NANOPARTICLE

The present invention relates to diblock polymers, nanoparticles comprising the diblock polymers and a therapeutic agent, in particular the therapeutic agent is attached to a magnetic moiety, and methods to prepare the diblock polymers and nanoparticles.

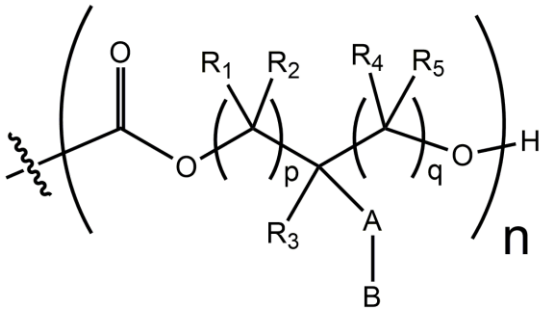
Most anticancer drugs are unable to discriminate healthy cells and cancer cells, and thus cause severe harmful side-effects to patients. In addition, many anticancer agents are hydrophobic, and clinical administration of these drugs typically requires dissolution using organic solvents. For example, paclitaxel is a widely used small molecular drug effective against an extensive range of solid tumours. However, its clinical applications have been mostly impeded by its extremely low aqueous solubility (0.3 μg/mL in water), which limits its administration to the use of a formulation comprising of 50:50 mixture of Cremophor EL (polyethoxylated castor oil) and ethanol. Although this formulation is able to increase the solubility and bioavailability of paclitaxel, it leads to hypersensitivity reactions and other severe side effects.

As such, substantial efforts have been invested in devising alternative formulations for improving the solubility of paclitaxel without the use of the organic solvents, which include liposomes, microspheres, nanospheres and polymeric micelles. Over the past few decades, the development of polymeric micelles as carriers of hydrophobic drugs has attracted significant attention due to their unique properties. Polymeric micelles can significantly enhance the solubility of hydrophobic drugs to improve bioavailability and serve as drug reservoirs to allow sustained release to prevent or decrease tissue damage upon accidental extravasations. Polymeric micelles typically have excellent stability in the blood stream and long circulation time as the polymers can be designed to have low critical micelle concentration. Such nanocarriers can also alter drug biodistribution profiles and allow preferential targeting to the tumour sites *via* the “Enhanced Permeability and Retention (EPR)” effect while evading renal clearance and the non-specific reticuloendothelial uptake. It has been established that nanocarriers formed from polymers bearing poly(ethylene glycol) (PEG) are particularly useful in reducing plasma protein adsorption and prolonging blood circulation time. Many anticancer drugs have

been physically incorporated into PEG-bearing polymeric micelles. The loading capacity of polymeric micelles largely depends on the interactions between the drugs and the micellar core. Paclitaxel has a rigid chemical structure with 11 stereocenters (4 *R* and 7 *S*) and a strong tendency to self-assemble into long fibres. As a result, it is difficult to load paclitaxel into existing nanoparticles.

Furthermore, functionalised paclitaxel is likely to be as, or more, difficult to administer to patients, as the additional functionality introduces additional pharmacokinetic variables, for example aqueous solubility, cell permeability, and metabolism.

In accordance with a first aspect of the invention, there is provided a nanoparticle comprising: a therapeutic agent; and a diblock polymer, the polymer comprising: a first block polymer comprising of a polyether repeating unit; and a second block polymer comprising of a repeating unit of a general Formula I



(I),

wherein R_1, R_2, R_3, R_4, and R_5 is each independently selected from the group comprising: hydrogen, halogen, alkyl group comprising 1 to 20 carbon atoms, and aryl group comprising 6 to 20 carbon atoms, p and q is each independently an integer from 0 to 6, wherein p and q cannot both be 0, A is a linker or linking group, and B is an aromatic moiety with two adjacent hydroxyl groups. Preferably, the nanoparticle has an average particle size of 20 nm to 400 nm.

The term “alkyl” used alone or as part of a larger moiety, such as alkoxy, haloalkyl, arylalkyl, alkylamine, cycloalkyl, dialkyamine, alkylamino, dialkyamino alkylcarbonyl, alkoxycarbonyl and the like, includes as used herein means saturated straight-chain, cyclic or branched aliphatic group. As used herein, an alkyl group may comprise 1 to 20

carbon atoms (“C1-C20”), 1 to 10 carbon atoms (“C1-C10”), 1 to 6 carbon atoms (“C1-C6”).

The term “aryl” refers to an aromatic ring system having any suitable number of ring atoms and any suitable number of rings. Aryl groups can include any suitable number of ring atoms, such as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 ring atoms, as well as from 6 to 10, 6 to 12, or 6 to 14 ring members. Aryl groups can be monocyclic, fused to form bicyclic or tricyclic groups, or linked by a bond to form a biaryl group. Representative aryl groups include phenyl, naphthyl and biphenyl. Other aryl groups include benzyl, having a methylene linking group. Some aryl groups have from 6 to 12 ring members, such as phenyl, naphthyl or biphenyl. Other aryl groups have from 6 to 10 ring members, such as phenyl or naphthyl. Some other aryl groups have 6 ring members, such as phenyl. Aryl groups can be substituted or unsubstituted.

The aryl group may include one or more substituents, wherein each substituent is independently selected from halogen, cyano, hydroxy, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{3-5} cycloalkyl- alkoxy, amino, C_{1-6} alkylamino, di-C_{1-6} alkylamino, and -$C(O)R_x$, wherein each R_x is a member independently selected from H, hydroxy, C_{1-6} alkyl, C_{1-6} alkoxy, amino, C_{1-6} alkylamino and di- C_{1-6} alkylamino.

The wavy line across the bond herein indicates the point of attachment to the next atom or rest of the molecule.

The term polyether refers to any polymer comprising ether repeating units, in particular linear polymers of the ether repeating units. An example of a polyether is polyethylene glycol, or a mono endcapped polyethylene glycol. In an embodiment, the endcapped polyethylene glycol is an alkyl group endcapped polyethylene glycol, wherein the alkyl group comprises 1 to 6 carbon atoms. Preferably, the first polymer block is a methoxy polyethylene glycol (i.e. the alkyl group of the mono endcapped polyethylene glycol is methyl).

Preferably, the nanoparticle made from polymer C has an average particle size of between 20 to 400 nm.

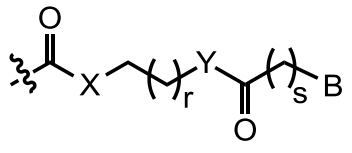
Preferably, the diblock polymer has a number average molecular weight (M_n) of between 3 kDa to 40 kDa.

Preferably, the first block polymer is a mono endcapped polyethylene glycol the endcapped polyethylene glycol is an alkyl group capped polyethylene glycol, wherein the alkyl group comprises 1 to 6 carbon atoms.

Preferably, the first block polymer has a number average molecular weight (M_n) of between 2 kDa to 10 kDa. More preferably, the mean number of repeating units in the second block polymer is between 5 to 50.

Preferably, the linker is any one selected from the group comprising: alkyl group comprising 1 to 20 carbon atoms, aryl group comprising 1 to 20 carbon atoms, a ketone, an ester, an amide, a urea, a carbomate, and a carbonate.

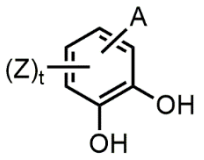
More preferably, the linker or linking group has a general Formula (II)

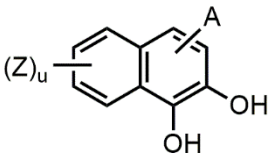


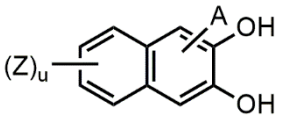
(II)

wherein r is an integer from 1 to 6, and s is an integer from 0 to 5, X and Y is each independently selected from the group comprising: O, NH, or NR_6; and wherein R_6 is an alkyl group comprising 1 to 6 carbon atoms.

Preferably, the aromatic moiety is selected from the group comprising:



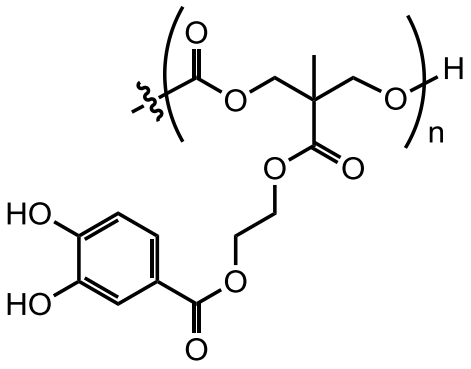
,


, and


wherein Z is a hydrogen, an electron donating group, or an electron withdrawing group; and wherein t is an integer from 0 to 3, u is an integer from 0 to 5.

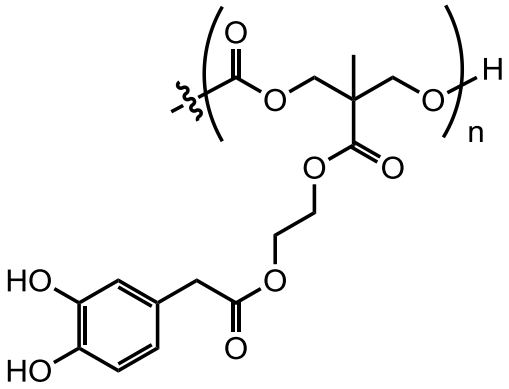
Preferably, the electron donating group is selected from the group comprising: OR_7, NR_8R_9, and R_{10}, wherein R_7, R_8, R_9, and R_{10} are each independently selected from the group comprising: hydrogen, an alkyl comprising 1 to 20 carbon atoms, and aryl group comprising 1 to 20 carbon atoms. The electron withdrawing group may be selected from the group comprising: halogen, trihalogencarbon, carbonyl, nitrile, sulfonyl, and nitro.

Preferably, the repeating unit of the second polymer block is Polymer B having a general formula (III)



(III).

