and efficiency to tumor cells. The silk-*b*-PLL block copolymer containing Lyp1 (ML-Lyp1) showed significant differences in cytotoxicity to MCF10A cells from the block copolymer containing F3 (ML-F3), indicating that ML-F3 was the best candidate for target delivery into tumorigenic cells (see Fig~~ure~~. 15) [347].



Fig~~ure.~~ 15 Schematic representation of the formation of pDNA nanocomplexes based on block copolymers of silk fibroin with poly(~~L-LYSINE) DOMAINS FOR TUMOR CELL-SPECIFIC D~~L-lysine) domains for tumor cell-specific delivery.

5 C~~ONCLUSIONS AND PERSPECTIVES FOR THE FUTURE~~onclusions and perspectives for the future

The challenge of the application of drugs for cancer and anti-inflammatory therapies is the preparation and application of selective drugs and drug delivery systems with high efficacy and selectivity for the tumor cells. The design of polymer systems with the specific functionality and equilibrated balance of hydrophobic and hydrophilic components offers exceptional possibilities for the preparation of bioactive formulations of high efficacy and low toxicity. In addition, the incorporation of signaling functions and targeting ligands to the macromolecular matrices offers very interesting approaches for the specific targeting of the bioactive systems with the guarantee of very low toxicity while are moving to the target site.

The application of resorbable or biodegradable polymers and composites, as well as bioactive “polymer drugs”, should involve the development of novel and advanced methodologies for treatments of high efficacy, selectivity and low toxicity, which would offer new alternatives to those traditionally used in cancer therapies. It is probably more necessary to find the best, more active and less toxic way to apply bioactive compounds and specific drugs, than the discovery of new drugs.

From a biomimetic point of view, there is a challenge for the design, preparation and application of polymer therapeutics with the design and morphology offered by self-assembling bioactive polymer systems, which can offer the appropriate conditions of low toxicity, targeting and selectivity by the incorporation of specific compounds such as cell signaling (ligands) and a core~~-~~shell morphology that guarantees the circulation and accumulation in the site of action. In addition, the combination of diagnostic and treatment (theranostic) is of great importance in new developments. The application of well-known polymer systems with the appropriate balance of hydrophobic/hydrophilic components in a biomimetic scenario, together with the incorporation of effective drugs applied in the clinics, offers a very interesting alternative to classical treatments, and facilitates the development of cheap and effective treatments for cancer therapies and anti-inflammatory strategies.

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References

[1]

S. Nazir, T. Hussain, A. Ayub, U. Rashid and A.J. MacRobert, Nanomaterials in combating cancer: ~~T~~therapeutic applications and developments, *Nanomedicine* **10**, 2014, 19–34.

[2]

I.F. Uchegbu and A. Siew, Nanomedicines and nanodiagnostics come of age, *J Pharm Sci* **102**, 2013, 305–310.

[3]

J.A. MacKay, A. Almutairi, W. Hennink and A.S. Hoffman, NanoDDS 2013: ~~T~~the 11th International Nano Drug Delivery Symposium, *J Controlled Release* **191**, 2014, 1–3.

[4]

M.C. Branco and J.P. Schneider, Self-assembling materials for therapeutic delivery, *Acta Biomater* **5**, 2009, 817–831.

[5]

F. Alexis, E.M. Pridgen, R. Langer and O.C. Farokhzad, Nanoparticle technologies for cancer therapy, In: M. Schäfer-Korting, (Ed), *Drug delivery*, 2010, Springer; Berlin Heidelberg, 55–86.

[6]

L. Mei, Z. Zhang, L. Zhao, L. Huang, X.L. Yang, J. Tang and S.S. Feng, Pharmaceutical nanotechnology for oral delivery of anticancer drugs, *Adv Drug Deliv Rev* **65**, 2013, 880–890.

[7]

Y. Lu and K. Park, Polymeric micelles and alternative nanonized delivery vehicles for poorly soluble drugs, *Int J Pharm* **453**, 2013, 198–214.

[8]

N. Nishiyama and K. Kataoka, Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery, *Pharmacol Ther* **112**, 2006, 630–648.

[9]

C. Oerlemans, W. Bult, M. Bos, G. Storm, J.F.W. Nijsen and W.E. Hennink, Polymeric micelles in anticancer therapy: targeting, imaging and triggered release, *Pharm Res* **27**, 2010, 2569–2589.

[10]

R. Wang, P.S. Billone and W.M. Mullett, Nanomedicine in action: an overview of cancer nanomedicine on the market and in clinical trials, *J Nanomater* **1**, 2013, 1–12.

[11]

J. Shaji and M. Lal, Nanocarriers for targeting in inflammation, *Asian J Pharm Clin Res* **6**, 2013, 3–12.

[12]

A.Z. Wang, R. Langer and O.C. Farokhzad, Nanoparticle delivery of cancer drugs, *Annu Rev Med* **63**, 2012, 185–198.

[13]

S.P. Egusquiaguirre, M. Igartua, R.M. Hernández and J.L. Pedraz, Nanoparticle delivery systems for cancer therapy: advances in clinical and preclinical research, *Clin Transl Oncol* **14**, 2012, 83–93.

[14]

M.E. Davis, Nanoparticle therapeutics: an emerging treatment modality for cancer, *Nat Rev Drug Discov* **7**, 2008, 771–782.

[15]

R. Misra, M. Upadhyay and S. Mohanty, Nanoparticles as ~~Carriers for Chemotherapeutic Drugs: A R~~carriers for chemotherapeutic drugs: a review, *J Nanopharm Drug Deliv* **1**, 2013, 103–137.

[16]

J.H. Park, S. Lee, J.H. Kim, K. Park, K. Kim and I.C. Kwon, Polymeric nanomedicine for cancer therapy, *Prog Polym Sci* **33**, 2008, 113–137.

[17]

S. Parveen, R. Misra and S.K. Sahoo, ~~Nanoparticles:~~Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging, *Nanomedicine* **8**, 2012, 147–166.

[18]

U. Kedar, P. Phutane, S. Shidhaye and V. Kadam, Advances in polymeric micelles for drug delivery and tumor targeting, *Nanomedicine* **6**, 2010, 714–729.

[19]

A. Rösler, G.W.M. Vandermeulen and H.A. Klok, Advanced drug delivery devices via self-assembly of amphiphilic block copolymers, ~~. Adv Drug Deliv Rev 2012; 64, Supplement:270-9.~~ *Adv Drug Deliv Rev* **64** (Suppl.), 2012, 270–279.

[20]

R. Duncan, Polymer therapeutics as nanomedicines: new perspectives, *Curr Opin Biotechnol* **22**, 2011, 492–501.

[21]

R. Duncan and M.J. Vicent, Polymer therapeutics-prospects for 21st century: the end of the beginning, *Adv Drug Deliv Rev* **65**, 2013, 60–70.

[22]

S.A. Mousa and D.J. Bharali, Nanotechnology-based detection and targeted therapy in cancer: nano-bio paradigms and applications, *Cancers* **3**, 2011, 2888–2903.

[23]

A. Kumari, S.K. Yadav and S.C. Yadav, Biodegradable polymeric nanoparticles based drug delivery systems, *Colloids Surf ~~B~~, B: Biointerfaces* **75**, 2010, 1–18.

[24]

C. Deng, Y. Jiang, R. Cheng, F. Meng and Z. Zhong, Biodegradable polymeric micelles for targeted and controlled anticancer drug delivery: ~~P~~promises, progress and prospects, *Nano Today* **7**, 2012, 467–480.

[25]

L.N. Hassani, F. Hendra and K. Bouchemal, Auto-associative amphiphilic polysaccharides as drug delivery systems, *Drug Discov Today* **17**, 2012, 608–614.

[26]

K. Miyata, R.J. Christie and K. Kataoka, Polymeric micelles for nano-scale drug delivery, *React Funct Polym* **71**, 2011, 227–234.

[27]

W.R. Gombotz and A.S. Hoffman, B.6—polymeric micelles, In: B.D. Ratner, A.S. Hoffman, F.J. Schonene and J.E. Lemons, (Eds.), *Biomaterials science*, 3rd ed., 2013, Academic Press; Madison, WI, 1041–1045.

[28]

H. Ding, X.J. Wang, S. Zhang and X. Liu, Applications of polymeric micelles with tumor targeted in chemotherapy, ~~. J Nanopart Res 2012; 14:1254/1-13.~~ *J Nanopart Res* **14** (1254), 2012, 1–13.

[29]

N. Rapoport, Physical stimuli-responsive polymeric micelles for anti-cancer drug delivery, *Prog Polym Sci* **32**, 2007, 962–990.

[30]

M.C. Jones and J.C. Leroux, Polymeric micelles~~- -~~—a new generation of colloidal drug carriers, *Eur J Pharm Biopharm* **48**, 1999, 101–111.

[31]

I. Brigger, C. Dubernet and P. Couvreur, Nanoparticles in cancer therapy and diagnosis, *Adv Drug Deliv Rev* **64** (Supplement), 2012, 24–36.

[32]

J.H. Choi, J.Y. Jang, Y.K. Joung, M.H. Kwon and K.D. Park, Intracellular delivery and anti-cancer effect of self-assembled heparin~~-~~–Pluronic nanogels with RNase A, *J Controlled Release* **147**, 2010, 420–427.

[33]

C. Mora-Huertas, H. Fessi and A. Elaissari, Polymer-based nanocapsules for drug delivery, *Int J Pharm* **385**, 2010, 113–142.

[34]

V. Percec, D.A. Wilson, P. Leowanawat, C.J. Wilson, A.D. Hughes, M.S. Kaucher, D.A. Hammer, D.H. Levine, A.J. Kim, F.S. Bates, K.P. Davis, T.P. Lodge, M.L. Klein, R.H. DeVane, E. Aqad, B.M. Rosen, A.O. Argintaru, M.J. Sienkowska, K. Rissanen, S. Nummelin and J. Ropponen, Self-~~Assembly of Janus Dendrimers into Uniform Dendrimersomes and Other Complex A~~assembly of Janus dendrimers into uniform dendrimersomes and other complex architectures,

Science **328**, 2010, 1009–1014.

[35]

W. She, N. Li, K. Luo, C. Guo, G. Wang, Y. Geng and Z. Gu, Dendronized heparin–doxorubicin conjugate based nanoparticle as pH-responsive drug delivery system for cancer therapy, *Biomaterials* **34**, 2013, 2252–2264.

[36]

A.D. Schlüter and J.P. Rabe, Dendronized polymers: synthesis, characterization, assembly at interfaces, and manipulation, *Angew Chem Int Ed* **39**, 2000, 864–883.

[37]

H. Frauenrath, Dendronized polymers—building a new bridge from molecules to nanoscopic objects, *Prog Polym Sci* **30**, 2005, 325–384.

[38]

A.O. Saeed, J.P. Magnusson, E. Moradi, M. Soliman, W. Wang, S. Stolnik, K.J. Thurecht, S.M. Howdle and C. Alexander, Modular construction of multifunctional bioresponsive cell-targeted nanoparticles for gene delivery, *Bioconjugate Chem* **22**, 2011, 156–168.

[39]

V. Sanna, I.A. Siddiqui, M. Sechi and H. Mukhtar, Resveratrol-loaded nanoparticles based on poly(epsiloncaprolactone) and poly(D,L-lactic-co-glycolic acid)-poly(ethylene glycol) blend for prostate cancer treatment, *Mol Pharm* **10**, 2013, 3871–3881.

[40]

B. Li, Q. Wang, X. Wang, C. Wang and X. Jiang, Preparation, drug release and cellular uptake of doxorubicin-loaded dextran-*b*-poly(ε-caprolactone) nanoparticles, *Carbohydr Polym* **93**, 2013, 430–437.

[41]

A. Cirstoiu-Hapca, F. Buchegger, L. Bossy, M. Kosinski, R. Gurny and F. Delie, Nanomedicines for active targeting: physico-chemical characterization of paclitaxel-loaded anti-HER2 immunonanoparticles and in vitro functional studies on target cells, *Eur J Pharm Sci* **38**, 2009, 230–237.

[42]

H. Koo, K.H. Min, S.C. Lee, J.H. Park, K. Park, S.Y. Jeong, K. Choi, I.C. Kwon and K. Kim, Enhanced drug-loading and therapeutic efficacy of hydrotropic oligomer-conjugated glycol chitosan nanoparticles for tumor-targeted paclitaxel delivery, *J Controlled Release* **172**, 2013, 823–831.

[43]

C. Zhang, G. Li, Y. Wang, F. Cui, J. Zhang and Q. Huang, Preparation and characterization of 5-fluorouracil-loaded PLLA-PEG/PEG nanoparticles by a novel supercritical CO_2 technique, *Int J Pharm* **436**, 2012, 272–281.

[44]

S.A. Meenach, K.W. Anderson, J. Zach Hilt, R.C. McGarry and H.M. Mansour, Characterization and aerosol dispersion performance of advanced spray-dried chemotherapeutic PEGylated phospholipid particles for dry powder inhalation delivery in lung cancer, *Eur J Pharm Sci* **49**, 2013, 699–711.