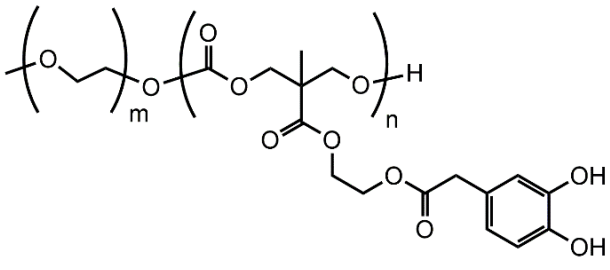
Preferably, the repeating unit of the second polymer block is Polymer C or D having a general Formula (IV)



(IV).

Preferably, the mean number of repeating units (n) in the second block polymer is 10, or 20. The nanoparticle may have an average particle size of between 20 to 400 nm.

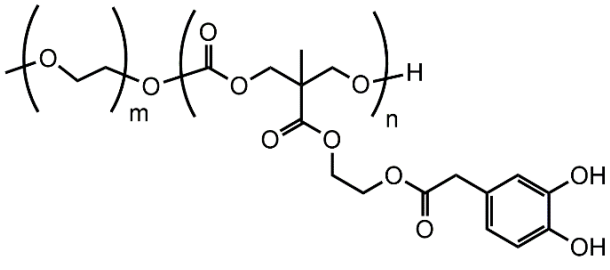
More preferably, Polymer C has a general Formula (VI)



(VI),

where the mean number of repeating units (m) in the first block polymer is 113, and the mean number of repeating units (n) in the second block polymer is 10.

Polymer D has a general Formula (VII)



(VII),

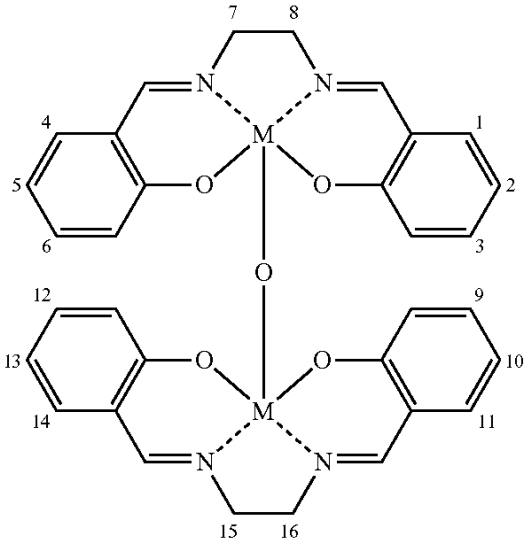
where the mean number of repeating units (m) in the first block polymer is 113, and the mean number of repeating units (n) in the second block polymer is 20.

Preferably, the first polymer block is a methoxy polyethylene glycol.

Preferably, the therapeutic agent is selected from the group comprising: paclitaxel, docetaxel, and cabazitaxel. Preferably, the therapeutic agent is chemotherapeutic.

The therapeutic agent may comprise any hydrophobic agents, hydrophilic agents, and combinations thereof. In various embodiments, the therapeutic agent is an analgesic agent, an anti-inflammatory agent, an anti-arrhythmic agent, an anti-asthma agent, an anti-bacterial agent, an anti-viral agent, an anti-coagulant, an anti-depressant, an anti-diabetic, an anti-epileptic, an anti-fungal agent, an anti-hypertensive agent, an anti-malarial, an anti-migraine agent, an anti-muscarinic agent, an anti-neoplastic agent, an immunosuppressant, an anti-protozoal agent, an anti-thyroid agent, an anxiolytic agent, a sedative, a hypnotic agent, a neuroleptic agent, a beta-Blocker, a cardiac inotropic agent, a corticosteroid, a diuretic agent, an anti-Parkinsonian agent, a gastrointestinal agent, an anti-histamine, a histamine-receptor antagonist, a lipid regulating agent, a muscle relaxant, nitrate and other anti-anginal agent, a nutritional agent, an opioid analgesic, sex hormone, stimulant, cytokine, peptidomimetic, peptide, protein, toxoid, sera, antibody, vaccine, nucleoside, nucleotide, nucleic acid and peptidyl-nucleic acid.

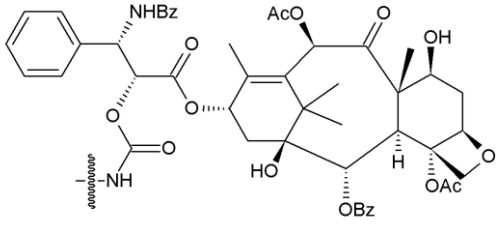
Preferably, the therapeutic agent is attached to a magnetic moiety. More preferably, the magnetic moiety has a general Formula (V):



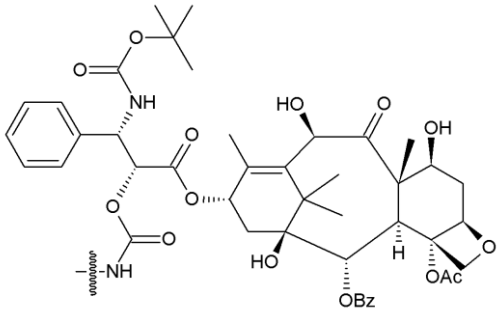
(V),

wherein M is selected from Fe, Cr, Mn, Co, Ni, Mo, Ru, Rh, Pd, W, Re, Os, Ir, Pt, Nd, Sm, Eu, and Gd,

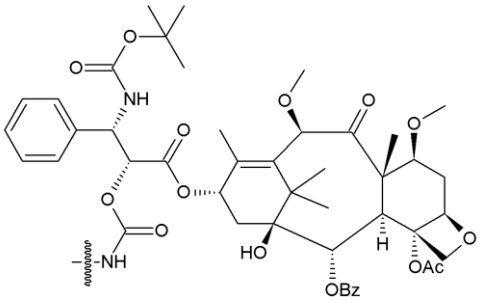
wherein the therapeutic agents are bonded to locations 5 and 10, or locations 2 and 13, and the therapeutic agents are independently selected from any one of the group comprising: paclitaxel, docetaxel, cabazitaxel,



,



, and

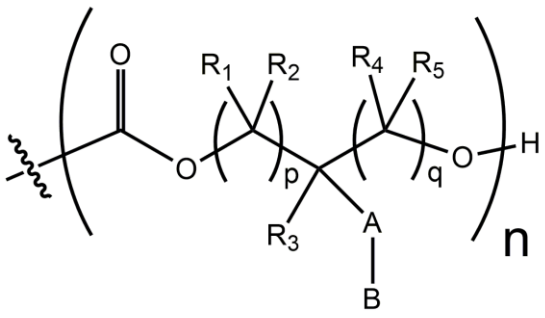


.

In various embodiments, the nanoparticle comprises 1 to 20 weight of the therapeutic agent; and 80 to 99 weight percent of the diblock polymer.

In various embodiments, the therapeutic agent may be encapsulated, surface conjugated or adsorbed on the diblock polymer.

In accordance with a second aspect of the invention, there is provided a diblock polymer comprising: a first block polymer comprising of a polyether repeating unit; and a second block polymer comprising of a repeating unit of a general formula I



(I),

wherein R_1, R_2, R_3, R_4, and R_5 is each independently selected from the group comprising: hydrogen, halogen, alkyl group comprising 1 to 20 carbon atoms, and aryl group comprising 6 to 20 carbon atoms, p and q is each independently an integer from 0 to 6, wherein p and q cannot both be 0, A is a linker, and B is an aromatic moiety with two adjacent hydroxyl groups.

Preferably, the diblock polymer has a number average molecular weight (M_n) of between 3 kDa to 40 kDa.

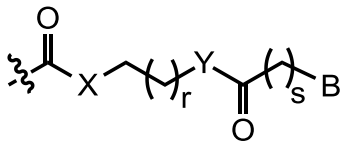
Preferably, the first block polymer is an endcapped polyethylene glycol, the endcapped polyethylene glycol is an alkyl group capped polyethylene glycol, wherein the alkyl group comprises 1 to 6 carbon atoms.

Preferably, the first block polymer (i.e. polyether) has a number average molecular weight (M_n) of between 2 kDa to 10 kDa.

Preferably, a mean number of repeating units in the second block polymer is between 5 to 50.

Preferably, the linker or linking group is any one selected from the group comprising: an alkyl group comprising 1 to 20 carbon atoms, an aryl group comprising 1 to 20 carbon atoms, a ketone, an ester, an amide, a urea, a carbamate, and a carbonate. The linker may be made of combinations of the functional groups.

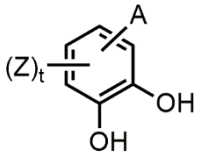
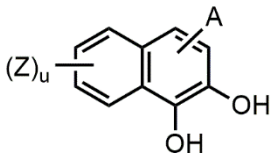
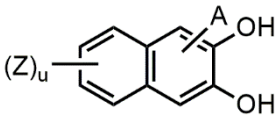
More preferably, the linking group is of a general Formula (II)



(II)

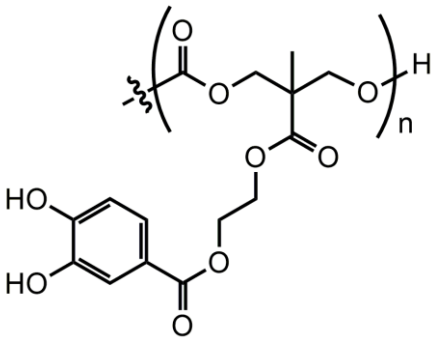
wherein r is an integer from 1 to 6, and s is an integer from 0 to 5, X and Y is each independently selected from the group comprising: O, NH, or NR_6; and wherein R_6 is an alkyl group comprising 1 to 6 carbon atoms.