[45]

A. Shalviri, H.K. Chan, G. Raval, M.J. Abdekhodaie, Q. Liu, H. Heerklotz and X.Y. Wu, Design of pH-responsive nanoparticles of terpolymer of poly(methacrylic acid), polysorbate 80 and starch for delivery of doxorubicin, *Colloids Surf B, B: Biointerfaces* **101**, 2013, 405–413.

[46]

A. Shalviri, G. Raval, P. Prasad, C. Chan, Q. Liu, H. Heerklotz, A.M. Rauth and X.Y. Wu, PH-Dependent doxorubicin release from terpolymer of starch, polymethacrylic acid and polysorbate 80 nanoparticles for overcoming multi-drug resistance in human breast cancer cells, *Eur J Pharm Biopharm* **82**, 2012, 587–597.

[47]

Y. Bae and K. Kataoka, Intelligent polymeric micelles from functional poly(ethylene glycol)-poly(amino acid) block copolymers, *Adv Drug Deliv Rev* **61**, 2009, 768–784.

[48]

H. Rettig, E. Krause and H.G. Börner, Atom transfer radical polymerization with polypeptide initiators: ~~A~~a general approach to block copolymers of sequence-defined polypeptides and synthetic polymers, *Macromol Rapid Commun* **25**, 2004, 1251–1256.

[49]

J. Ding, C. Xiao, C. He, M. Li, D. Li, X. Zhuang and X. Chen, Facile preparation of a cationic poly(amino acid) vesicle for potential drug and gene co-delivery, *Nanotechnology* **22**, 2011, 1–9.

[50]

J. Ding, C. Xiao, Z. Tang, X. Zhuang and X. Chen, Highly ~~Efficient "Grafting From" an α-Helical Polypeptide Backbone by Atom Transfer Radical P~~efficient "grafting from" an α-helical polypeptide backbone by atom transfer radical polymerization, *Macromol Biosci* **11**, 2011, 192–198.

[51]

M.L. Lopez-~~-~~Donaire, J. Parra-Caceres, B. Vazquez-Lasa, I. Garcia-Alvarez, A. Fernandez-Mayoralas, A. Lopez-Bravo and J. San Roman, Polymeric drugs based on bioactive glycosides for the treatment of brain tumours, *Biomaterials* **30**, 2009, 1613–1626.

[52]

M.L. Lopez-Donaire, E.M. Sussman, M. Fernandez-Gutierrez, A. Mendez-Vilas, B.D. Ratner, B. Vazquez-Lasa and J. San Roman, Amphiphilic self-assembled "polymeric drugs": ~~M~~morphology, properties, and biological behavior of nanoparticles, *Biomacromolecules* **13**, 2012, 624–635.

[53]

L. Garc~~ia-Fernaía-Fernán~~dez, M.R. Aguilar, M.M. Fernandez, R.M. Lozano, G. Gimenez, S. Valverde and J. San Roman, Structure, morphology, and bioactivity of biocompatible systems derived from functionalized acrylic polymers based on 5-amino-2-naphthalene sulfonic acid, *Biomacromolecules* **11**, 2010, 1763–1772.

[54]

L. Garc~~ia-Fernaía-Fernán~~dez, M.R. Aguilar, L. Ochoa-Callejero, C. Abradelo, A. Martinez and J. San Roman, BFGF interaction and in vivo angiogenesis inhibition by self-assembling sulfonic acid-based copolymers, *J Mater Sci Mater Med* **23**, 2012, 129–135.

[55]

L. Garcia-Fernandez, S. Halstenberg, R.E. Unger, M.R. Aguilar, C.J. Kirkpatrick and J. San Roman, Anti-angiogenic activity of heparin-like polysulfonated polymeric drugs in 3D human cell culture, *Biomaterials* **31**, 2010, 7863–7872.

[56]

J. Nicolas, Y. Guillaneuf, C. Lefay, D. Bertin, D. Gigmes and B. Charleux, Nitroxide-mediated polymerization, *Prog Polym Sci* **38**, 2013, 63–235.

[57]

D.J. Siegwart, J.K. Oh and K. Matyjaszewski, ATRP in the design of functional materials for biomedical applications, *Prog Polym Sci* **37**, 2012, 18–37.

[58]

V.M.C. Coessens and K. Matyjaszewski, Fundamentals of ~~Atom Transfer Radical P~~atom transfer radical polymerization, *J Chem Educ* **87**, 2010, 916–919.

[59]

C. Giacomelli, V. Schmidt and R. Borsali, Nanocontainers formed by self-assembly of poly~~-(ethylene oxide)-b-poly-~~(ethylene oxide)-*b*-poly(glycerol monomethacrylate)~~--~~drug conjugates, *Macromolecules* **40**, 2007, 2148–2157.

[60]

X. Jiang, G. Zhang, R. Narain and S. Liu, Covalently stabilized temperature and pH responsive four-layer nanoparticles fabricated from surface 'clickable'~~-~~shell cross-linked micelles, *Soft Matter* **5**, 2009, 1530–1538.

[61]

A. Gregory and M.H. Stenzel, Complex polymer architectures via RAFT polymerization: ~~F~~from fundamental process to extending the scope using click chemistry and nature's building blocks, *Prog Polym Sci* **37**, 2012, 38–105.

[62]

J.T. Sun, C.Y. Hong and C.Y. Pan, Recent advances in RAFT dispersion polymerization for preparation of block copolymer aggregates, *Polym Chem* **4**, 2013, 873–881.

[63]

P. De, S.R. Gondi and B.S. Sumerlin, Folate-conjugated thermoresponsive block copolymers: highly efficient conjugation and solution self-assembly, *Biomacromolecules* **9**, 2008, 1064–1070.

[64]

C.Y. Quan, D.Q. Wu, C. Chang, G.B. Zhang, S.X. Cheng, X.Z. Zhang and R.X. Zhuo, Synthesis of thermo-sensitive micellar aggregates self-assembled from biotinylated PNAS-~~*b-PNIPAAm-b*~~*b*-PNIPAAm-*b*-PCL triblock copolymers for tumor targeting, *J Phys Chem C* **113**, 2009, 11262–11267.

[65]

B.M. Blunden, H. Lu and M.H. Stenzel, Enhanced delivery of the RAPTA-C macromolecular chemotherapeutic by conjugation to degradable polymeric micelles, *Biomacromolecules* **14**, 2013, 4177–4188.

[66]

J. Zhao, J. Liu, S. Han, H. Deng, L. Deng, J. Liu, A. Meng, A. Dong and J. Zhang, Acid-induced disassemblable nanoparticles based on cyclic benzylidene acetal-functionalized graft copolymer via sequential RAFT and ATRP polymerization, *Polym Chem* **5**, 2014, 1852–1856.

[67]

Y.C. Xu and C.M. Dong, Dendron-like poly(e-benzyloxycarbonyl-~~L~~-L-lysine)/linear PEO block copolymers: ~~S~~synthesis, physical characterization, self-assembly, and drug-release behavior, *J Polym Sci ~~Part A,~~ A: Polym Chem* **50**, 2012, 1216–1225.

[68]

J. Lu, M. Shi and M.S. Shoichet, Click chemistry functionalized polymeric nanoparticles target corneal epithelial cells through RGD-cell surface receptors, *Bioconj~~ugate~~ Chem* **20**, 2008, 87–94.

[69]

D.P.Y. Chan, G.F. Deleavey, S.C. Owen, M.J. Damha and M.S. Shoichet, Click conjugated polymeric immuno-nanoparticles for targeted siRNA and antisense oligonucleotide delivery, *Biomaterials* **34**, 2013, 8408–8415.

[70]

A.E. Van ~~D~~der Ende, V. Sathiyakumar, R. Diaz, D.E. Hallahan and E. Harth, Linear release nanoparticle devices for advanced targeted cancer therapies with increased efficacy, *Polym Chem* **1**, 2010, 93–96.

[71]

R.K. Shukla and A. Tiwari, Carbohydrate molecules: ~~A~~an expanding horizon in drug delivery and biomedicine, *Crit Rev Ther Drug Carrier Syst* **28**, 2011, 255–292.

[72]

Bc. Bae and K. Na, Self-quenching polysaccharide-based nanogels of pullulan/folate-~~–~~photosensitizer conjugates for photodynamic therapy, *Biomaterials* **31**, 2010, 6325–6335.

[73]

H. Lee, K. Lee and T.G. Park, Hyaluronic ~~Acid–Paclitaxel Conjugate Micelles: Synthesis, Characterization, and Antitumor A~~acid–paclitaxel conjugate micelles: synthesis, characterization, and antitumor activity, *Bioconj~~ugate~~ Chem* **19**, 2008, 1319–1325.

[74]

S. Kim, S.Y. Chae, K. Na, S.W. Kim and Y.H. Bae, Insulinotropic activity of sulfonylurea/pullulan conjugate in rat islet microcapsule, *Biomaterials* **24**, 2003, 4843–4851.

[75]

S. Kim, K.M. Park, J.Y. Ko, I.C. Kwon, H.G. Cho, D. Kang, I.T. Yu, K. Kim and K. Na, Minimalism in fabrication of self-organized nanogels holding both anti-cancer drug and targeting moiety, *Colloids Sur~~f B,~~ B: Biointerfaces* **63**, 2008, 55–63.

[76]

L. Li, J.K. Kim, K.M. Huh, Y.k. Lee and S.Y. Kim, Targeted delivery of paclitaxel using folate-conjugated heparin-poly(β-benzyl-~~L~~L-aspartate) self-assembled nanoparticles, *Carbohydr Polym* **87**, 2012, 2120–2128.

[77]

K. Park, G.Y. Lee, Y.S. Kim, M. Yu, R.W. Park, I.S. Kim, S.Y. Kim and Y. Byun, Heparin–deoxycholic acid chemical conjugate as an anticancer drug carrier and its antitumor activity, *J Controlled Release* **114**, 2006, 300–306.

[78]

D. Bellini and A. Topai, Amides of hyaluronic acid and the derivatives thereof and a process for their preparation, ~~. US PCT Int Appl Wo,~~ US PCT Int Appl Wo, 2013, Fidia Farmaceutici SPA.

~~2013.~~**[79]**

L. Huang and R.J. Kerns, Diversity-oriented chemical modification of heparin: ~~i~~identification of charge-reduced ~~*N*~~*N*-acyl heparin derivatives having increased selectivity for heparin-binding proteins, *Biorg Med Chem* **14**, 2006, 2300–2313.

[80]

C.E. Schanté, G. Zuber, C. Herlin and T.F. Vandamme, Chemical modifications of hyaluronic acid for the synthesis of derivatives for a broad range of biomedical applications, *Carbohydr Polym* **85**, 2011, 469–489.

[81]

C. Fernández, C.M. Hattan and R.J. Kerns, Semi-synthetic heparin derivatives: chemical modifications of heparin beyond chain length, sulfate substitution pattern and ~~*N-sulfo/N*~~*N*-sulfo/*N*-acetyl groups, *Carbohydr Res* **341**, 2006, 1253–1265.

[82]

W. Park, K.s. Kim, B.c. Bae, Y.H. Kim and K. Na, Cancer cell specific targeting of nanogels from acetylated hyaluronic acid with low molecular weight, *Eur J Pharm Sci* **40**, 2010, 367–375.

[83]

K.K. Upadhyay, J.F.L. Meins, A. Misra, P. Voisin, V. Bouchaud, E. Ibarboure, C. Schatz and S. Lecommandoux, Biomimetic ~~Doxorubicin-Loaded Polymersomes from Hyaluronan-block-P~~doxorubicin loaded polymersomes from hyaluronan-block-poly((-benzyl glutamate) ~~C~~copolymers, *Biomacromolecules* **10**, 2009, 2802–2808.

[84]

M. Kong, H. Park, C. Feng, L. Hou, X. Cheng and X. Chen, Construction of hyaluronic acid noisome as functional transdermal nanocarrier for tumor therapy, *Carbohydr Polym* **94**, 2013, 634–641.

[85]

Y. Liu, J. Sun, W. Cao, J. Yang, H. Lian, X. Li, Y. Sun, Y. Wang, S. Wang and Z. He, Dual targeting folate-conjugated hyaluronic acid polymeric micelles for paclitaxel delivery, *Int J Pharm* **421**, 2011, 160–169.

[86]

J.L. Wu, C.G. Liu, X.L. Wang and Z.H. Huang, Preparation and characterization of nanoparticles based on histidine-~~H~~-hyaluronic acid conjugates as doxorubicin carriers, *J Mater Sci Mater Med* **23**, 2012, 1921–1929.

[87]

S. Ganesh, A.K. Iyer, D.V. Morrissey and M.M. Amiji, Hyaluronic acid based self-assembling nanosystems for CD44 target mediated siRNA delivery to solid tumors, *Biomaterials* **34**, 2013, 3489–3502.

[88]

H.S. Han, J. Lee, H.R. Kim, S.Y. Chae, M. Kim, G. Saravanakumar, H.Y. Yoon, D.G. You, H. Ko, K. Kim, I.C. Kwon, J.C. Park and J.H. Park, Robust PEGylated hyaluronic acid nanoparticles as the carrier of doxorubicin: ~~M~~mineralization and its effect on tumor targetability in vivo, *J Controlled Release* **168**, 2013, 105–114.

[89]

J. Li, M. Huo, J. Wang, J. Zhou, J.M. Mohammad, Y. Zhang, Q. Zhu, A.Y. Waddad and Q. Zhang, Redox-sensitive micelles self-assembled from amphiphilic hyaluronic acid-deoxycholic acid conjugates for targeted intracellular delivery of paclitaxel, *Biomaterials* **33**, 2012, 2310–2320.

[90]

G. Saravanakumar, K.Y. Choi, H.Y. Yoon, K. Kim, J.H. Park, I.C. Kwon and K. Park, Hydrotropic hyaluronic acid conjugates: Ssynthesis, characterization, and implications as a carrier of paclitaxel, *Int J Pharm* **394**, 2010, 154–161.

[91]

T.H. Tran, B.c. Bae, Y.k. Lee, K. Na and K.M. Huh, Heparin-folate- –folate–retinoic acid bioconjugates for targeted delivery of hydrophobic photosensitizers, *JT Carbohydr PolymCarbohydr Polym* **92**, 2013, 1615–1624.

[92]

I.h. Oh, K. Cho, T. Tran, K. Huh and Yk. Lee, Biofuntional nanoparticle formation and folate-targeted antitumor effect of heparin- –retinoic acid conjugates, *Macromol Res* **20**, 2012, 520–527.

[93]

I.K. Park, T.H. Tran, I.H. Oh, Y.J. Kim, K.J. Cho, K.M. Huh and Y.K. Lee, Ternary biomolecular nanoparticles for targeting of cancer cells and anti-angiogenesis, *- Eur J Pharm Sci 2010;41:148-55.Eur J Pharm Sci* **41**, 2010, 148–155.

[94]

D. Xin, Y. Wang and J. Xiang, The use of amino acid linkers in the conjugation of paclitaxel with hyaluronic acid as drug delivery system: Ssynthesis, self-assembled property, drug release, and in vitro efficiency, *Pharm Res* **27**, 2010, 380–389.

[95]

I.K. Park, Y.J. Kim, T.H. Tran, K.M. Huh and Y.k. Lee, Water-soluble heparin–PTX conjugates for cancer targeting, *Polymer* **51**, 2010, 3387–3393.

[96]

Z. Khatun, M. Nurunnabi, G.R. Reeck, K.J. Cho and Y.K. Lee, Oral delivery of taurocholic acid linked heparin–docetaxel conjugates for cancer therapy, *- J Controlled Release 2013;170:74-82.J Controlled Release* **170**, 2013, 74–82.

[97]

S. Manju and K. Sreenivasan, Conjugation of curcumin onto hyaluronic acid enhances its aqueous solubility and stability, *J Colloid Interface Sci* **359**, 2011, 318–325.

[98]

Basu S, Das A. Curcumin-Hyaluronan Compounds. US 11/966,398 Wo, Kent State University; 2009.

[99]

P. Opanasopit, T. Ngawhirunpat, T. Rojanarata, C. Choochottiros and S. Chirachanchai, *N*-Phthaloylchitosan-g-mPEG design for all-trans retinoic acid-loaded polymeric micelles, *Eur J Pharm Sci* **30**, 2007, 424–431.

[100]

Y.Z. Du, Q. Weng, H. Yuan and F.Q. Hu, Synthesis and antitumor activity of stearate-g-g-dextran micelles for intracellular doxorubicin delivery, *ACS Nano* **4**, 2010, 6894–6902.

[101]

L. Hou, J. Yao, J. Zhou and Q. Zhang, Pharmacokinetics of a paclitaxel-loaded low molecular weight heparin-all-*trans*trans-retinoid acid conjugate ternary nanoparticulate drug delivery system, *Biomaterials* **33**, 2012, 5431–5440.

[102]

J.S. Yang, Q.Q. Zhou and W. He, Amphipathicity and self-assembly behavior of amphiphilic alginate esters, *Carbohydr Polym* **92**, 2013, 223–227.

[103]

C. Zhang, W. Wang, C. Wang, Q. Tian, W. Huang, Z. Yuan and X. Chen, Cytotoxicity of liver targeted drug-loaded alginate nanoparticles, *Sci China Ser B* **52**, 2009, 1382–1387.

[104]

R. Guo, L. Zhang, Z. Jiang, Y. Cao, Y. Ding and X. Jiang, Synthesis of alginic acid-poly[2-(diethylamino)ethyl methacrylate] monodispersed nanoparticles by a polymer- –monomer pair reaction system, *Biomacromolecules* **8**, 2007, 843–850.

[105]

M. Chen, Y. Liu, W. Yang, X. Li, L. Liu, Z. Zhou, Y. Wang, R. Li and Q. Zhang, Preparation and characterization of self-assembled nanoparticles of 6-*O*-cholesterol-modified chitosan for drug delivery, *Carbohydr Polym* **84**, 2011, 1244–1251.

[106]

K. Kurita, Controlled functionalization of the polysaccharide chitin, *Prog Polym Sci* **26**, 2001, 1921–1971.

[107]

H. Sashiwa and S.I. Aiba, Chemically modified chitin and chitosan as biomaterials, *Prog Polym Sci* **29**, 2004, 887–908.

[108]

Y. Zhang, M. Huo, J. Zhou, D. Yu and Y. Wu, Potential of amphiphilically modified low molecular weight chitosan as a novel carrier for hydrophobic anticancer drug: Ssynthesis, characterization, micellization and cytotoxicity evaluation, *Carbohydr Polym* **77**, 2009, 231–238.

[109]

Y.Z. Du, L. Wang, H. Yuan, X.H. Wei and F.Q. Hu, Preparation and characteristics of linoleic acid-grafted chitosan oligosaccharide micelles as a carrier for doxorubicin, *Colloids Surf B, B: Biointerfaces* **69**, 2009, 257–263.

[110]

F.Q. Hu, G.F. Ren, H. Yuan, Y.Z. Du and S. Zeng, Shell cross-linked stearic acid grafted chitosan oligosaccharide self-aggregated micelles for controlled release of paclitaxel, *Colloids Surf B, B: Biointerfaces* **50**, 2006, 97–103.

[111]

J. You, F.Q. Hu, Y.Z. Du and H. Yuan, Polymeric micelles with glycolipid-like structure and multiple hydrophobic domains for mediating molecular target delivery of paclitaxel, *Biomacromolecules* **8**, 2007, 2450–2456.

[112]

F.Q. Hu, Wu Xl, Y.Z. Du, J. You and H. Yuan, Cellular uptake and cytotoxicity of shell crosslinked stearic acid-grafted chitosan oligosaccharide micelles encapsulating doxorubicin, *Eur J Pharm Biopharm* **69**, 2008, 117–125.

[113]

Z. Pan, Y. Gao, L. Heng, Y. Liu, G. Yao, Y. Wang and Y. Liu, Amphiphilic *N*-(2,3-dihydroxypropyl)-chitosan–cholic acid micelles for paclitaxel delivery, *Carbohydr Polym* **94**, 2013, 394–399.

[114]

J.H. Kim, S.M. Bae, M.H. Na, H. Shin, Y.J. Yang, K.H. Min, K.Y. Choi, K. Kim, R.W. Park and I.C. Kwon, Facilitated intracellular delivery of peptide-guided nanoparticles in tumor tissues, *J Controlled Release* **157**, 2012, 493–499.

[115]

W.z. Yang, H.l. Chen, F.p. Gao, M.m. Chen, X.m. Li, M.m. Zhang, Q.q. Zhang, L.r. Liu, Q. Jiang and Ys. Wang, Self-aggregated nanoparticles of cholesterol-modified pullulan conjugate as a novel carrier of mitoxantronep, *Curr Nanosci* **6**, 2010, 298–306.

[116]

W. Yang, M. Wang, L. Ma, H. Li and L. Huang, Synthesis and characterization of biotin modified cholesteryl pullulan as a novel anticancer drug carrier, *Carbohydr Polym* **99**, 2014, 720–727.

[117]

H. Zhang, F. Li, J. Yi, C. Gu, L. Fan, Y. Qiao, Y. Tao, C. Cheng and H. Wu, Folate-decorated maleilated pullulan–doxorubicin conjugate for active tumor-targeted drug delivery, *Eur J Pharm Sci* **42**, 2011, 517–526.

[118]

A. Scomparin, S. Salmaso, S. Bersani, R. Satchi-Fainaro and P. Caliceti, Novel folated and non-folated pullulan bioconjugates for anticancer drug delivery, *Eur J Pharm Sci* **42**, 2011, 547–558.

[119]

Y. Sasaki, W. Asayama, T. Niwa, S.I. Sawada, T. Ueda, H. Taguchi and K. Akiyoshi, Amphiphilic Polysaccharide Nanogels as Artificial Chaperones in Cell-Free Protein Spolysaccharide nanogels as artificial chaperones in cell-free protein synthesis, *Macromol Biosci* **11**, 2011, 814–820.

[120]

A. Besheer, G. Hause, J. Kressler and K. Mäder, Hydrophobically modified hydroxyethyl starch: Ssynthesis, characterization, and aqueous self-assembly into nano-sized polymeric micelles and vesicles, *Biomacromolecules* **8**, 2007, 359–367.

[121]

Q. Liu, X. Yang, H. Xu, K. Pan and Y. Yang, Novel nanomicelles originating from hydroxyethyl starch-g-polylactide and their release behavior of docetaxel modulated by the PLA chain length, *Eur Polym J* **49**, 2013, 3522–3529.

[122]

Q. Luo, P. Wang, Y. Miao, H. He and X. Tang, A novel 5-fluorouracil prodrug using hydroxyethyl starch as a macromolecular carrier for sustained release, *Carbohydr Polym* **87**, 2012, 2642–2647.

[123]

K. Na, K.H. Park, S.W. Kim and Y.H. Bae, Self-assembled hydrogel nanoparticles from curdlan derivatives: characterization, anti-cancer drug release and interaction with a hepatoma cell line (HepG2), *J Controlled Release* **69**, 2000, 225–236.

[124]

W.M. Li, D.M. Liu and S.Y. Chen, Amphiphilically-modified gelatin nanoparticles: Sself-assembly behavior, controlled biodegradability, and rapid cellular uptake for intracellular drug delivery, *J Mater Chem* **21**, 2011, 12381–12388.

[125]

P.H.L. Tran, T.T.D. Tran, T.V. Vo, C.L.N. Vo and B.J. Lee, Novel multifunctional biocompatible gelatin–oleic acid conjugate: Sself-assembled nanoparticles for drug delivery, *J Biomed Nanotechnol* **9**, 2013, 1416–1431.

[126]

M. Wareechuensook, Y. Tabata and S. Kanokpanont, Characteristics of cholesterol-grafted gelatin micelles, *Adv Mater Res* **93–94**, 2010, 595–598.

[127]

Z. Cao, Y. Jin, B. Zhang, Q. Miao and C. Ma, A novel temperature- and pH-responsive Ppolymer-biomolecule conjugate composed of Casein and Poly(Ncasein and poly(*N*-isopropylacrylamide), *Iran Polym J* **19**, 2010, 689–698.

[128]

M.A. Serban and D.L. Kaplan, PH-sensitive ionomeric particles obtained via chemical conjugation of silk with poly(amino acid)s, *Biomacromolecules* **11**, 2010, 3406–3412.

[129]

J. Drbohlavova, J. Chomoucka, V. Adam, M. Ryvolova, T. Eckschlager, J. Hubalek and R. Kizek, Nanocarriers for anticancer drugs—N—new trends in nanomedicine, *Curr Drug Metab* **14**, 2013, 547–564.

[130]

D.M. Webster, P. Sundaram and M.E. Byrne, Injectable nanomaterials for drug delivery: Ccarriers, targeting moieties, and therapeutics, *Eur J Pharm Biopharm* **84**, 2013, 1–20.

[131]

N. Stephanopoulos, J.H. Ortony and S.I. Stupp, Self-assembly for the synthesis of functional biomaterials, *Acta Mater* **61**, 2013, 912–930.

[132]

S. Lv, M. Li, Z. Tang, W. Song, H. Sun, H. Liu and X. Chen, Doxorubicin-loaded amphiphilic polypeptide-based nanoparticles as an efficient drug delivery system for cancer therapy, *Acta Biomater* **9**, 2013, 9330–9342.