The aromatic moiety with two adjacent hydroxyl groups refers to hydroxyl groups bonded to adjacent carbon atoms of the aromatic moiety. Preferably, the aromatic moiety is any one selected from the group comprising:


,

, and

,

wherein Z is a hydrogen, an electron donating group, or an electron withdrawing group; and wherein t is an integer from 0 to 3, u is an integer from 0 to 5. In an embodiment, the electron donating group is selected from the group comprising: OR_7, NR_8R_9, and R_{10}, wherein R_7, R_8, R_9, and R_{10} are each independently selected from the group comprising: hydrogen, an alkyl comprising 1 to 20 carbon atoms, and aryl group comprising 1 to 20 carbon atoms. In an embodiment, the electron withdrawing group is selected from the group comprising: halogen, trihalogencarbon, carbonyl, nitrile, sulfonyl, and nitro.

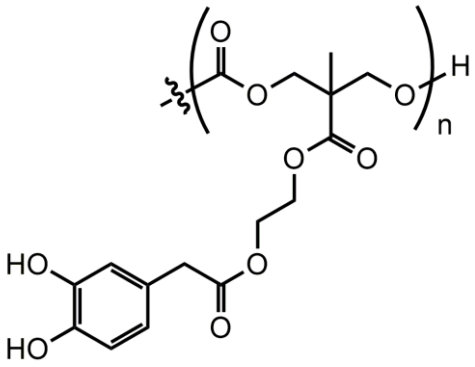
In an embodiment, the repeating unit of the second polymer block is Polymer B having a general formula (III)



(III).

In various embodiments, the first polymer block is a methoxy polyethylene glycol with a number average molecular weight of 10 kDa. The mean number of repeating units in the second block polymer is 15, and the diblock polymer has a number average molecular weight of 15 kDa.

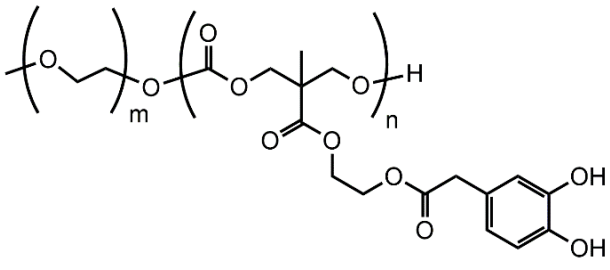
In an embodiment, the repeating unit of the second polymer block is Polymer C or D having a general formula (IV)



(IV).

In an embodiment, the mean number of repeating units in the second block polymer is about 10. In an embodiment, the mean number of repeating units in the second block polymer is 20.

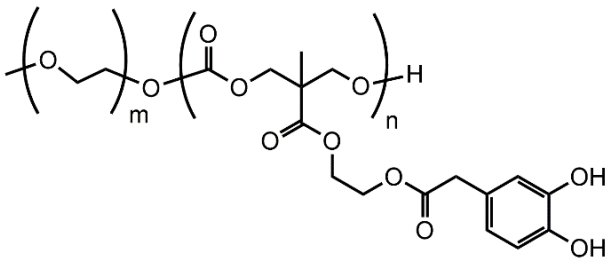
In an embodiment, Polymer C has a general formula (VI)



(VI),

the mean number of repeating units in the first block polymer is 113, and the mean number of repeating units in the second block polymer is 10.

In an embodiment, Polymer D has a general formula (VII)



(VII),

the mean number of repeating units in the first block polymer is 113, and the mean number of repeating units in the second block polymer is 20.

Preferably, in various embodiments, the block copolymer has a number average molecular weight of 8.5 kDa. The first polymer block is a methoxy polyethylene glycol with a number average molecular weight of 5 kDa.

In various embodiments, the diblock polymer further comprises a therapeutic agent. The the therapeutic agent is selected from the group comprising: paclitaxel, docetaxel, and cabazitaxel. In various embodiments, the therapeutic agent is attached to a magnetic moiety. The therapeutic agent may be encapsulated, surface conjugated or adsorbed on the diblock polymer.

In accordance with a third aspect of the invention, there is provided a method to prepare a therapeutic nanoparticle, the method comprising: (a) dissolving a therapeutic agent and a diblock polymer, according to an aspect of the invention, in a water miscible solvent to form a solution; and (b) formulating the therapeutic agent with the diblock polymer to form the therapeutic nanoparticle.

Preferably, dissolving the biologically active agent and diblock polymer is by sonication.

Preferably, the therapeutic agent is attached to a magnetic moiety.

Preferably, the therapeutic agent is selected from the group comprising: paclitaxel, deocetaxel, and cabazitaxel.

Preferably, formulating the therapeutic agent with the diblock polymer comprises adding the solution of the polymer and therapeutic agent to water under sonication.

Preferably, the method further comprises dialysing the solution with a dialysis membrane to remove the solvent.

More preferably, the ratio of the volume of the solution to water is 1: 0.3 to 2.

More preferably, adding water to the solution is performed at a temperature of between 0 °C to 10 °C.

In an embodiment, the water miscible solvent is any one selected from the group comprising dimethyl sulfoxide, dimethylformamide, and dimethylacetamide.

Preferably, formulating the therapeutic agent with the diblock polymer comprises removing the water miscible solvent under low pressure conditions to form a thin film, adding water or saline solution to the thin film, and sonicating the mixture.

Preferably, sonicating the mixture is performed for 3 hours.

In an embodiment, the water miscible solvent is an alcohol. The alcohol may be any one selected from the comprising ethanol, methanol, and isopropyl alcohol.

In accordance with a fourth aspect of the invention, there is provided a drug delivery system comprising the diblock polymer according to an aspect of the present invention.

Preferably, the drug delivery system further comprises one or more therapeutic agents. More preferably, the therapeutic agent is chemotherapeutic. Even more preferably, the therapeutic agent is selected from the group comprising: paclitaxel, docetaxel, and cabazitaxel.

Preferably, the therapeutic agent is attached to a magnetic moiety.

In accordance with a fifth aspect of the invention, there is provided the polymer according to the first aspect, or the nanoparticle according to the second aspect, or the drug delivery system according to the fourth aspect for use in medicine.

Alternatively, there is provided a method for treating a disease, the method comprising administering the nanoparticle according to the second aspect to a subject in need thereof.

Alternatively, use of a nanoparticle according to the second aspect in the manufacture of a medicament for the treatment of a disease.

Preferably, the disease to be treated is cancer, in particular breast cancer.

The present invention allows a therapeutic agent, in particular when attached to a magnetic moiety, to be loaded in greater amounts and efficiency in polymeric nanoparticles. This provides a pharmaceutical composition that allows for treatment of diseases by selective targeting of diseased cells, in particular tumour cells. The present invention allows for the reduction of side effects from the use of excipients, the reduction of the amount of therapeutic agent required, and a controlled or delayed release profile of the therapeutic agent.

In the Figures:

Figure 1A shows the *in vitro* cytotoxicity of Magptx and Magptx-loaded nanoparticles of polymer B against BT-474 cells in the absence and presence of a magnetic field;

Figure 1B shows the *in vitro* cytotoxicity of Magptx and Magptx-loaded nanoparticles of polymer B1 against BT-474 cells in the absence and presence of a magnetic field;

Figure 1C shows the *in vitro* cytotoxicity of Magptx and Magptx-loaded nanoparticles of polymer C against BT-474 cells in the absence and presence of a magnetic field;

Figure 2 shows a proton NMR spectra of polymer A [PEG-P(TMC)] in $CDCl_3$;

Figure 3 shows a proton NMR spectra of polymer B [PEG-P(MTC-Cat)] in DMSO-d6;

Figure 4. *In vivo* antitumor effect. (A) Antitumor activity of various formulations and the effect of magnetic field in nude mice bearing BT-474 xenografts: 0.9% saline, Magptx and Magptx-loaded nanoparticles B1 (40 mg/kg), (B) Student t test p value between different groups;

Figure 5. Changes of mouse body weight during the treatments of various formulation; and

Figure 6. Percentage of cancer stem cells in cells obtained from tumors at the end of the treatments. (Magptx nanoparticles B1 vs. Control without magnet, $p < 0.05$).

In the following description, numerous specific details are set forth in order to provide a thorough understanding of various illustrative embodiments of the invention. It will be understood, however, to one skilled in the art, that embodiments of the invention may be practiced without some or all of these specific details. In other instances, well known process operations have not been described in detail in order not to unnecessarily obscure pertinent aspects of embodiments being described.

It is generally contemplated that the compounds according to the inventive subject matter will be formulated for administration to a mammal, and especially to a human with a condition that is responsive to the administration of such compounds. Therefore, where contemplated compounds are administered in a pharmacological composition, it is contemplated that contemplated compounds can be formulated in admixture with a pharmaceutically acceptable carrier. For example, contemplated compounds can be administered orally as pharmacologically acceptable salts, or intravenously in a physiological saline solution (e.g., buffered to a pH of about 7.2 to 7.5). Conventional buffers such as phosphates, bicarbonates or citrates can be used for this purpose. Of

course, one of ordinary skill in the art may modify the formulations within the teachings of the specification to provide numerous formulations for a particular route of administration. In particular, contemplated compounds may be modified to render them more soluble in water or other vehicle, which for example, may be easily accomplished with minor modifications (salt formulation, esterification, etc.) that are well within the ordinary skill in the art. It is also well within the ordinary skill of the art to modify the route of administration and dosage regimen of a particular compound in order to manage the pharmacokinetics of the present compounds for maximum beneficial effect in a patient.

In certain pharmaceutical dosage forms, prodrug forms of contemplated compounds may be formed for various purposes, including reduction of toxicity, increasing the organ or target cell specificity, etc. Among various prodrug forms, acylated (acetylated or other) derivatives, pyridine esters and various salt forms of the present compounds are preferred. One of ordinary skill in the art will recognize how to readily modify the present compounds to prodrug forms to facilitate delivery of active compounds to a target site within the host organism or patient. One of ordinary skill in the art will also take advantage of favorable pharmacokinetic parameters of the prodrug forms, where applicable, in delivering the present compounds to a targeted site within the host organism or patient to maximize the intended effect of the compound. Similarly, it should be appreciated that contemplated compounds may also be metabolized to their biologically active form (e.g., via hydroxylation, glycolsylation, oxidation etc.), and all metabolites of the compounds herein are therefore specifically contemplated.