[133]

M. Li, W. Song, Z. Tang, S. Lv, L. Lin, H. Sun, Q. Li, Y. Yang, H. Hong and X. Chen, Nanoscaled Poly(l-glutamic acid)/Doxorubicin-Amphiphile Cpoly(L-glutamic acid)/doxorubicin–amphiphile complex as pH-responsive Drug Delivery System for Effective Treatment of Nonsmall Cell Lung Cdrug delivery system for effective treatment of nonsmall cell lung cancer, *ACS Appl Mater Interfaces* **5**, 2013, 1781–1792.

[134]

X. Li, H. Li, W. Yi, J. Chen and B. Liang, Acid-triggered core cross-linked nanomicelles for targeted drug delivery and magnetic resonance imaging in liver cancer cells, *Int J Nanomed* **8**, 2013, 3019–3031.

[135]

T. Hamaguchi, Y. Matsumura, M. Suzuki, K. Shimizu, R. Goda, I. Nakamura, I. Nakatomi, M. Yokoyama, K. Kataoka and T. Kakizoe, NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend in vivo antitumour activity and reduce the neurotoxicity of paclitaxel, *Br J Cancer* **92**, 2005, 1240–1246.

[136]

Y. Matsumura, Poly-(amino acid) micelle nanocarriers in preclinical and clinical studies, *Adv Drug Deliv Rev* **60**, 2008, 899–914.

[137]

J. Gong, M. Chen, Y. Zheng, S. Wang and Y. Wang, Polymeric micelles drug delivery system in oncology, *J Controlled Release* **159**, 2012, 312–323.

[138]

T. Hamaguchi, K. Kato, H. Yasui, C. Morizane, M. Ikeda, H. Ueno, K. Muro, Y. Yamada, T. Okusaka and K. Shirao, A phase I and pharmacokinetic study of NK105, a paclitaxel-incorporating micellar nanoparticle formulation, *Br J Cancer* **97**, 2007, 170–176.

[139]

Y. Matsumura and K. Kataoka, Preclinical and clinical studies of anticancer agent-incorporating polymer micelles, *Cancer Sci* **100**, 2009, 572–579.

[140]

K. Kato, T. Hamaguchi, H. Yasui, T. Okusaka, H. Ueno, M. Ikeda, K. Shirao, Y. Shimada, H. Nakahama and K. Muro, Phase I study of NK105, a paclitaxel-incorporating micellar nanoparticle, in patients with advanced cancer, *J Clin Oncol* **24**, 2006, 2018.

[141]

A.M. Eckman, E. Tsakalozou, N.Y. Kang, A. Ponta and Y. Bae, Drug release patterns and cytotoxicity of PEG–poly(aspartate) block copolymer micelles in cancer cells, *Pharm Res* **29**, 2012, 1755–1767.

[142]

S.S. Desale, S.M. Cohen, Y. Zhao, A.V. Kabanov and T.K. Bronich, Biodegradable hybrid polymer micelles for combination drug therapy in ovarian cancer, *J Controlled Release* **171**, 2013, 339–348.

[143]

T. Thambi, H.Y. Yoon, K. Kim, I.C. Kwon, C.K. Yoo and J.H. Park, Bioreducible block copolymers based on poly(ethylene glycol) and poly(γ-Benzyl L-Gbenzyl L-glutamate) for intracellular delivery of camptothecin, *Bioconjugate Chem* **22**, 2011, 1924–1931.

[144]

T. Araki, Y. Kono, K.I. Ogawara, T. Watanabe, T. Ono, T. Kimura and K. Higaki, Formulation and evaluation of paclitaxel-loaded polymeric nanoparticles composed of polyethylene glycol and polylactic acid block copolymer, *Biol Pharm Bull* **35**, 2012, 1306–1313.

[145]

D. Kim, S. Kim, H. Kim, S. Kim, S. Shin, J. Kim, K. Park, M. Lee and D. Heo, Multicenter phase II trial of Genexol-PM, a novel Cremophor-free, polymeric micelle formulation of paclitaxel, with cisplatin in patients with advanced non-small-cell lung cancer, *Ann Oncol* **18**, 2007, 2009–2014.

[146]

K.S. Lee, H.C. Chung, S.A. Im, Y.H. Park, C.S. Kim, S.B. Kim, S.Y. Rha, M.Y. Lee and J. Ro, Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer, *Breast Cancer Res Treat* **108**, 2008, 241–250.

[147]

W. Lim, E. Tan, C. Toh, S. Hee, S. Leong, P. Ang, N. Wong and B. Chowbay, Phase I pharmacokinetic study of a weekly liposomal paclitaxel formulation (Genexol®-PM) in patients with solid tumors, *Ann Oncol* **21**, 2010, 382–388.

[148]

W.Y. Ayen, K. Garkhal and N. Kumar, Doxorubicin-loaded (PEG) 3-PLA nanopolymersomes: Eeffect of solvents and process parameters on formulation development and in vitro study, *Mol Pharm* **8**, 2011, 466–478.

[149]

J.P. Jain, W.Y. Ayen and N. Kumar, Self assembling polymers as polymersomes for drug delivery, *Curr Pharm Des* **17**, 2011, 65–79.

[150]

D.H. Levine, P.P. Ghoroghchian, J. Freudenberg, G. Zhang, M.J. Therien, M.I. Greene, D.A. Hammer and R. Murali, Polymersomes: a new multi-functional tool for cancer diagnosis and therapy, *Methods* **46**, 2008, 25–32.

[151]

E. Blanco, E.A. Bey, C. Khemtong, S.G. Yang, J. Setti-Guthi, H. Chen, C.W. Kessinger, K.A. Carnevale, W.G. Bornmann and D.A. Boothman, β-Lapachone micellar nanotherapeutics for non–small cell lung cancer therapy, *Cancer Res* **70**, 2010, 3896–3904.

[152]

C. Tan, Y. Wang and W. Fan, Exploring polymeric micelles for improved delivery of anticancer agents: Rrecent developments in preclinical studies, *Pharmaceutics* **5**, 2013, 201–219.

[153]

I.A. Siddiqui, V.M. Adhami, D.J. Bharali, B.B. Hafeez, M. Asim, S.I. Khwaja, N. Ahmad, H. Cui, S.A. Mousa and H. Mukhtar, Introducing nanochemoprevention as a novel approach for cancer control: proof of principle with green tea polyphenol epigallocatechin-3-gallate, *Cancer Res* **69**, 2009, 1712–1716.

[154]

D.J. Bharali and S.A. Mousa, Emerging nanomedicines for early cancer detection and improved treatment: current perspective and future promise, *Pharmacol Ther* **128**, 2010, 324–335.

[155]

C.F. Mu, P. Balakrishnan, F.D. Cui, Y.M. Yin, Y.B. Lee, H.G. Choi, C.S. Yong, S.J. Chung, C.K. Shim and D.D. Kim, The effects of mixed MPEG–PLA/Pluronic copolymer micelles on the bioavailability and multidrug resistance of docetaxel, *Biomaterials* **31**, 2010, 2371–2379.

[156]

H.C. Tsai, W.H. Chang, C.L. Lo, C.H. Tsai, C.H. Chang, T.W. Ou, T.C. Yen and G.H. Hsiue, Graft and diblock copolymer multifunctional micelles for cancer chemotherapy and imaging, *Biomaterials* **31**, 2010, 2293–2301.

[157]

Z.G. Gao, L. Tian, J. Hu, I.S. Park and Y.H. Bae, Prevention of metastasis in a 4T1 murine breast cancer model by doxorubicin carried by folate conjugated pH sensitive polymeric micelles, *J Controlled Release* **152**, 2011, 84–89.

[158]

D. Kim, Z.G. Gao, E.S. Lee and Y.H. Bae, In vivo evaluation of doxorubicin-loaded polymeric micelles targeting folate receptors and early endosomal pH in drug-resistant ovarian cancer, *Mol Pharm* **6**, 2009, 1353–1362.

[159]

C. Zhan, B. Gu, C. Xie, J. Li, Y. Liu, W. Lu and R.G.D. Cyclic, conjugated poly(ethylene glycol)-co-poly(ethylene glycol)-co-poly(lactic acid) micelle enhances paclitaxel anti-glioblastoma effect, *J Controlled Release* **143**, 2010, 136–142.

[160]

X. Wei, C. Gong, M. Gou, S. Fu, Q. Guo, S. Shi, F. Luo, G. Guo, L. Qiu and Z. Qian, Biodegradable poly(ε-caprolactone)-poly(ε-caprolactone)–poly(ethylene glycol) copolymers as drug delivery system, *Int J Pharm* **381**, 2009, 1–18.

[161]

Y. Wang, C. Wang, S. Fu, Q. Liu, D. Dou, H. Lv, M. Fan, G. Guo, F. Luo and Z. Qian, Preparation of tacrolimus loaded micelles based on poly(ε-caprolactone)-poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone), *Int J Pharm* **407**, 2011, 184–189.

[162]

L. Liu, L. Sun, Q. Wu, W. Guo, L. Li, Y. Chen, Y. Li, C. Gong, Z. Qian and Y. Wei, Curcumin loaded polymeric micelles inhibit breast tumor growth and spontaneous pulmonary metastasis, *Int J Pharm* **443**, 2013, 175–182.

[163]

X. Gao, B.L. Wang, X.W. Wei, M.L. Gou, K. Men, X.Y. Liu, G. Guo, Z.Y. Qian and M.J. Huang, Preparation and characterization of monomethoxy poly(ethylene glycol)–poly(ε-caprolactone) micelles for the solubilization and in vivo delivery of luteolin, *Int J Nanomed* **8**, 2013, 3061–3069.

[164]

H. Cho, T.C. Lai and G.S. Kwon, Poly(ethylene glycol)-block-poly(ε-caprolactone) micelles for combination drug delivery: Eevaluation of paclitaxel, cyclopamine and gossypol in intraperitoneal xenograft models of ovarian cancer, *J Controlled Release* **166**, 2013, 1–9.

[165]

C.L. Peng, P.S. Lai, F.H. Lin, S. Yueh Hsiu Wu and M.J. Shieh, Dual chemotherapy and photodynamic therapy in an HT-29 human colon cancer xenograft model using SN-38-loaded chlorin-core star block copolymer micelles, *Biomaterials* **30**, 2009, 3614–3625.

[166]

Q. Hu, X. Gao, T. Kang, X. Feng, D. Jiang, Y. Tu, Q. Song, L. Yao, X. Jiang and H. Chen, CGKRK-modified nanoparticles for dual-targeting drug delivery to tumor cells and angiogenic blood vessels, *Biomaterials* **34**, 2013, 9496–9508.

[167]

F. Danhier, E. Ansorena, J.M. Silva, R. Coco, A. Le Breton and V. Préat, PLGA-based nanoparticles: an overview of biomedical applications, *J Controlled Release* **161**, 2012, 505–522.

[168]

H. Sah, L.A. Thoma, H.R. Desu, E. Sah and G.C. Wood, Concepts and practices used to develop functional PLGA-based nanoparticulate systems, *Int J Nanomed* **8**, 2013, 747–765.

[169]

H. Ding, R. Mora, J. Gao and B.D. Sumer, Characterization and optimization of mTHPP nanoparticles for photodynamic therapy of head and neck cancer, *Otolaryngol Head Neck Surg* **145**, 2011, 612–617.

[170]

Z. Song, R. Feng, M. Sun, C. Guo, Y. Gao, L. Li and G. Zhai, Curcumin-loaded PLGA-PEG--PEG–PLGA triblock copolymeric micelles: Ppreparation, pharmacokinetics and distribution in vivo, *J Colloid Interface Sci* **354**, 2011, 116–123.

[171]

N. Graf, D.R. Bielenberg, N. Kolishetti, C. Muus, J. Banyard, O.C. Farokhzad and S.J. Lippard, αVβ3 Integrin-Targeted PLGA-PEG Nanoparticles for Enhanced Anti-tumor Efficacy of a Pt(IV) Pintegrin-targeted PLGA–PEG nanoparticles for enhanced anti-tumor efficacy of a Pt(IV) prodrug, *ACS Nano* **6**, 2012, 4530–4539.

[172]

J. Chen, S. Li and Q. Shen, Folic acid and cell-penetrating peptide conjugated PLGA--PEG bifunctional nanoparticles for vincristine sulfate delivery, *Eur J Pharm Sci* **47**, 2012, 430–443.

[173]

J. Chen, S. Li, Q. Shen, H. He and Y. Zhang, Enhanced cellular uptake of folic acid-conjugated PLGA--PEG nanoparticles loaded with vincristine sulfate in human breast cancer, *Drug Dev Ind Pharm* **37**, 2011, 1339–1346.

[174]

P. Liu, H. Wang, Q. Wang, Y. Sun, M. Shen, M. Zhu, Z. Wan and Y. Duan, cRGD conjugated mPEG-PLGA--PLGA–PLL nanoparticles for SGC-7901 gastric cancer cells-targeted Ddelivery of fluorouracil, *J Nanosci Nanotechnol* **12**, 2012, 4467–4471.