Certain compounds of the present invention may possess asymmetric carbon atoms (i.e., chiral centers) or cis/trans stereoisomers (i.e., geometric isomers). In general, the racemates, diastereomers, geometric isomers, and individual isomers (e.g., separate enantiomers) are all intended to be encompassed within the scope of the present invention. In certain aspects, a composition is enriched in one isomer (e.g., entiomerically or diastereomerically enriched).

The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes at greater than natural abundance, such as deuterium (^{2}H), tritium (^{3}H), iodine-125 (^{125}I), phosphorous-32 (^{32}P), or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

The term “pharmaceutically acceptable” means compatible with the treatment of animals, and in particular, humans.

The term “subject” as used herein includes all members of the animal kingdom, such as mammals, e.g., humans.

[0001] The term "treating" or “treatment” as used herein (and as well understood in the art) means an approach for obtaining beneficial or desired results in a subject’s condition, including clinical results. Beneficial or desired clinical results can include, but are not limited to, one or more of the following: alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of a disease, stabilizing (i.e., not worsening) the state of disease, prevention of a disease’s transmission or spread, delaying or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission, whether partial or total and whether detectable or undetectable.

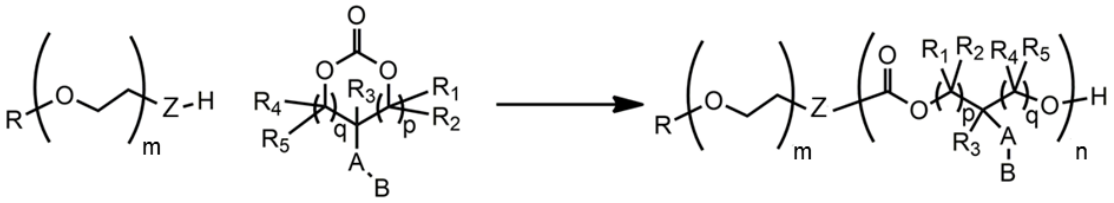
Treatment methods comprise administering to a subject a therapeutically effective amount of an active agent. In certain aspects, the administering step may consist of a single administration or may comprise a series of administrations. The length of the treatment period depends on a variety of factors, such as the severity of the condition, the age of the patient, the concentration of active agent, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment or prophylaxis may increase or decrease over the course of a particular treatment or prophylaxis regime. Changes in

dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration may be required. For example, the compositions are administered to the subject in an amount and for a duration sufficient to treat the patient.

The term "w/w" or "wt/wt" means a percentage expressed in terms of the weight of the ingredient or agent over the total weight of the composition multiplied by 100.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

The present invention provides diblock polymers of general formula (X) comprising of a first block polymer (a polyether) and a second block polymer (a polycarbonate) (Scheme 1). The diblock polymers may form nanoparticles and used to load a therapeutic agent, thereby functioning as a drug delivery system.



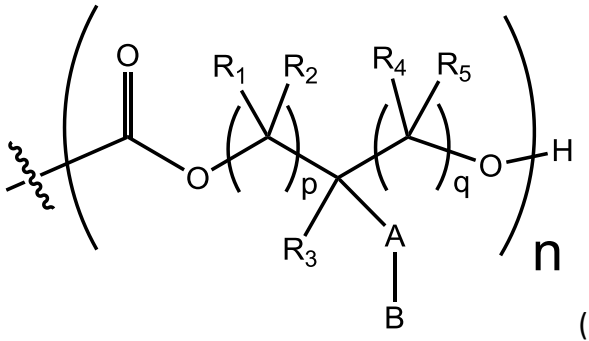
(X)

Scheme 1. General method to prepare diblock polymers of general formula (X).

The first block polymer comprises a polyether block polymer, examples of which include a linear polyether like polyethylene glycol (PEG), in particular a mono endcapped polyethylene glycol (PEG). The mono endcapped PEG is particularly useful as it allows greater control in the polymerisation process as only one free hydroxyl group is available

in the PEG polymer block to act as the polymerisation initiator. The endcap group (R) may be any suitable functional group, like ethers, and esters. Examples of suitable endcap groups (R) include alkyl groups comprising 1 to 6 carbon atoms. Methoxypolyethylene glycol (mPEG) is used to exemplify the present invention herein. mPEG may be prepared by known methods or is commercially available with different number average molecular weights (M_n). The first polymer block may have a M_n of between 2 kDa to 10 kDa. For example, mPEG with a M_n range of 5 kDa to 10 kDa may be used. PEG generally refers to the ether backbone and thus the terminal hydroxyl group may possibly be replaced by other nucleophilic atoms, for example nitrogen or sulphur (i.e. in Scheme 1 Z may be O, NH, N-alkyl, or S). For example, methoxypolyethylene glycol amine and methoxypolyethylene glycol thiol with varying Mn are also commercially available. Therefore, polyether as used herein only refers to the backbone of the first polymer block and does not restrict the free nucleophilic end.

The second block polymer comprises a polycarbonate block polymer attached to an aromatic moiety with two adjacent hydroxyl groups attached. In particular, the aromatic moiety is attached in every single repeating unit of the second block polymer, i.e. the aromatic moiety is present in the monomer of the second block polymer. Therefore, the second block polymer comprises a repeating unit of a general formula I



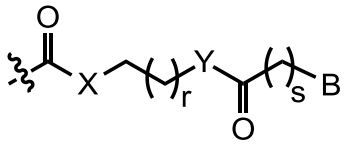
(I),

wherein R_1, R_2, R_3, R_4, and R_5 is each independently selected from the group comprising: hydrogen, halogen, alkyl group comprising 1 to 20 carbon atoms, and aryl group comprising 6 to 20 carbon atoms, p and q is each independently an integer from 0 to 6, wherein p and q cannot both be 0, A is a linker, and B is an aromatic moiety with two adjacent hydroxyl groups.

The diblock polymers may be prepared by an organocatalyzed ring-opening polymerisation method as described in US patent 8,834,861, and which is herein incorporated by reference in its entirety. The second block polymer of the diblock polymers prepared herein have regularly spaced aromatic moieties with two adjacent hydroxyl groups. This provides the diblock polymer different properties from similar existing diblock polymers, for example in US patent 8,834,861.

A linker (A) may be provided to attach the aromatic moiety to the polycarbonate backbone. Examples of suitable linkers include any one selected from: an alkyl group comprising 1 to 20 carbon atoms, an aryl group comprising 1 to 20 carbon atoms, a ketone, an ester, an amide, a urea, a carbamate, and a carbonate. The linker may contain more than one of these functional groups to allow for different linker lengths and types to be constructed. The linkers may be varied and combined as required to allow rapid and convergent assembly of the monomers, to vary the linker length and shape.

In an embodiment, the linker is of a general formula (II)

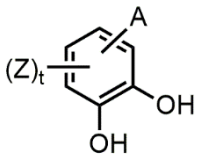


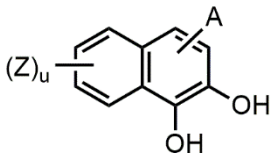
(II)

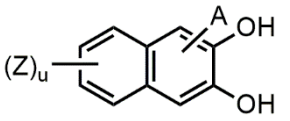
wherein r is an integer from 1 to 6, and s is an integer from 0 to 5, X and Y is each independently selected from the group comprising: O, NH, or NR_6; and R_6 is an alkyl group comprising 1 to 6 carbon atoms.

Examples of aromatic moieties with two adjacent hydroxyl groups (B) include 1,2-dihydroxybenzene (catechol), 1,2-dihydroxynaphthalene, and 2,3-dihydroxynaphthalene. The aromatic moieties may be attached at various positions to the linker (A) which changes the numbering of the two adjacent hydroxyl groups as a matter of nomenclature only. The aromatic moiety may be further substituted with other functional groups to vary the electron density or steric effects of the aromatic moiety to provide different properties and interactions for the aromatic moiety and resultant polymer and nanoparticle formed. Examples of functional groups include electron donating and electron withdrawing groups.

Structurally, the aromatic moiety may be represented by the following group:



, 

, and 

,

wherein Z is a hydrogen, an electron donating group, or an electron withdrawing group; and t is an integer from 0 to 3, u is an integer from 0 to 5.

Examples of electron donating groups include OR_7, NR_8R_9, and R_{10}, wherein R_7, R_8, R_9, and R_{10} are each independently selected from the group comprising: hydrogen, an alkyl comprising 1 to 20 carbon atoms, and aryl group comprising 1 to 20 carbon atoms. The alkyl or aryl groups may contain carbonyl groups for attachment to the heteroatom (i.e. reverse esters or amides which are still generally regarded as electron donating groups).