[175]

V. Saxena, Y. Naguib and M.D. Hussain, Folate receptor targeted 17-allylamino-17-demethoxygeldanamycin (17-AAG) loaded polymeric nanoparticles for breast cancer, *Colloids Surf B, B: Biointerfaces* **94**, 2012, 274–280.

[176]

Y. Wang, P. Liu, Y. Duan, X. Yin, Q. Wang, X. Liu, X. Wang, J. Zhou, W. Wang, L. Qiu and W. Di, Specific cell targeting with APRPG conjugated PEG--PLGA nanoparticles for treating ovarian cancer, *Biomaterials* **35**, 2014, 983–992.

[177]

Y. Lei, Y. Lai, Y. Li, S. Li, G. Cheng, D. Li, H. Li, B. He and Z. Gu, Anticancer drug delivery of PEG based micelles with small lipophilic moieties, *Int J Pharm* **453**, 2013, 579–586.

[178]

Y. Liang, Y. Lai, D. Li, B. He and Z. Gu, Novel polymeric micelles with cinnamic acid as lipophilic moiety for 9-Nitro-20 (S)-camptothecin delivery, *Mater Lett* **97**, 2013, 4–7.

[179]

Y. Lai, Y. Long, Y. Lei, X. Deng, B. He, M. Sheng, M. Li and Z. Gu, A novel micelle of coumarin derivative monoend-functionalized PEG for anti-tumor drug delivery: in vitro and in vivo study, *J Drug Target* **20**, 2012, 246–254.

[180]

H.S. Oberoi, F.C. Laquer, L.A. Marky, A.V. Kabanov and T.K. Bronich, Core cross-linked block ionomer micelles as pH-responsive carriers for cis-diamminedichloroplatinum-(II), *J Controlled Release* **153**, 2011, 64–72.

[181]

J.O. Kim, G. Sahay, A.V. Kabanov and T.K. Bronich, Polymeric micelles with ionic cores containing biodegradable cross-links for delivery of chemotherapeutic agents, *Biomacromolecules* **11**, 2010, 919–926.

[182]

N.V. Nukolova, H.S. Oberoi, S.M. Cohen, A.V. Kabanov and T.K. Bronich, Folate-decorated nanogels for targeted therapy of ovarian cancer, *Biomaterials* **32**, 2011, 5417–5426.

[183]

H.S. Oberoi, N.V. Nukolova, F.C. Laquer, L.Y. Poluektova, J. Huang, Y. Alnouti, M. Yokohira, L.L. Arnold, A.V. Kabanov and S.M. Cohen, Cisplatin-loaded core cross-linked micelles: comparative pharmacokinetics, antitumor activity, and toxicity in mice, *Int J Nanomed* **7**, 2012, 2557.

[184]

M. Talelli, M. Iman, A.K. Varkouhi, C.J. Rijcken, R.M. Schiffelers, T. Etrych, K. Ulbrich, C.F. van Nostrum, T. Lammers and G. Storm, Core-crosslinked polymeric micelles with controlled release of covalently entrapped doxorubicin, *Biomaterials* **31**, 2010, 7797–7804.

[185]

K.K. Gill, A. Kaddoumi and S. Nazzal, Mixed micelles of PEG2000-DSPE and vitamin-E TPGS for concurrent delivery of paclitaxel and parthenolide: Eenhanced chemosenstization and antitumor efficacy against non-small cell lung cancer (NSCLC) cell lines, *Eur J Pharm Sci* **46**, 2012, 64–71.

[186]

S.W. Tong, B. Xiang, D.W. Dong and X.R. Qi, Enhanced antitumor efficacy and decreased toxicity by self-associated docetaxel in phospholipid-based micelles, *Int J Pharm* **434**, 2012, 413–419.

[187]

S. Zhu, P.D.S. Lansakara, X. Li and Z. Cui, Lysosomal delivery of a lipophilic gemcitabine prodrug using novel acid-sensitive micelles improved its antitumor activity, *Bioconjugate Chem* **23**, 2012, 966–980.

[188]

L. Bildstein, C. Dubernet and P. Couvreur, Prodrug-based intracellular delivery of anticancer agents, *Adv Drug Deliv Rev* **63**, 2011, 3–23.

[189]

J. Kopeček, Polymer–drug conjugates: Oorigins, progress to date and future directions, *Adv Drug Deliv Rev* **65**, 2012, 49–59.

[190]

F. Canal, J. Sanchis and M.J. Vicent, Polymer–drug conjugates as nano-sized medicines, *Curr Opin Biotechnol* **22**, 2011, 894–900.

[191]

C. Li and S. Wallace, Polymer-–drug conjugates: recent development in clinical oncology, *Adv Drug Deliv Rev* **60**, 2008, 886–898.

[192]

A. Ponta and Y. Bae, PEG-–poly(amino acid) block copolymer micelles for tunable drug release, *Pharm Res* **27**, 2010, 2330–2342.

[193]

S. Svenson, Clinical translation of nanomedicines, *Curr Opin Solid State Mater Sci* **16**, 2012, 287–294.

[194]

H. Uchino, Y. Matsumura, T. Negishi, F. Koizumi, T. Hayashi, T. Honda, N. Nishiyama, K. Kataoka, S. Naito and T. Kakizoe, Cisplatin-incorporating polymeric micelles (NC-6004) can reduce nephrotoxicity and neurotoxicity of cisplatin in rats, *Br J Cancer* **93**, 2005, 678–687.

[195]

R. Plummer, R. Wilson, H. Calvert, A. Boddy, M. Griffin, J. Sludden, M. Tilby, M. Eatock, D. Pearson and C. Ottley, A Phase I clinical study of cisplatin-incorporated polymeric micelles (NC-6004) in patients with solid tumours, *Br J Cancer* **104**, 2011, 593–598.

[196]

H. Cabral, N. Nishiyama, S. Okazaki, H. Koyama and K. Kataoka, Preparation and biological properties of dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt)-loaded polymeric micelles, *J Controlled Release* **101**, 2005, 223–232.

[197]

H. Cabral, N. Nishiyama and K. Kataoka, Optimization of (1,2-diamino-cyclohexane)platinum(II)-loaded polymeric micelles directed to improved tumor targeting and enhanced antitumor activity, *J Controlled Release* **121**, 2007, 146–155.

[198]

G. Pasut and F.M. Veronese, PEG conjugates in clinical development or use as anticancer agents: ~~A~~an overview, *Adv Drug Deliv Rev* **61**, 2009, 1177–1188.

[199]

V. Bala, S. Rao, B.J. Boyd and C.A. Prestidge, Prodrug and nanomedicine approaches for the delivery of the camptothecin analogue SN38, *J Controlled Release* **172**, 2013, 48–61.

[200]

F. Koizumi, M. Kitagawa, T. Negishi, T. Onda, Matsumoto Si, T. Hamaguchi and Y. Matsumura, Novel SN-38~~-Incorporating Polymeric Micelles, NK012, Eradicate Vascular Endothelial Growth Factor–Secreting Bulky T~~-incorporating polymeric micelles, NK012, eradicate vascular endothelial growth factor-secreting bulky tumors, *Cancer Res* **66**, 2006, 10048–10056.

[201]

T. Nagano, M. Yasunaga, K. Goto, H. Kenmotsu, Y. Koga, Ji Kuroda, Y. Nishimura, T. Sugino, Y. Nishiwaki and Y. Matsumura, Synergistic antitumor activity of the SN-38-incorporating polymeric micelles NK012 with S-1 in a mouse model of non-small cell lung cancer, *Int J Cancer* **127**, 2010, 2699–2706.

[202]

T. Hamaguchi, T. Doi, T. Eguchi-Nakajima, K. Kato, Y. Yamada, Y. Shimada, N. Fuse, A. Ohtsu, Si Matsumoto and M. Takanashi, Phase I ~~S~~study of NK012, a Novel SN-38~~-Incorporating Micellar Nanoparticle, in Adult Patients with Solid T~~-incorporating micellar nanoparticle, in adult patients with solid tumors, *Clin Cancer Res* **16**, 2010, 5058–5066.

[203]

Y. Matsumura, Preclinical and clinical studies of NK012, an SN-38-incorporating polymeric micelles, which is designed based on EPR effect, *Adv Drug Deliv Rev* **63**, 2011, 184–192.

[204]

T. Nakanishi, S. Fukushima, K. Okamoto, M. Suzuki, Y. Matsumura, M. Yokoyama, T. Okano, Y. Sakurai and K. Kataoka, Development of the polymer micelle carrier system for doxorubicin, *J Controlled Release* **74**, 2001, 295–302.

[205]

Y. Tsukioka, Y. Matsumura, T. Hamaguchi, H. Koike, F. Moriyasu and T. Kakizoe, Pharmaceutical and biomedical differences between micellar doxorubicin (NK911) and liposomal doxorubicin (Doxil), *Cancer Sci* **93**, 2002, 1145–1153.

[206]

Y. Matsumura, T. Hamaguchi, T. Ura, K. Muro, Y. Yamada, Y. Shimada, K. Shirao, T. Okusaka, H. Ueno and M. Ikeda, Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin, *Br J Cancer* **91**, 2004, 1775–1781.

[207]

H. Xiao, H. Song, Q. Yang, H. Cai, R. Qi, L. Yan, S. Liu, Y. Zheng, Y. Huang, T. Liu and X. Jing, A prodrug strategy to deliver cisplatin(IV) and paclitaxel in nanomicelles to improve efficacy and tolerance, *Biomaterials* **33**, 2012, 6507–6519.

[208]

W. Zhang, Y. Shi, Y. Chen, J. Ye, X. Sha and X. Fang, Multifunctional Pluronic P123/F127 mixed polymeric micelles loaded with paclitaxel for the treatment of multidrug resistant tumors, *Biomaterials* **32**, 2011, 2894–2906.

[209]

Y. Chen, X. Sha, W. Zhang, W. Zhong, Z. Fan, Q. Ren, L. Chen and X. Fang, Pluronic mixed micelles overcoming methotrexate multidrug resistance: in vitro and in vivo evaluation, *Int J Nanomed* **8**, 2013, 1463–1476.

[210]

Y. Wang, Y. Li, L. Zhang and X. Fang, Pharmacokinetics and biodistribution of paclitaxel-loaded pluronic P105 polymeric micelles, *Arch Pharm Res* **31**, 2008, 530–538.

[211]

S. Danson, D. Ferry, V. Alakhov, J. Margison, D. Kerr, D. Jowle, M. Brampton, G. Halbert and M. Ranson, Phase I dose escalation and pharmacokinetic study of pluronic polymer-bound doxorubicin (SP1049C) in patients with advanced cancer, *Br J Cancer* **90**, 2004, 2085–2091.

[212]

J.W. Valle, A. Armstrong, C. Newman, V. Alakhov, G. Pietrzynski, J. Brewer, S. Campbell, P. Corrie, E.K. Rowinsky and M. Ranson, A phase 2 study of SP1049C, doxorubicin in ~~F~~*P*-glycoprotein-targeting pluronics, in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction, *Invest New Drugs* **29**, 2011, 1029–1037.

[213]

D.Y. Alakhova, Y. Zhao, S. Li and A.V. Kabanov, Effect of Doxorubicin/Pluronic SP1049C on ~~Tumorigenicity, Aggressiveness, DNA Methylation and Stem Cell Markers in Murine LeukemiaPloS one~~tumorigenicity, aggressiveness, DNA methylation and stem cell markers in murine leukemia, *PLoS ONE* **8**, 2013, 1–14.

[214]

H. Song, R. He, K. Wang, J. Ruan, C. Bao, N. Li, J. Ji and D. Cui, Anti-HIF-1α antibody-conjugated pluronic triblock copolymers encapsulated with Paclitaxel for tumor targeting therapy, *Biomaterials* **31**, 2010, 2302–2312.

[215]

M. Talelli, C. Rijcken, C. Van Nostrum, G. Storm and W. Hennink, Micelles based on HPMA copolymers, *Adv Drug Deliv Rev* **62**, 2010, 231–239.

[216]

E. Segal and R. Satchi-Fainaro, Design and development of polymer conjugates as anti-angiogenic agents, *Adv Drug Deliv Rev* **61**, 2009, 1159–1176.

[217]

J. Kopecek and P. Kopecková, HPMA copolymers: ~~O~~origins, early developments, present, and future, *Adv Drug Deliv Rev* **62**, 2010, 122–149.

[218]

Y. Yang, D. Pan, K. Luo, L. Li and Z. Gu, Biodegradable and amphiphilic block copolymer–doxorubicin conjugate as polymeric nanoscale drug delivery vehicle for breast cancer therapy, *Biomaterials* **34**, 2013, 8430–8443.

[219]

P. Chytil, T. Etrych, C. Konák, M. Sírová, T. Mrkvan, J. Boucek, B. Říhová and K. Ulbrich, New HPMA copolymer-based drug carriers with covalently bound hydrophobic substituents for solid tumour targeting, *J Controlled Release* **127**, 2008, 121–130.