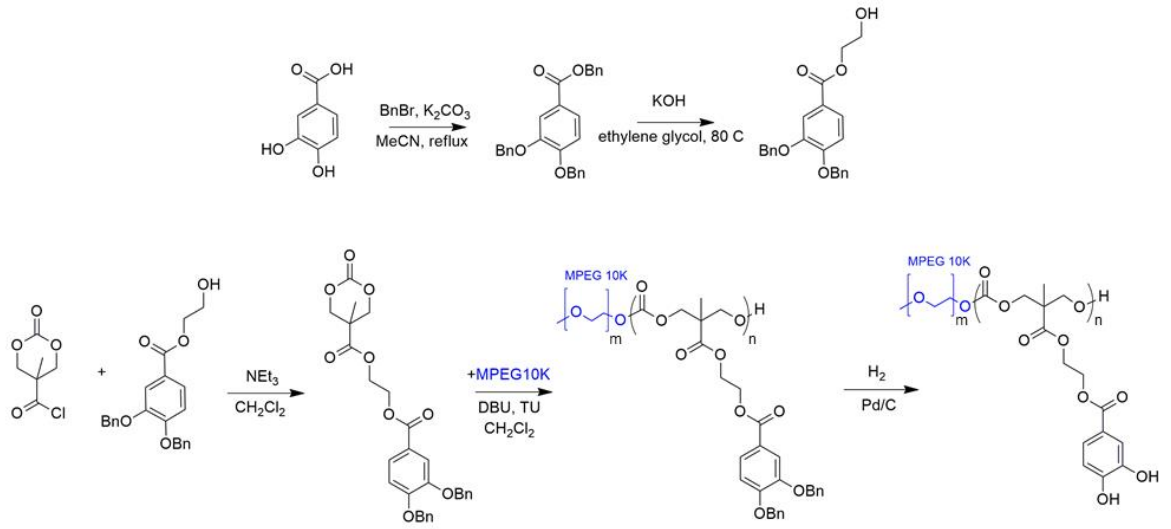
Examples of electron withdrawing groups include halogen, trihalogencarbon, carbonyl, nitrile, sulfonyl, and nitro.

In an embodiment, the mean number of repeating units in the second block polymer is between 5 and 50.

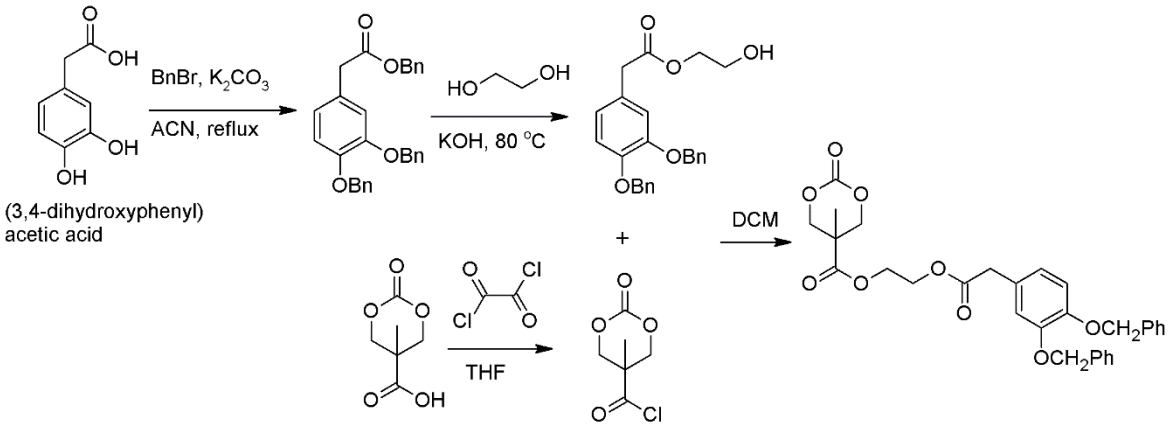
The number average molecular weight (M_n) of the resultant diblock polymer is thus dependent in part on the individual block polymers. In an embodiment, the diblock polymer has a Mn of between 3 kDa to 40 kDa.

Embodiments of the diblock polymers are prepared as follows: Polymers B [PEG-P(MCT-Cat)], C and D comprising polyethylene glycol (PEG) with a number average molecular weight (M_n) of 5 kDa, or 10 kDa, and catechol-functionalized polycarbonate were synthesized *via* organocatalytic ring-opening polymerization of the respective cyclic carbonate monomers (Schemes 2 – 4). The values of m and n for the first and second block polymer respectively may represent the mean number of repeating units in the respective block polymer. Polymer A [PEG-P(TMC-Cat)] with only the polycarbonate

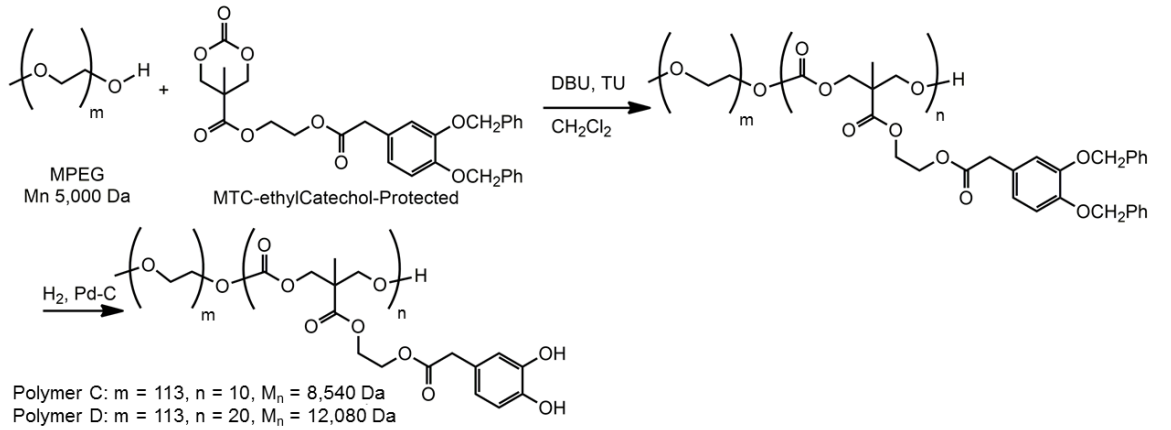
backbone was synthesized by ring-opening polymerization of cyclic TMC carbonate as a control (Scheme 5). The properties of the polymers prepared are shown in Table 1.



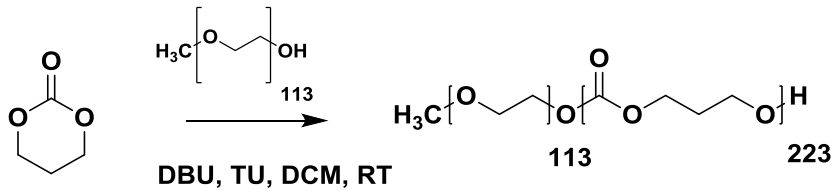
Scheme 2. Synthesis scheme of amphiphilic diblock copolymers of PEG and polycarbonate containing catechol groups (polymer B).



Scheme 3. Synthesis scheme of MTC-ethylCatechol-Protected monomer.



Scheme 4. Synthesis scheme of amphiphilic diblock copolymers of PEG and polycarbonate containing catechol groups (polymer C and polymer D).



Scheme 5. Synthesis scheme of amphiphilic diblock copolymers of PEG and polycarbonate without catechol groups (polymer A).

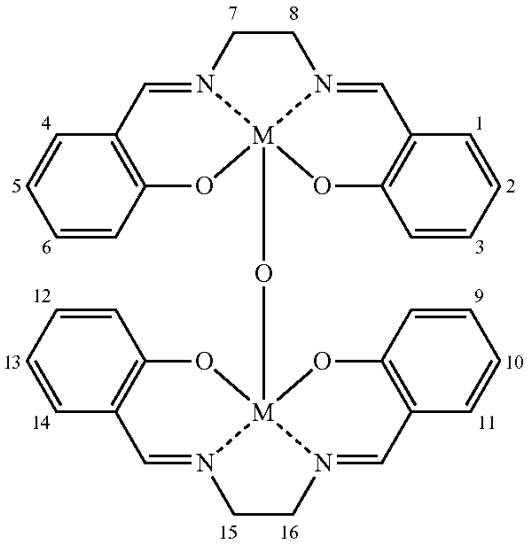
Table 1. **Composition, molecular weight and molecular weight distribution of Polymer A and Polymer B**

Example	Entry	PEG M_n	Polycarbonate block (DP)	M_n (Đ or PDI)
1	Polymer A	5000	223	27.75 kDa (1.39)
2	Polymer B	10000	15	15.0 kDa (1.25)
3	Polymer C	5000	10	8.54 kDa (1.11)
4	Polymer D	5000	20	12.08 kDa (1.25)

Without being limited by theory, the polyether block polymer is generally considered to be hydrophilic, in particular for a linear polyether like PEG or mPEG, while the polycarbonate block polymer is hydrophobic. Hence, a diblock polymer (X) prepared as such typically have amphiphilic properties, and is able to form a micelle. The micelle may have a particle size in the nanometre region and may also be known as nanoparticles.

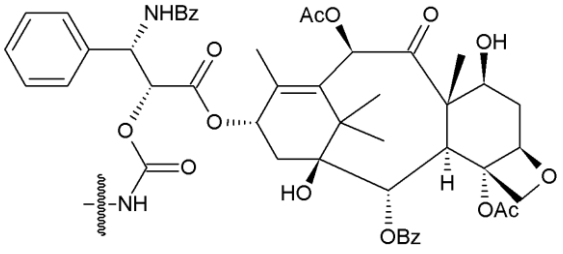
It has been found that the nanoparticles prepared from the diblock polymers (X) are able to load a therapeutic agent, in particular a therapeutic agent attached to a magnetic moiety with high efficiency and loading capacity. Examples of such a therapeutic agent is the taxane family of drugs alone, or attached to a magnetic moiety. Examples of taxane drugs include paclitaxel, docetaxel, and cabazitaxel. US patent 9,505,732, herein incorporated by reference in its entirety, describes drugs or medicinal molecules including paclitaxel bonded to a magnetic moiety, and methods to prepare them.

In an embodiment, the magnetic moiety is a metal-salen complex. The metal-salen complex may have the following general formula (V):

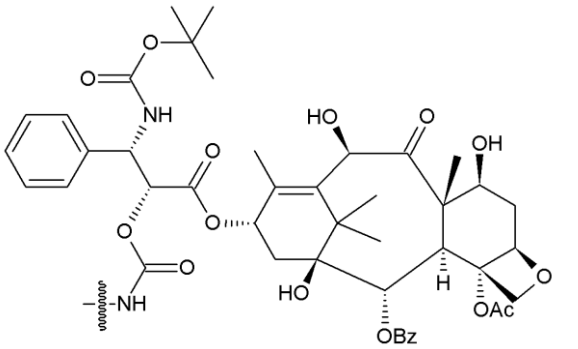


(V).