[220]

K. Miller, R. Erez, E. Segal, D. Shabat and R. Satchi-Fainaro, Targeting bone metastases with a bispecific anticancer and antiangiogenic polymer–alendronate–taxane conjugate, *Angew Chem Int Ed* **48**, 2009, 2949–2954.

[221]

K. Miller, A. Eldar-Boock, D. Polyak, E. Segal, L. Benayoun, Y. Shaked and R. Satchi-Fainaro, Antiangiogenic antitumor activity of HPMA copolymer–paclitaxel–alendronate conjugate on breast cancer bone metastasis mouse model, *Mol Pharm* **8**, 2011, 1052–1062.

[222]

E. Segal, H. Pan, L. Benayoun, P. Kopečková, Y. Shaked, J. Kopeček and R. Satchi-Fainaro, Enhanced anti-tumor activity and safety profile of targeted nano-scaled HPMA copolymer-alendronate-–alendronate–TNP-470 conjugate in the treatment of bone malignances, *Biomaterials* **32**, 2011, 4450–4463.

[223]

Z. Jia, L. Wong, T.P. Davis and V. Bulmus, One-pot conversion of RAFT-generated multifunctional block copolymers of HPMA to doxorubicin conjugated acid-and reductant-sensitive crosslinked micelles, *Biomacromolecules* **9**, 2008, 3106–3113.

[224]

M. Regehly, K. Greish, F. Rancan, H. Maeda, F. Böhm and B. Röder, Water-soluble polymer conjugates of ZnPP for photodynamic tumor therapy, *Bioconjugate Chem* **18**, 2007, 494–499.

[225]

A.K. Iyer, K. Greish, T. Seki, S. Okazaki, J. Fang, K. Takeshita and H. Maeda, Polymeric micelles of zinc protoporphyrin for tumor targeted delivery based on EPR effect and singlet oxygen generation, *J Drug Target* **15**, 2007, 496–506.

[226]

H. Nakamura, L. Liao, Y. Hitaka, K. Tsukigawa, V. Subr, J. Fang, K. Ulbrich and H. Maeda, Micelles of zinc protoporphyrin conjugated to *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer for imaging and light-induced antitumor effects in vivo, *J Controlled Release* **165**, 2013, 191–198.

[227]

E. Jabbari, X. Yang, S. Moeinzadeh and X. He, Drug release kinetics, cell uptake, and tumor toxicity of hybrid VVVVVVKK peptide-assembled polylactide nanoparticles, *Eur J Pharm Biopharm* **84**, 2013, 49–62.

[228]

J. Xu, J.H. Zhao, Y. Liu, N.P. Feng and Y.T. Zhang, RGD-modifed poly(D,L-lactic acid) nanoparticles enhance tumor targeting of oridonin, *Int J Nanomed* **7**, 2012, 211–219.

[229]

R. Ortiz, J. Prados, C. Melguizo, J.L. Arias, M.A. Ruiz, P.J. Álvarez, O. Caba, R. Luque, A. Segura and A. Aránega, 5-Fluorouracil-loaded poly(ε-caprolactone) nanoparticles combined with phage E gene therapy as a new strategy against colon cancer, *Int J Nanomed* **7**, 2012, 95–107.

[230]

C. Huang, K.G. Neoh, L. Xu, E.T. Kang and E. Chiong, Polymeric nanoparticles with encapsulated superparamagnetic iron oxide and conjugated cisplatin for potential bladder cancer therapy, *Biomacromolecules* **13**, 2012, 2513–2520.

[231]

Y.F. Tan, P. Chandrasekharan, D. Maity, C.X. Yong, K.H. Chuang, Y. Zhao, S. Wang, J. Ding and S.S. Feng, Multimodal tumor imaging by iron oxides and quantum dots formulated in poly-(lactic acid)-d(lactic acid)–D-alpha-tocopheryl polyethylene glycol 1000 succinate nanoparticles, *Biomaterials* **32**, 2011, 2969–2978.

[232]

Y. Mi, X. Liu, J. Zhao, J. Ding and S.S. Feng, Multimodality treatment of cancer with herceptin conjugated, thermomagnetic iron oxides and docetaxel loaded nanoparticles of biodegradable polymers, *Biomaterials* **33**, 2012, 7519–7529.

[233]

J.R. McDaniel, J. Bhattacharyya, K.B. Vargo, W. Hassouneh, D.A. Hammer and A. Chilkoti, Self-Assembly of Thermally Responsive Nanoparticles of a Genetically Encoded Peptide Polymer by Drug G assembly of thermally responsive nanoparticles of a genetically encoded peptide polymer by drug conjugation, *Angew Chem Int Ed* **52**, 2013, 1683–1687.

[234]

M.C. Branco, D.M. Sigano and J.P. Schneider, Materials from peptide assembly: towards the treatment of cancer and transmittable disease, *Curr Opin Chem Biol* **15**, 2011, 427–434.

[235]

H. Cui, M.J. Webber and S.I. Stupp, Self-assembly of peptide amphiphiles: ~~F~~from molecules to nanostructures to biomaterials, *Pept Sci* **94**, 2010, 1–18.

[236]

R.J. Mart, R.D. Osborne, M.M. Stevens and R.V. Ulijn, Peptide-based stimuli-responsive biomaterials, *Soft Matter* **2**, 2006, 822–835.

[237]

E. Kokkoli, A. Mardilovich, A. Wedekind, E.L. Rexeisen, A. Garg and J.A. Craig, Self-assembly and applications of biomimetic and bioactive peptide~~-~~ amphiphiles, *Soft Matter* **2**, 2006, 1015–1024.

[238]

D.J. Toft, T.J. Moyer, S.M. Standley, Y. Ruff, A. Ugolkov, S.I. Stupp and V.L. Cryns, Coassembled cytotoxic and pegylated peptide amphiphiles form filamentous nanostructures with potent antitumor activity in models of breast cancer, *ACS Nano* **6**, 2012, 7956–7965.

[239]

S. Aluri, S.M. Janib and J.A. Mackay, Environmentally responsive peptides as anticancer drug carriers, *Adv Drug Deliv Rev* **61**, 2009, 940–952.

[240]

S.M. Standley, D.J. Toft, H. Cheng, S. Soukasene, J. Chen, S.M. Raja, V. Band, H. Band, V.L. Cryns and S.I. Stupp, Induction of cancer cell death by self-assembling nanostructures incorporating a cytotoxic peptide, *Cancer Res* **70**, 2010, 3020–3026.

[241]

M.J. Webber, C.J. Newcomb, R. Bitton and S.I. Stupp, Switching of self-assembly in a peptide nanostructure with a specific enzyme, *Soft Matter* **7**, 2011, 9665–9672.

[242]

S. Soukasene, D.J. Toft, T.J. Moyer, H. Lu, H.K. Lee, S.M. Standley, V.L. Cryns and S.I. Stupp, Antitumor activity of peptide amphiphile nanofiber-encapsulated camptothecin, *ACS Nano* **5**, 2011, 9113–9121.

[243]

J.A. MacKay, M. Chen, J.R. McDaniel, W. Liu, A.J. Simnick and A. Chilkoti, Self-assembling chimeric polypeptide–doxorubicin conjugate nanoparticles that abolish tumours after a single injection, *Nat Mater* **8**, 2009, 993–999.

[244]

N. Wiradharma, Y.W. Tong and Y.Y. Yang, Self-assembled oligopeptide nanostructures for co-delivery of drug and gene with synergistic therapeutic effect, *Biomaterials* **30**, 2009, 3100–3109.

[245]

J.B. Matson and S.I. Stupp, Drug release from hydrazone-containing peptide amphiphiles, *Chem Commun* **47**, 2011, 7962–7964.

[246]

M.J. Webber, J.B. Matson, V.K. Tamboli and S.I. Stupp, Controlled release of dexamethasone from peptide nanofiber gels to modulate inflammatory response, *Biomaterials* **33**, 2012, 6823–6832.

[247]

R. Hoogenboom, Poly (2-oxazoline)~~s: A Polymer Class with Numerous Potential A~~s: a polymer class with numerous potential applications, *Angew Chem Int Ed* **48**, 2009, 7978–7994.

[248]

H. Schlaad, C. Diehl, A. Gress, M. Meyer, A.L. Demirel, Y. Nur and A. Bertin, Poly~~-(2-oxazoline)-s as Smart Bioinspired P~~(2-oxazoline)s as smart bioinspired polymers, *Macromol Rapid Commun* **31**, 2010, 511–525.

[249]

R. Hoogenboom and H. Schlaad, Bioinspired ~~Poly-~~poly(2-oxazoline)s, *Polymers* **3**, 2011, 467–488.

[250]

S.C. Lee, C. Kim, I.C. Kwon, H. Chung and S.Y. Jeong, Polymeric micelles of poly (2-ethyl-2-oxazoline)-block-poly (ε-caprolactone) copolymer as a carrier for paclitaxel, *J Controlled Release* **89**, 2003, 437–446.

[251]

G.H. Hsiue, C.H. Wang, C.L. Lo, C.H. Wang, J.P. Li and J.L. Yang, Environmental-sensitive micelles based on poly (2-ethyl-2-oxazoline)-*b poly (l-b*-poly (L-lactide) diblock copolymer for application in drug delivery, *Int J Pharm* **317**, 2006, 69–75.

[252]

R. Luxenhofer, A. Schulz, C. Roques, S. Li, T.K. Bronich, E.V. Batrakova, R. Jordan and A.V. Kabanov, Doubly amphiphilic poly(2-oxazoline)s as high-capacity delivery systems for hydrophobic drugs, *Biomaterials* **31**, 2010, 4972–4979.

[253]

J. Tong, R. Luxenhofer, X. Yi, R. Jordan and A.V. Kabanov, Protein modification with amphiphilic block copoly (2-oxazoline) s as a new platform for enhanced cellular delivery, *Mol Pharm* **7**, 2010, 984–992.

[254]

M.J. Shieh, C.L. Peng, W.L. Chiang, C.H. Wang, C.Y. Hsu, S.J.J. Wang and P.S. Lai, Reduced skin photosensitivity with meta-tetra (hydroxyphenyl) chlorin-loaded micelles based on a poly-(2-ethyl-2-oxazoline)-*b poly (d, l-b*-poly(D,L-lactide) diblock copolymer in vivo, *Mol Pharm* **7**, 2010, 1244–1253.

[255]

W.J. Syu, H.P. Yu, C.Y. Hsu, Y.C. Rajan, Y.H. Hsu, Y.C. Chang, W.Y. Hsieh, C.H. Wang and P.S. Lai, Improved photodynamic cancer treatment by folate-conjugated polymeric micelles in a KB xenografted animal model, *Small* **8**, 2012, 2060–2069.

[256]

S.J. Huang, S.L. Sun, C.C. Chiu and L.F. Wang, Retinol-encapsulated water-soluble succinated chitosan nanoparticles for antioxidant applications, *J Biomater Sci Polym Ed* **24**, 2013, 315–329.

[257]

N. Goodarzi, R. Varshochian, G. Kamalinia, F. Atyabi and R. Dinarvand, A review of polysaccharide cytotoxic drug conjugates for cancer therapy, *Carbohydr Polym* **92**, 2013, 1280–1293.

[258]

Y. Luo, N.J. Bernshaw, Z.R. Lu, J. Kopecek and G.D. Prestwich, Targeted delivery of doxorubicin by HPMA copolymer-–hyaluronan bioconjugates, *Pharm Res* **19**, 2002, 396–402.

[259]

S. Patel and M.J. Page, The *Role of Hyaluronan in G*role of hyaluronan in cancer, In: G.G. Hari and C.A. Hales, (Eds.), *Chemistry and Biology of H*biology of h*yaluronan,* 2004, Elsevier Science Ltd; Oxford, 285–305.

[260]

M. Götte and G.W. Yip, Heparanase, hyaluronan, and CD44 in cancers: a breast carcinoma perspective, *Cancer Res* **66**, 2006, 10233–10237.

[261]

A. Bartolazzi, R. Peach, A. Aruffo and I. Stamenkovic, Interaction between CD44 and hyaluronate is directly implicated in the regulation of tumor development, *J Exp Med* **180**, 1994, 53–66.

[262]

K.Y. Choi, H. Chung, K.H. Min, H.Y. Yoon, K. Kim, J.H. Park, I.C. Kwon and S.Y. Jeong, Self-assembled hyaluronic acid nanoparticles for active tumor targeting, *Biomaterials* **31**, 2010, 106–114.

[263]

A. Szarpak, D. Cui, Fdr Dubreuil, B.G. De Geest, L.J. De Cock, C. Picart and R. Auzély-Velty, Designing hyaluronic acid-based layer-by-layer capsules as a carrier for intracellular drug delivery, *Biomacromolecules* **11**, 2010, 713–720.

[264]

G.M. Campo, A. Avenoso, S. Campo, A. D'Ascola, G. Nastasi and A. Calatroni, Molecular size hyaluronan differently modulates toll-like receptor-4 in LPS-induced inflammation in mouse chondrocytes, *Biochimie* **92**, 2010, 204–215.