The metal M is selected from Fe, Cr, Mn, Co, Ni, Mo, Ru, Rh, Pd, W, Re, Os, Ir, Pt, Nd, Sm, Eu, and Gd. The therapeutic agent or medicinal molecules are bonded to the metal-salen complex at locations 5 and 10, and/or locations 2 and 13 and the medicinal molecules are independently selected from: paclitaxel, docetaxel, cabazitaxel,

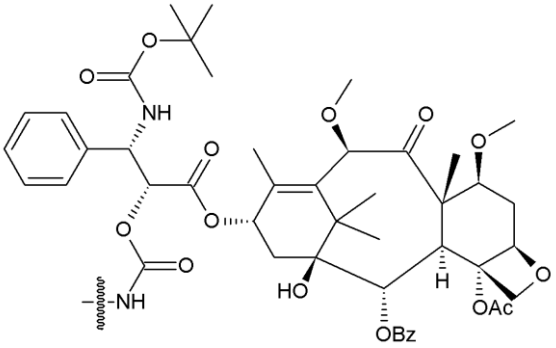


,



,

and



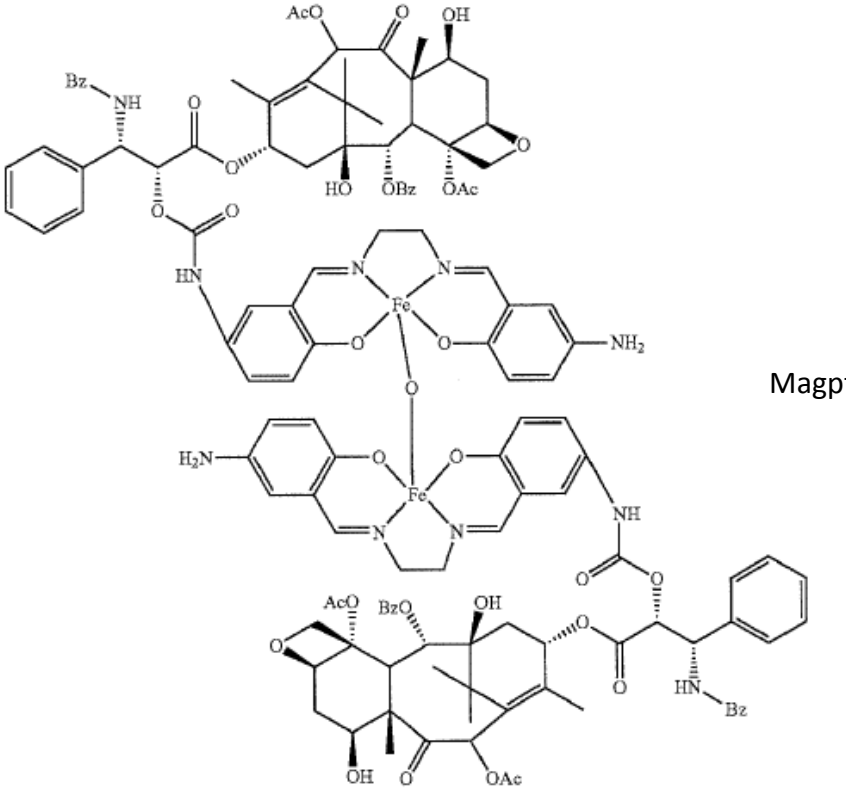
.

The terms “Ac” and “Bz” refer to acetyl, and benzoyl respectively.

The taxane family of drugs may be attached to the metal-salen complex via the free hydroxyl group of what is commonly called the “tail” of paclitaxel. The hydroxyl group is

similarly present in docetaxel and cabazitaxel, and the attachment of these compounds to the metal-salen complex may be done in a similar manner. An example of paclitaxel attached to an iron-salen complex is shown below, and is referred to as Magptx herein. The attachment between the magnetic moiety and paclitaxel is via an amide or more specifically the carbamate group but other functional groups are possible as well.

Magptx comprise paclitaxel bounded to locations 5 and 10, and/or 2 and 13 of the iron-salen complex.



Magptx

The solubility of Magptx in various solvents was studied. It was found that Magptx had limited solubility in water and numerous commonly used organic solvents (e.g. methanol, ethanol, acetonitrile, dichloromethane, dimethyl ether, dimethylacetamide (DMA), toluene, dimethylformamide (DMF)) even after sonication for 48 h. A clear Magptx solution was obtained through dissolving Magptx in dimethyl sulfoxide (DMSO) at 0.2 mg/mL after 48 h sonication. It may also be possible to dissolve Magptx in DMF and DMA.

The limited solubility of Magptx may be attributed to the size and complex structure of Magptx, and may pose a problem in the pharmaceutical formulation of Magptx. The pharmaceutical formulation affects the bioavailability of the therapeutic agent, and ultimately the clinical efficacy of the therapeutic agent in treating the disease.

The effect of the polymer compositions on drug loading efficiency, particle size and size distribution (PDI) was studied and shown in Table 2. Drug-loaded nanoparticles were prepared using a sonication/dialysis method or a thin film/hydration method. A fixed amount of initial Magptx loading (1 mg and 2 mg for the sonication/dialysis method and the thin film/hydration method, respectively) was used. As described above, in an embodiment, Magptx was dissolved in DMSO (clear solution) at 0.2 mg/mL for the sonication method. In another embodiment, for the thin film method, the Magptx was dissolved in ethanol (suspension) at 0.4 mg/mL.

Using the sonication/dialysis method, the loading capacity was significantly improved for catechol-containing polymer B (Example 7) as compared to the conventional polycarbonate polymer A (Example 6) (16.8% *vs.* 2.9%). The enhanced drug loading capacity for the catechol-functionalized polymer might be due to non-covalent interactions (π-π stacking and hydrogen-bonding) formed between catechol and Magptx. The particle size of the polymer B nanoparticles was 214 nm with a narrow PDI of 0.12 and it was 194 nm with PDI of 0.12 after centrifugation. Precipitation was observed during the preparation process when polymers C and D were used, giving rise to lower drug loading capacity as compared to polymer B. This was most likely due to the shorter PEG length, which might lead to higher hydrophobicity of polymers C and D.

Using the thin film/hydration method, the drug loading level was further increased to 20% (see Table 2, Examples 8 and 11 for polymers B1 and C1 respectively; B1 and C1 denoting the respective polymer made by the sonication/thin film method). The drug loading level is defined as the wt% of Magptx in the nanoparticles relative to the weight of the polymers and Magptx in the nanoparticles.

Table 2. Physical Properties of Magptx formulation and Magptx loaded nanoparticles

Example	Entry	Method	Particle size (nm)	PDI	Drug loading level (%)
5	Magptx	Sonication (12.5% ethanol / 12.5% Cremophor EL / 75% saline	1651	0.78	
6	Polymer A-Magptx	Sonication/dialysis (0.2 mg Magptx)	167	0.22	2.9
7	Polymer B-Magptx	Sonication/dialysis	214 194 (after centrifugation)	0.12 0.12	16.8
8	Polymer B1-Magptx	Thin film/hydration	354	0.2	20.0
9	Polymer C-Magptx	Sonication/dialysis	467 (before centrifugation)	0.20	5.0
10	Polymer C-Magptx	Sonication/dialysis	138 (after centrifugation)	0.74	4.0
11	Polymer C1-Magptx	Thin film/hydation	460	0.22	20.0
12	Polymer D-Magptx	Sonication/dialysis	686 (before centrifugation)	0.12	5.5
13	Polymer D-Magptx	Sonication/dialysis	297 (after centrifugation)	0.42	3.1
14	Polymer D1-Magptx	Thin film/hydation	487	0.25	20.0

In vitro cytotoxicity of Magptx and Magptx-loaded nanoparticles against the BT-474 cell line was investigated, wherein the untreated cell line was used as a control. As shown in

Figures 1A – 1C, and Table 3, the IC_{50} value of Magptx-loaded nanoparticles B was slightly higher than that of free Magptx. The Magptx-loaded nanoparticles B1 and C had higher cytotoxicity and higher anti-proliferation ability than free Magptx. The greater inhibitory effect of Magptx-loaded nanoparticles over free Magptx was possibly due to greater cellular uptake of Magptx-loaded nanoparticles.

Cytotoxicity of Magptx against BT-474 cells in the presence of external magnetic field over 72 hour treatment was studied. The presence of magnetic field enhanced the cytotoxicity of all formulations at certain concentrations (Figures 1A – 1C and Table 3). Table 3 shows the IC_{50} values of Magptx-loaded nanoparticles with equal concentration of Magptx. The Magptx-loaded nanoparticles C was prepared using the sonication/dialysis method. A possible reason might be that both free drug and drug-loaded nanoparticles were sensitive to external magnetic field, leading to greater cellular uptake.

Table 3. **IC_{50} (mg/L) of Magptx and Magptx-loaded nanoparticles.**

IC_{50} (mg/L)	**Magptx**	**Magptx-loaded nanoparticles B**	**Magptx-loaded nanoparticles B1**	**Magptx-loaded nanoparticles C**
No Magnetic Field	5.0	8.0	2.5	1.5
Strong Magnetic Field (3 Magnet Bar)	2.5	7.0	2.1	1.0
Magnetic Field (1 magnet bar)	2.6	-	2.3	-

The *in vitro* cellular data shows that Magptx loaded nanoparticles as described herein are effective in delivering the therapeutic agents to the tumour cells.

The nanoparticles, in particular with a therapeutic agent, may be used in medicine, as a method of treatment, or the manufacture of a medicament for the treatment of a disease, in particular cancer. The disease to be treated may be any disease that the therapeutic agent is known to be able to treat. For example, some indications (i.e. clinical use) of paclitaxel include ovarian cancer, breast cancer, lung cancer (non-small cell lung

cancer), Kaposi sarcoma, cervical cancer, and pancreatic cancer. These indications may be as a first or second line treatment, and may be in combination with other drugs. In particular, the disease may be breast cancer.