[265]

G. Cheng, S. Swaidani, M. Sharma, M.E. Lauer, V.C. Hascall and M.A. Aronica, Hyaluronan deposition and correlation with inflammation in a murine ovalbumin model of asthma, *Matrix Biol* **30**, 2011, 126–134.

[266]

G.M. Campo, A. Avenoso, A. D'Ascola, M. Scuruchi, V. Prestipino, A. Calatroni and S. Campo, Hyaluronan in part mediates IL-1beta-induced inflammation in mouse chondrocytes by up-regulating CD44 receptors, *Gene* **494**, 2012, 24–35.

[267]

X. Jiang and D.J. Shapiro, The immune system and inflammation in breast cancer, *Mol Cell Endocrinol* **382**, 2014, 673–682.

[268]

E.M. August, T. Nguyen, N.M. Malinowski and R.L. Cysyk, Non-steroidal anti-inflammatory drugs and tumor progression: inhibition of fibroblast hyaluronic acid production by indomethacin and mefenamic acid, *Cancer Lett* **82**, 1994, 49–54.

[269]

D.A. Ossipov, Nanostructured hyaluronic acid-based materials for active delivery to cancer, *Expert Opin Drug Deliv* **7**, 2010, 681–703.

[270]

Y.I. Jeong, D.H. Kim, C.W. Chung, J.J. Yoo, K.H. Choi, C.H. Kim, S.H. Ha and D.H. Kang, Self-assembled nanoparticles of hyaluronic acid/poly(DL-LACTIDE-CODL-lactide-*co*-glycolide) block copolymer, *Colloids Surf B, B: Biointerfaces* **90**, 2012, 28–35.

[271]

L. Ray, P. Kumar and K.C. Gupta, The activity against Ehrlich's ascites tumors of doxorubicin contained in self assembled, cell receptor targeted nanoparticle with simultaneous oral delivery of the green tea polyphenol epigallocatechin-3-gallate, *Biomaterials* **34**, 2013, 3064–3076.

[272]

K.Y. Choi, K.H. Min, J.H. Na, K. Choi, K. Kim, J.H. Park, I.C. Kwon and S.Y. Jeong, Self-assembled hyaluronic acid nanoparticles as a potential drug carrier for cancer therapy: Ssynthesis, characterization, and in vivo biodistribution, *J Mater Chem* **19**, 2009, 4102–4107.

[273]

J. Huang, H. Zhang, Y. Yu, Y. Chen, D. Wang, G. Zhang, G. Zhou, J. Liu, Z. Sun, D. Sun, Y. Lu and Y. Zhong, Biodegradable self-assembled nanoparticles of poly(d,l-lactide-co(D,L-lactide-*co*-glycolide)/hyaluronic acid block copolymers for target delivery of docetaxel to breast cancer, *Biomaterials* **35**, 2014, 550–566.

[274]

B. Zhou, J.A. Weigel, L. Fauss and P.H. Weigel, Identification of the hyaluronan receptor for endocytosis (HARE), *J Biol Chem* **275**, 2000, 37733–37741.

[275]

H. Kim, H.T. Park, Y.M. Tae, W.H. Kong, D.K. Sung, B.W. Hwang, K.S. Kim, Y.K. Kim and S.K. Hahn, Bioimaging and pulmonary applications of self-assembled Flt1 peptide–hyaluronic acid conjugate nanoparticles, *Biomaterials* **34**, 2013, 8478–8490.

[276]

Y. Luo and G.D. Prestwich, Synthesis and selective cytotoxicity of a hyaluronic acid–antitumor bioconjugate, *Bioconjugate Chem* **10**, 1999, 755–763.

[277]

Y. Luo, M.R. Ziebell and G.D. Prestwich, A hyaluronic acid–taxol antitumor bioconjugate targeted to cancer cells, *Biomacromolecules* **1**, 2000, 208–218.

[278]

A. Rosato, A. Banzato, G. De Luca, D. Renier, F. Bettella, C. Pagano, G. Esposito, P. Zanovello and P. Bassi, HYTAD1-p20: A new paclitaxel-a new paclitaxel–hyaluronic acid hydrosoluble bioconjugate for treatment of superficial bladder cancer, *Urol Oncol* **24**, 2006, 207–215.

[279]

E. Auzenne, Hyaluronic acid-paclitaxel: antitumor efficacy against CD44 (+) human ovarian carcinoma xenografts, *Neoplasia* **9**, 2007, 479–486.

[280]

H.Y. Yoon, H. Koo, K.Y. Choi, S.J. Lee, K. Kim, I.C. Kwon, J.F. Leary, K. Park, S.H. Yuk, J.H. Park and K. Choi, Tumor-targeting hyaluronic acid nanoparticles for photodynamic imaging and therapy, *Biomaterials* **33**, 2012, 3980–3989.

[281]

T. Ito, I.P. Fraser, Y. Yeo, C.B. Highley, E. Bellas and D.S. Kohane, Anti-inflammatory function of an in situ cross-linkable conjugate hydrogel of hyaluronic acid and dexamethasone, *Biomaterials* **28**, 2007, 1778–1786.

[282]

S.M. Smorenburg and C.J. Van Noorden, The complex effects of heparins on cancer progression and metastasis in experimental studies, *Pharmacol Rev* **53**, 2001, 93–106.

[283]

B. Casu, I. Vlodavsky and R.D. Sanderson, Non-anticoagulant heparins and inhibition of cancer, *Pathophysiol Haemost Thromb* **36**, 2009, 195–203.

[284]

H.D. Han, A. Lee, C.K. Song, T. Hwang, H. Seong, C.O. Lee and B.C. Shin, In vivo distribution and antitumor activity of heparin-stabilized doxorubicin-loaded liposomes, *Int J Pharm* **313**, 2006, 181–188.

[285]

L. Hou, Y. Fan, J. Yao, J. Zhou, C. Li, Z. Fang and Q. Zhang, Low molecular weight heparin-all-~~*trans*~~*trans*-retinoid acid conjugate as a drug carrier for combination cancer chemotherapy of paclitaxel and all-~~*trans*~~*trans*-retinoid acid, *Carbohydr Polym* **86**, 2011, 1157–1166.

[286]

M. Larsson, W.C. Huang, M.H. Hsiao, Y.J. Wang, M. Nydén, S.H. Chiou and D.M. Liu, Biomedical applications and colloidal properties of amphiphilically modified chitosan hybrids, *Prog Polym Sci* **38**, 2013, 1307–1328.

[287]

K.V. Harish Prashanth and R.N. Tharanathan, Depolymerized products of chitosan as potent inhibitors of tumor-induced angiogenesis, *Biochim Biophys Acta Gen Subj* **1722**, 2005, 22–29.

[288]

J.H. Park, G. Saravanakumar, K. Kim and I.C. Kwon, Targeted delivery of low molecular drugs using chitosan and its derivatives, *Adv Drug Deliv Rev* **62**, 2010, 28–41.

[289]

C. Zhang, Q. Ping, H. Zhang and J. Shen, Preparation of ~~*N-alkyl-C*~~*N*-alkyl-*C*-sulfate chitosan derivatives and micellar solubilization of taxol, *Carbohydr Polym* **54**, 2003, 137–141.

[290]

C. Zhang, P. Qineng and H. Zhang, Self-assembly and characterization of paclitaxel-loaded ~~*N-octyl-O*~~*N*-octyl-*C*-sulfate chitosan micellar system, *Colloids Surf ~~B~~, B: Biointerfaces* **39**, 2004, 69–75.

[291]

C. Zhang, G. Qu, Y. Sun, T. Yang, Z. Yao, W. Shen, Z. Shen, Q. Ding, H. Zhou and Q. Ping, Biological evaluation of ~~*N-octyl-C*~~*N*-octyl-*C*-sulfate chitosan as a new nano-carrier of intravenous drugs, *Eur J Pharm Sci* **33**, 2008, 415–423.

[292]

G. Qu, Z. Yao, C. Zhang, X. Wu and Q. Ping, PEG conjugated ~~*N-octyl-C*~~*N*-octyl-*C*-sulfate chitosan micelles for delivery of paclitaxel: ~~i~~in vitro characterization and in vivo evaluation, *Eur J Pharm Sci* **37**, 2009, 98–105.

[293]

C. Dufes, J.M. Muller, W. Couet, J.C. Olivier, I.F. Uchegbu and A.G. Schätzlein, Anticancer ~~Drug Delivery with Transferrin Targeted Polymeric Chitosan V~~drug delivery with transferrin targeted polymeric chitosan vesicles, *Pharm Res* **21**, 2004, 101–107.

[294]

J. You, X. Li, F. De Cui, Y.Z. Du, H. Yuan and F.Q. Hu, Folate-conjugated polymer micelles for active targeting to cancer cells: Ppreparation, in vitro evaluation of targeting ability and cytotoxicity, *Nanotechnology* **19**, 2008, 1–9.

[295]

K. Wang, T. Zhang, L. Liu, X. Wang, P. Wu, Z. Chen, C. Ni, J. Zhang, F. Hu and J. Huang, Novel micelle formulation of curcumin for enhancing antitumor activity and inhibiting colorectal cancer stem cells, *Int J Nanomed* **7**, 2012, 4487–4497.

[296]

G.M. Mekhail, A.O. Kamel, G.A.S. Awad and N.D. Mortada, Anticancer effect of atorvastatin nanostructured polymeric micelles based on stearyl-grafted chitosan, *Int J Biol Macromol* **51**, 2012, 351–363.

[297]

Z. Zhao, M. He, L. Yin, J. Bao, L. Shi, B. Wang, C. Tang and C. Yin, Biodegradable nanoparticles based on linoleic acid and poly(β′-malic acid) double grafted chitosan derivatives as carriers of anticancer drugs, *Biomacromolecules* **10**, 2009, 565–572.

[298]

J.H. Kim, Y.S. Kim, S. Kim, J.H. Park, K. Kim, K. Choi, H. Chung, S.Y. Jeong, R.W. Park, I.S. Kim and I.C. Kwon, Hydrophobically modified glycol chitosan nanoparticles as carriers for paclitaxel, *J Controlled Release* **111**, 2006, 228–234.

[299]

Y.H. Jin, H.Y. Hu, M.X. Qiao, J. Zhu, J.W. Qi, C.J. Hu, Q. Zhang and D.W. Chen, PH-sensitive chitosan-derived nanoparticles as doxorubicin carriers for effective anti-tumor activity: Ppreparation and in vitro evaluation, *Colloids Surf B, B: Biointerfaces* **94**, 2012, 184–191.

[300]

K. Yang, T. Gao, Z. Bao, J. Su and X. Chen, Preparation and characterization of a novel thermosensitive nanoparticle for drug delivery in combined hyperthermia and chemotherapy, *J Mater Chem B* **1**, 2013, 6442–6448.

[301]

J.S. Park, T.H. Han, K.Y. Lee, S.S. Han, J.J. Hwang, D.H. Moon, S.Y. Kim and Y.W. Cho, *N*N-acetyl histidine-conjugated glycol chitosan self-assembled nanoparticles for intracytoplasmic delivery of drugs: Eendocytosis, exocytosis and drug release, *J Controlled Release* **115**, 2006, 37–45.

[302]

U. Termsarasab, H.J. Cho, D.H. Kim, S. Chong, S.J. Chung, C.K. Shim, H.T. Moon and D.D. Kim, Chitosan oligosaccharide–arachidic acid-based nanoparticles for anti-cancer drug delivery, *Int J Pharm* **441**, 2013, 373–380.

[303]

H. Bouterfa, T. Picht, D. Keß, C. Herbold, E. Noll, P.M. Black, K. Roosen and J.C. Tonn, Retinoids inhibit human glioma cell proliferation and migration in primary cell cultures but not in established cell lines, *Neurosurgery* **46**, 2000, 419–430.

[304]

P. Opanasopit, T. Ngawhirunpat, T. Rojanarata, C. Choochottiros and S. Chirachanchai, Camptothecin-incorporating *N*N-phthaloylchitosan-g-mPEG self-assembly micellar system: Eeffect of degree of deacetylation, *Colloids Surf B, B: Biointerfaces* **60**, 2007, 117–124.

[305]

L. Fan, H. Wu, H. Zhang, F. Li, Th. Yang, Ch. Gu and Q. Yang, Novel super pH-sensitive nanoparticles responsive to tumor extracellular pH, *Carbohydr Polym* **73**, 2008, 390–400.

[306]

J.G. Marques, V.M. Gaspar, E. Costa, C.M. Paquete and I.J. Correia, Synthesis and characterization of micelles as carriers of non-steroidal anti-inflammatory drugs (NSAID) for application in breast cancer therapy, *Colloids Surf B, B: Biointerfaces* **113**, 2014, 375–383.