The nanoparticles loaded with the therapeutic agent attached to the magnetic moiety demonstrates a synergistic effect. The therapeutic agent may be encapsulated, surface conjugated, or adsorbed on the diblock polymer. The size of the nanoparticles allows it to exploit the enhanced permeability and retention effect in tumour tissues through the leaky blood vessels in the tumour. This allows the transportation and accumulation of the nanoparticles, and hence therapeutic agent in the tumour (i.e. the nanoparticles serve as a drug delivery system). The use of magnetic fields enhances the tumour targeting capacity of the nanoparticles by directing the therapeutic agent bonded to the magnetic moiety. In addition, the nanoparticles potentially overcome the possible cell permeability and pharmacokinetic problems of Magptx. By directing and concentrating the therapeutic agent in the tumour, the effect on healthy normal cells and corresponding side effects due to the therapeutic agent may be reduced. The dosage of the therapeutic agent may be reduced due to higher bioavailability, and lower loss through the action on non-targeted cells.

In addition, the use of the nanoparticles may avoid the use of other excipients like Cremophor EL, thereby reducing the side effects due to the excipient, while maintaining or increasing the bioavailability of the therapeutic agent, in particular for the taxane class drugs like paclitaxel, docetaxel and cabazitaxel.

In particular, it is possible that the aromatic moiety with two adjacent hydroxyl groups may interact with the magnetic moiety attached to the therapeutic agent, for example Magptx, leading to a higher and more efficient loading of the therapeutic agent in the nanoparticle. The interaction may be one or more of the following: van der Waals, π-π stacking, for example between the aromatic moiety and the metal-salen complex, and hydrogen-bonding interactions. Therefore, by varying the substitution pattern on the aromatic moiety, it may be possible to modulate the interaction between the nanoparticle and the therapeutic agent, in particular with an attached magnetic moiety.

The non-covalent nature of the interaction possibly allows for the therapeutic agent to be released in a controlled manner over a period of time. This may allow different dosing regimens to be provided to patients without requiring strict patient compliance beyond the initial administration.

The present invention allows for more selective targeting of, and accumulation of the therapeutic agent in, specific cancer cells. This may possibly reduce the required dosage and side effects of the therapeutic agent and pharmaceutical composition to be administered.

Examples

Materials

Unless, specifically mentioned, all materials were purchased from Sigma-Aldrich or TCI and all other solvents were of analytical grade, purchased from Fisher Scientific or J. T.Baker and used as received. Macroinitiator, mPEG-OH (Mn of 5 kDa) was purchased from RAAP Polymere GmbH (Tuebingen, Germany). Trimethylene carbonate (TMC) was purchased from Boehringer Ingelheim (Ingelheim, Germany), and dried extensively by freeze-drying under high vacuum. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide used in MTT assay was purchased from Promega, U.S.A. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was distilled from CaH_2 under dry N_2 and transferred to a glove box. *N*-(3,5-Trifluoromethyl)phenyl-*N*'-cyclohexylthiourea (TU) catalyst was prepared as described in the literature (*Macromolecules* 2006**,** 39, 7863-7871). Before transferring into the glove box, monomers and other reagents (like mPEG-OH) were dried extensively by freeze-drying under high vacuum. BT-474 cell line was obtained from ATCC, U.S.A. RPMI-1460 was used to culture the cell, which was supplemented with 10% fetal bovine serum and 1% antibiotic penicillin. Paclitaxel attached to the iron-salen complex (Magptx) was prepared according to US patent number 9,505,732.

The measurement for Mn values (dynamic laser scattering method) is calculated based on the NMR results and PDI is measured by GPC. The particle size and zeta potential of the micelles in PBS were measured by Zetasizer (Malvern Instrument Ltd., Malvern, UK) equipped with a He-Ne laser beam at 658 nm (scattering angle: 90°). Each measurement was repeated 3 times. The size measurements were performed by multimodel analysis.

Polymer Synthesis

Example 1: Polymer A synthesis

In a 7 mL vial containing a magnetic stir bar, in a glovebox, mPEG-OH (5 kDa, 185 mg, 37.0 µmol, 1.0 equiv.), TMC (1.29 g, 12.6 mmol, 341.7 equiv.), and TU (165 mg, 446 µmol, 12.0 equiv.) were dissolved in dichloromethane (5.0 mL). To this solution, DBU (64.5 µL, 165.7 mg, 432 µmol, 11.7 equiv.) was added to initiate polymerization. The reaction mixture was allowed to stir at room temperature. After 7 h and 45 min, the reaction was quenched by the addition of about 70 mg of benzoic acid, and the reaction mixture was precipitated twice into ice-cold diethyl ether : hexane mixture (80:20 mixture; 2 × 50 mL). Polymer A [PEG-P(TMC)] was dried in a tared vial for about 1−2 days until a constant sample mass was obtained as a white powder (1.14 g).

$M_n^{\text{SEC, PS Calib}}$ = 24560 Da; Đ = 1.39; M_n^{NMR} = 27750 Da; DP_n^{NMR} TMC = 223. ^{1}H NMR (400 MHz, CDCl3, δ, ppm): 4.15 - 4.35 (t, 891H, CH_2CH$_2$CH_2OCOO), 3.75 - 3.55 (s, 451H, CH_2CH_2O of mPEG), 3.38 (s, 3H, CH_3 of mPEG), 2.12–1.98 (quintet, 449 H, CH$_2$CH_2CH$_2$OCOO) (Figure 2).

Example 2: Polymer B synthesis

Synthesis of 2-hydroxyethyl 3,4-bis(benzyloxy)benzoate

The first step involves mixing 3,4-dihydroxy benzoic acid (5 g, 32.44 mmol), benzyl bromide (15.41 mL, 129.76 mmol), and potassium carbonate (17.93 g, 129.73 mmol) in 100 mL of acetonitrile (MeCN or ACN) under reflux for 18 h. The reaction solution was cooled to room temperature and the salts were filtered out. The solution was concentrated down to about 20 mL and diluted with 100 mL of hexane. A solid was formed which was removed by filtration. The solid was recrystallized out of tetrahydrofuran (THF)/Hexane to yield a pure product (7.88 g, 57.3% yield). ^{1}H NMR (400

MHz, $CDCl_3$, 22 °C): δ 7.70-7.68 (m, 2H, -COOCC*H*-), 7.47-7.28 (m, 15H, -$CH_2C_6H_5$), 6.96-6.93 (m, 1H, -COOCCHCC*H*C-), 5.34 (s, 2H, -COOC$H_2C_6H_5$), 5.25-5.21 (d, *J* = 20 Hz, 4H, -$C_2COCH_2C_6H_5$).

In the second step, benzyl protected 3,4-dihydroxy benzoic acid (4 g, 9.43 mmol) was suspended in ethylene glycol (50 mL) and the solution was heated to 80 °C. A catalytic amount of KOH (0.106 g, 1.86 mmol) was added and the solution was left to react until all solids were dissolved (typically 8 h – 24 h). The solution was precipitated into cold water (500 mL) and left to stir for several hours, during which a white solid was formed. The solid was filtered out, rinsed with water and subsequently lyophilized to remove the remaining water to give the product (2.81 g, 78.71% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 22 ºC): δ 7.69-7.66 (m, 2H, -COOCC*H*-), 7.45-7.28 (m, 10H, -$CH_2C_6H_5$), 6.97-6.95 (m, 1H, -COOCCHCC*H*C-), 5.26-5.23 (d, *J* = 12 Hz, 4H, -$C_2COCH_2C_6H_5$), 4.45-4.43 (m, 2H, -COOCH_2CH_2-), 3.95 (s, 2H, -$COOCH_2CH_2OH$).

Synthesis of MTC-ProtCat monomer.

In a dry two-neck 500 mL round bottom flask equipped with a stir bar, 5-methyl-2-oxo-1,3-dioxane-5-carboxylic acid (MTC-OH) (3.08 g, 19.3 mmol) was first dissolved in dry THF (50 mL) with 5-8 drops of dimethylformamide (DMF). Subsequently, oxalyl chloride (3.3 mL) was added in one shot (pure form), followed by an additional 20 mL of THF. The solution was stirred for 90 min, after which volatiles were removed under a strong flow of nitrogen to yield a pale yellow solid intermediate (5-chlorocarboxy-5-methyl-1,3-dioxan-2-one, MTC-Cl). The solid was subjected to heat at 60 °C for 2-3 min for the removal of residual solvent, and re-dissolved in dry dichloromethane (CH_2Cl_2, 50 mL), followed by immersing the flask in an ice bath at 0 °C. A mixture of 2-hydroxyethyl 3,4-bis(benzyloxy)benzoate (4.19 g, 17.88 mmol) and triethylamine (1.77 mL, 19.3 mmol) were dissolved in dry CH_2Cl_2 (50 mL), and added dropwise to the flask containing MTC-Cl over a duration of 30 min, and allowed to stir at room temperature (~ 22 °C) for an additional 2.5 h immediately after complete addition. The reacted mixture was quenched by addition of 50 mL of brine, and the organic solvent was collected after separation. After removal of solvent, the crude product was purified by silica-gel flash column chromatography *via* a dichloromethane-ethyl acetate solvent system (gradient elution up

to 20% vol. ethyl acetate) to yield MTC-ProtCat as a white solid. The crude product was further purified by recrystallization. The solid was dissolved in 1 ml of diethyl ether and 1 mL of ethyl acetate, followed by addition of 50 mL of hexane. The crystals were allowed to form at room temperature for 1 day, and are subsequently obtained by washing the crystals with cold hexane. ^{1}H NMR (400 MHz, $CDCl_3$, 22 °C): δ 7.65-7.63 (m, 2H, -COOCC*H*-), 7.46-7.28 (m, 10H, -$CH_2C_6H_5$), 6.98-6.96 (m, 1H, - $COOCH_2CH$-), 5.26-5.23 (d, *J* = 12 Hz, 4H, -$C_2COCH_2C_6H_5$), 4.70-4.67 (m, 2H, -$COOCH_2CH_2$-), 4.54 (m, 4H, $COOCH_2CC2$-), 4.21-4.18 (m, 2H, -$COOCH_2CH_2OH$), 1.32 (s, 3H, -CH_3).