[307]

J.H. Park, Y.W. Cho, Y.J. Son, K. Kim, H. Chung, S.Y. Jeong, K. Choi, C.R. Park, R.W. Park, I.S. Kim and I.C. Kwon, Preparation and characterization of self-assembled nanoparticles based on glycol chitosan bearing adriamycin, *Colloid Polym*

Sci **284**, 2006, 763–770.

[308]

Y. Tao, J. Han and H. Dou, Paclitaxel-loaded tocopheryl succinate-conjugated chitosan oligosaccharide nanoparticles for synergistic chemotherapy, *J Mater Chem* **22**, 2012, 8930–8937.

[309]

Z.X. Yuan, X. Sun, T. Gong, H. Ding, Y. Fu and Z.R. Zhang, Randomly 50% ~~N~~*N*-acetylated low molecular weight chitosan as a novel renal targeting carrier, *J Drug Target* **15**, 2007, 269–278.

[310]

M. Wang, H. Hu, Y. Sun, L. Qiu, J. Zhang, G. Guan, X. Zhao, M. Qiao, L. Cheng and L. Cheng, A pH-sensitive gene delivery system based on folic acid-PEG-chitosan–PAMAM-plasmid DNA complexes for cancer cell targeting, *Biomaterials* **34**, 2013, 10120–10132.

[311]

T.D. Leathers, Biotechnological production and applications of pullulan, *Appl Microbiol Biotechnol* **62**, 2003, 468–473.

[312]

M. Li, J.W. Lam, F. Mahtab, S. Chen, W. Zhang, Y. Hong, J. Xiong, Q. Zheng and B.Z. Tang, Biotin-decorated fluorescent silica nanoparticles with aggregation-induced emission characteristics: ~~F~~fabrication, cytotoxicity and biological applications, *J Mater Chem B* **1**, 2013, 676–684.

[313]

Y. Ochi, Y. Shiose, H. Kuga and E. Kumazawa, A possible mechanism for the long-lasting antitumor effect of the macromolecular conjugate DE-310: ~~M~~mediation by cellular uptake and drug release of its active camptothecin analog DX-8951, *Cancer Chemother Pharmacol* **55**, 2005, 323–332.

[314]

S.A. Veltkamp, E.O. Witteveen, A. Capriati, A. Crea, F. Animati, M. Voogel-Fuchs, I.J.G.M. Van Den Heuvel, J.H. Beijnen, E.E. Voest and J.H.M. Schellens, Clinical and pharmacologic study of the novel prodrug delimotecan (MEN 4901/T-0128) in patients with solid tumors, *Clin Cancer Res* **14**, 2008, 7535–7544.

[315]

J. Varshosaz, F. Hassanzadeh, H. Sadeghi, F. Firozian and M. Mirian, Effect of molecular weight and molar ratio of dextran on self-assembly of dextran stearate polymeric micelles as nanocarriers for etoposide, *J Nanomater* **2012**, 2012, 1–10.

[316]

Y.I. Jeong, K.D. Chung and K.C. Choi, Doxorubicin release from self-assembled nanoparticles of deoxycholic acid-conjugated dextran, *Arch Pharm Res* **34**, 2011, 159–167.

[317]

J.H. Hwang, C.W. Choi, H.W. Kim, D.H. Kim, T.W. Kwak, H.M. Lee, C.H. Kim, C.W. Chung, Y.I. Jeong and D.H. Kang, Dextran-~~*b-poly(L*~~*b*-poly(L-histidine) copolymer nanoparticles for pH-responsive drug delivery to tumor cells, *Int J Nanomed* **8**, 2013, 3197–3207.

[318]

H. Sun, B. Guo, X. Li, R. Cheng, F. Meng, H. Liu and Z. Zhong, Shell-sheddable micelles based on dextran-SS-poly(ε-caprolactone) diblock copolymer for efficient intracellular release of doxorubicin, *Biomacromolecules* **11**, 2010, 848–854.

[319]

P. Prabu, A.A. Chaudhari, S. Aryal, N. Dharmaraj, S.Y. Park, W.D. Kim and H.Y. Kim, In vitro evaluation of poly(caporlactone) grafted dextran (PGD) nanoparticles with cancer cell, *J Mater Sci Mater Med* **19**, 2008, 2157–2163.

[320]

P. Prabu, A.A. Chaudhari, J.A. Ko, N. Dharmaraj, S.Y. Park, H.Y. Kim and M.S. Khil, Cellular uptake and in vitro drug release studies on paclitaxel-loaded poly(caprolactone)-grafted dextran copolymeric nanoparticles, *Nanobiotechnology* **5**, 2009, 42–49.

[321]

P. Liu, B. Shi, C. Yue, G. Gao, P. Li, H. Yi, M. Li, B. Wang, Y. Ma and L. Cai, Dextran-based redox-responsive doxorubicin prodrug micelles for overcoming multidrug resistance, *Polym Chem* **4**, 2013, 5793–5799.

[322]

S. Tan, D. Zhao, D. Yuan, H. Wang, K. Tu and L.Q. Wang, Influence of indomethacin-loading on the micellization and drug release of thermosensitive dextran-graft-poly(~~*N*~~*N*-isopropylacrylamide), *React Funct Polym* **71**, 2011, 820–827.

[323]

K. Roberts, R. Spychal, M.J. Brookes, V. Strugala, P.W. Dettmar, L.M. Johnstone, I.G. Jolliffe and C. Tselepis, M2016 ~~Potential Therapeutic Role of Alginates in the Management of Oesophageal A~~potential therapeutic role of alginates in the management of oesophageal adenocarcinoma, *Gastroenterology* **1349** (Suppl 1), 2008, ~~A–A451~~(A–A451).

[324]

A.P.A. de Sousa, M.R. Torres, C. Pessoa, Moraes MOd, F.D.R. Filho, A.P.N.N. Alves and L.V. Costa-Lotufo, In vivo growth-inhibition of Sarcoma 180 tumor by alginates from brown seaweed Sargassum vulgare, *Carbohydr Polym* **69**, 2007, 7–13.

[325]

C. Zhang, W. Wang, T. Liu, Y. Wu, H. Guo, P. Wang, Q. Tian, Y. Wang and Z. Yuan, Doxorubicin-loaded glycyrrhetinic acid-modified alginate nanoparticles for liver tumor chemotherapy, *Biomaterials* **33**, 2012, 2187–2196.

[326]

C. Du, J. Zhao, J. Fei, L. Gao, W. Cui, Y. Yang and J. Li, Alginate-based microcapsules with a molecule recognition linker and photosensitizer for the combined cancer treatment, *Chem Asian J* **8**, 2013, 736–742.

[327]

A. Shalviri, P. Cai, A.M. Rauth, J.T. Henderson and X.Y. Wu, Evaluation of new bi-functional terpolymeric nanoparticles for simultaneous in vivo optical imaging and chemotherapy of breast cancer, *Drug Deliv Transl Res* **2**, 2012, 437–453.

[328]

S. Svenson, M. Wolfgang, J. Hwang, J. Ryan and S. Eliasof, Preclinical to clinical development of the novel camptothecin nanopharmaceutical CRLX101, *J Controlled Release* **153**, 2011, 49–55.

[329]

C. Young, T. Schluep, J. Hwang and S. Eliasof, CRLX101 (formerly IT-101)~~–A Novel Nanopharmaceutical of Camptothecin in Clinical D~~—A Novel nanopharmaceutical of camptothecin in clinical development, *Curr Bioact Compd* **7**, 2011, 8–14.

[330]

S. Eliasof, D. Lazarus, C.G. Peters, R.I. Case, R.O. Cole, J. Hwang, T. Schluep, J. Chao, J. Lin and Y. Yen, Correlating preclinical animal studies and human clinical trials of a multifunctional, polymeric nanoparticle, *Proc Natl Acad Sci USA* **110**, 2013, 15127–15132.

[331]

W.J. Gradishar, Albumin-bound paclitaxel: ~~A~~a next-generation taxane, *Expert Opin Pharmacother* **7**, 2006, 1041–1053.

[332]

N. Desai, V. Trieu, Z. Yao, L. Louie, S. Ci, A. Yang, C. Tao, T. De, B. Beals, D. Dykes, P. Noker, R. Yao, E. Labao, M. Hawkins and P. Soon-Shiong, Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel, *Clin Cancer Res* **12**, 2006, 1317–1324.

[333]

D.W. Nyman, K.J. Campbell, E. Hersh, K. Long, K. Richardson, V. Trieu, N. Desai, M.J. Hawkins, D.D. Von Hoff and I. Phase, and pharmacokinetics trial of ABI-007, a novel nanoparticle formulation of paclitaxel in patients with advanced nonhematologic malignancies, *J Clin Oncol* **23**, 2005, 7785–7793.

[334]

D. Cirstea, T. Hideshima, S. Rodig, L. Santo, S. Pozzi, S. Vallet, H. Ikeda, G. Perrone, G. Gorgun, K. Patel, N. Desai, P. Sportelli, S. Kapoor, S. Vali, S. Mukherjee, N.C. Munshi, K.C. Anderson and N. Raje, Dual ~~Inhibition of Akt/Mammalian Target of Rapamycin Pathway by N~~inhibition of akt/mammalian target of rapamycin pathway by nanoparticle Albumin-Bound~~–Rapamycin and Perifosine Induces Antitumor Activity in Multiple M~~—rapamycin and perifosine induces antitumor

activity in multiple myeloma, *Mol Cancer Ther* **9**, 2010, 963–975.

[335]

J.Y. Lee, K.H. Bae, J.S. Kim, Y.S. Nam and T.G. Park, Intracellular delivery of paclitaxel using oil-free, shell cross-linked HSA–M–multi-armed PEG nanocapsules, *Biomaterials* **32**, 2011, 8635–8644.

[336]

Z. Lu, T.K. Yeh, M. Tsai, J.L.S. Au and M.G. Wientjes, Paclitaxel-loaded gelatin nanoparticles for intravesical bladder cancer therapy, *Clin Cancer Res* **10**, 2004, 7677–7684.

[337]

J. Ge, E. Neofytou, J. Lei, R.E. Beygui and R.N. Zare, Protein-Polymer Hybrid Nanoparticles for Drug D–polymer hybrid nanoparticles for drug delivery, *Small* **8**, 2012, 3573–3578.

[338]

C.L. Tseng, T.W. Wang, G.C. Dong, S. Yueh-Hsiu Wu, T.H. Young, M.J. Shieh, P.J. Lou and F.H. Lin, Development of gelatin nanoparticles with biotinylated EGF conjugation for lung cancer targeting, *Biomaterials* **28**, 2007, 3996–4005.

[339]

C.L. Tseng, S.Y.H. Wu, W.H. Wang, C.L. Peng, F.H. Lin, C.C. Lin, T.H. Young and M.J. Shieh, Targeting efficiency and biodistribution of biotinylated-EGF-conjugated gelatin nanoparticles administered via aerosol delivery in nude mice with lung cancer, *Biomaterials* **29**, 2008, 3014–3022.

[340]

S. Kommareddy and M. Amiji, Biodistribution and pharmacokinetic analysis of long-circulating thiolated gelatin nanoparticles following systemic administration in breast cancer-bearing mice, *J Pharm Sci* **96**, 2007, 397–407.

[341]

S. Kommareddy and M. Amiji, Antiangiogenic gene therapy with systemically administered sFlt-1 plasmid DNA in engineered gelatin-based nanovectors, *Cancer Gene Ther* **14**, 2007, 488–498.

[342]

P.H.L. Tran, T.T.D. Tran and B.J. Lee, Enhanced solubility and modified release of poorly water-soluble drugs via self-assembled gelatin–oleic acid nanoparticles, *Int J Pharm* **455**, 2013, 235–240.

[343]

M. Boulet-Audet, A.E. Terry, F. Vollrath and C. Holland, Silk protein aggregation kinetics revealed by Rheo-IR, *Acta Biomater* 2013, 776–784.

[344]

W.H. Chiang, Y.J. Lan, Y.C. Huang, Y.W. Chen, Y.F. Huang, S.C. Lin, C.S. Chern and H.C. Chiu, Multi-scaled polymersomes from self-assembly of octadecanol-modified dextrans, *Polymer* **53**, 2012, 2233–2244.

[345]

Z. Zhao, A. Chen, Y. Li, J. Hu, X. Liu, J. Li, Y. Zhang, G. Li and Z. Zheng, Fabrication of silk fibroin nanoparticles for controlled drug delivery, *J Nanopart Res* **14**, 2012, 736/1–736/736.

[346]

K. Numata, M.R. Reagan, R.H. Goldstein, M. Rosenblatt and D.L. Kaplan, Spider silk-based gene carriers for tumor cell-specific delivery, *Bioconjugate Chem* **22**, 2011, 1605–1610.

[347]

K. Numata, A.J. Mieszawska-Czajkowska, L.A. Kvenvold and D.L. Kaplan, Silk-based nanocomplexes with tumor-homing peptides for tumor-specific gene delivery, *Macromol Biosci* **12**, 2012, 75–82.

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