Synthesis of PEG-P(MTC-Cat)

In a glove-box, 0.556 g (0.056 mmol) of 10 kDa MPEG-OH initiator and 0.376 g (1 mmol) of MTC-ProtCat were charged in a 20 mL glass vial equipped with a stir bar. Dichloromethane was added and the monomer concentration was adjusted to 2 M. Once the initiator and monomer were completely dissolved, 8.3 µL (0.06 mmol) of DBU was added to initiate the polymerization. After 1 h of stirring at room temperature, the reaction was quenched with 30 mg of benzoic acid. Subsequently, the polymer intermediate was purified *via* precipitation twice in cold diethyl ether, and was dried on a vacuum line until a constant weight was achieved.

The protected polymer was deprotected by hydrogenation. It was first dissolved in 14 mL of methanol and THF (1:1), followed by addition of 2 spatulas of Pd/C into a 50 ml glass vial. The glass vial was placed under hydrogen at room temperature with overnight stirring. After which, the polymer was filtered using THF/methanol (1:1) solvent mixture, and the collected polymer was dried under vacuum, followed by precipitating in cold diethyl ether twice.

Finally, the solvents were removed and the polymer was lyophilized to obtain an off-white polymer B [PEG-P(MTC-Cat)] (0.35g, 80% yield, PDI: 1.25). ^{1}H NMR (400 MHz, $CDCl_3$, 22 °C): δ 7.34-7.26 (m, 26H, -$COOC_2H_2$-), 6.83-6.72 (m, 13H, -$COOCH_2CH$-), 4.40-4.28 (m,

52H, -OC$_2$*H*$_4$O-), 4.23-4.05 (m, 52H, -COOC*H*$_2$-), 3.96-3.47 (-OC*H*$_2$C*H*$_2$- from 10kDa PEG), 3.23 (s, 3H, C*H*$_3$-PEG-), 1.15-1.03 (m, 39H, -C*H*$_3$)

(Figure 3).

Example 3: Polymer C synthesis

Synthesis of benzyl 2-(3,4-bis(benzyloxy)phenyl)acetate

3,4-Dihydroxyphenylacetic acid (10 g, 65 mmol), benzyl bromide (30.88 mL, 260 mmol), and potassium carbonate (35.86 g, 260 mmol) in 200 mL acetonitrile were refluxed for 16 hrs. The reaction solution was cooled to room temperature and the solvent was removed under vacuum. Ethyl acetate (300 mL), hexanes (300 mL), and water (100 mL) were added to the residue. The organic layer was retained, washed with water (100 mL) twice, dried over $MgSO_4$ and evaporated. The resulting solid was recrystallized in THF/Hexane to yield a pure product (19.28 g, 74.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 22 °C): δ 7.36 (m, 15H, H of -OCH$_2$Ph*H*), 6.89 (m, 2H, H of phenylacetate), 6.78 (m, 1H, H of phenylacetate), 5.11 (m, 6H, -OC*H*$_2$PhH), 3.56 (s, 2H, H of acetate).

Synthesis of 2-hydroxyethyl 2-(3,4-bis(benzyloxy)phenyl)acetate

Benzyl 2-(3,4-bis(benzyloxy)phenyl)acetate (9.64 g, 220 mmol) was suspended in ethylene glycol (120 mL) and the solution was heated to 85 °C. A catalytic amount of KOH (0.26 g, 4.64 mmol) was added and the solution was left to react until all solids were dissolved (typically 8 h to 24 h). The solution was poured into cold water (500 mL) and left to stir for several hours to form a yellowish sticky liquid. The liquid was centrifuged and washed with deionised (DI) water for three times. Finally, the liquid was freeze dried to remove the remaining water (6.73 g, 78.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 22 °C): δ 7.37 (m, 10H, H of -OCH$_2$Ph*H*), 6.90 (m, 2H, H of phenylacetate), 6.79 (m, 1H, H of phenylacetate), 5.15 (m, 4H, -OC*H*$_2$PhH), 4.18 (t, 2H, H of HOC*H*$_2$CH$_2$OC(O)-), 3.79 (t, 2H, H of HOCH$_2$C*H*$_2$OC(O)-), 3.56 (s, 2H, H of acetate).

Synthesis of 2-(2-(4-(benzyloxy)-3-hydroxyphenyl)acetoxy)ethyl 5-methyl-2-oxo- 1,3-dioxane-5-carboxylate (MTC-ethylCatechol)

In a dry three-neck round bottom flask equipped with a stir bar, MTC-OH (3 g, 18.64 mmol) was dissolved in dry THF (50 mL) with several drops of dry dimethylformamide (DMF). A solution of oxalyl chloride (1.77 mL, 20.62 mmol) in THF (50 mL) was subsequently added from a dropping funnel. Under N_2 gas atmosphere, the solution was stirred for 1 h, after which volatiles were removed under vacuum, yielding an off-white solid (i.e. 5-chlorocarboxy-5-methyl- 1,3-dioxan-2-one, MTC-Cl intermediate). The solid was heated to 62 °C for 2-3 min to remove any residual solvent, re-dissolved in dry CH_2Cl_2 (50 mL) and cooled down to 0 ºC via an ice bath. A mixture of 2-hydroxyethyl 2-(3,4-bis(benzyloxy) phenyl)acetate (6.73 g, 17.17 mmol) and triethylamine (3.12 mL, 22.38 mmol) dissolved in dry CH_2Cl_2 (50 mL) was added dropwise over a duration of 30 min, before the reaction was heated to 42 ºC and stirred overnight. The reaction solution was concentrated to a small volume (about 10 mL) and precipitated with 200 mL of dry THF. After filtering the precipitant (triethylamine salt), the solvent was removed and the crude product was subjected to purification by flash column chromatography using silica gel and a hexane-ethyl acetate solvent system as the eluent (gradient elution up to 80% vol. ethyl acetate) to yield MTC-ethylCatechol as a yellowish sticky liquid (6.14g, 80.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 22 °C): δ 7.36 (m, 10H, H of -OCH$_2$Ph*H*), 6.91 (m, 2H, H of phenylacetate), 6.78 (m, 1H, H of phenylacetate), 5.15 (d, 4H, -OC*H*$_2$PhH), 4.32 (dd, 4H, H of HOC*H*$_2$C*H*$_2$OC(O)-), 4.30 (dd, 4H, H of -OC*H*$_2$OCOO-), 3.54 (s, 2H, H of acetate), 1.18 (s, 3H, -C*H*$_3$).

General procedure for synthesis of PEG/Catechol-functionalized Polycarbonate Diblock Copolymers C

The detailed procedures for the ring-opening polymerization (ROP) of MTC-ethylCatechol with mPEG (M_n 5,000 Da) as a macroinitiator are given as a representative example. Using a glove box, mPEG (0.2 g, 0.04 mmol) was added to a reaction vial containing MTC-ethylCatechol (0.32 mg, 0.6 mmol) and DBU (9 µL, 0.06 mmol) dissolved in dry DCM (2 mL) and left to stir for about 3hrs. The reaction was stopped by quenching the catalyst using an excess of benzoic acid (10 mg, 0.08 mmol). The crude polymer was precipitated in diethyl ether, washed with diethyl ether for three times and dried under high vacuum, giving mPEG5K-P(MTC-ethylCatechol-Benzyl) as an off-white solid (0.34g, 83% yield). ^{1}H-NMR (400 MHz, $CDCl_3$, 22° C): δ 7.35 (m, 100H, H of -OCH$_2$Ph*H*), 6.87 (m, 20H, H of

phenylacetate), 6.75 (s, 10H, H of phenylacetate), 5.10 (s, 40H, -OCH_2PhH), 4.22 (m, 80H, H of HOCH_2CH_2OC(O)- and -OCH_2OCOO-), 3.64 (s, 455H, H of mPEG), 3.47 (s, 20H, H of acetate), 1.11 (s, 30H, -CH_3).

Deprotection of benzyl groups

The mixture of MPEG5K-P(MTC-ethylCatechol-Benzyl), MeOH (7.5 mL), THF (7.5 mL), and Pd−C (10%, w/w, 0.2 g) was swirled under H_2 (7 atm) overnight. After evacuation of H_2, the mixture was filtered using a syringe, and the filtrate was concentrated to dryness. The crude product was dissolved in 2 mL of DCM, precipitated in diethyl ether, centrifuged and washed with diethyl ether for three times before drying *in vacuo*. The yield of polymer C was more than 90% and ^{1}H-NMR (400 MHz, DMSO-d_6, 22° C): δ 8.83 (s, br, 14H, H of -PhO*H*), 6.62 (t, 20H, H of -Ph*H*), 6.47 (d, 10H, H of -Ph*H*), 4.20 (s, 80H, H of HOCH_2CH_2OC(O)- and -OCH_2OCOO-), 3.50 (s, 455H, H of mPEG), 3.41 (s, 20H, H of acetate), 1.11 (s, 30H, -CH_3).

Example 4: Polymer D synthesis

Polymer D was prepared as per Example 3 with double the amount of MTC-ethylCatechol. Polymer D, ^{1}H-NMR (400 MHz, DMSO-d_6, 22° C): δ 8.83 (s, br, 38H, H of -PhO*H*), 6.62 (t, 40H, H of -Ph*H*), 6.46 (d, 20H, H of -Ph*H*), 4.20 (s, 160H, H of HOCH_2CH_2OC(O)- and -OCH_2OCOO-), 3.50 (s,455H, H of mPEG), 3.41 (s, 40H, H of acetate), 1.11 (s, 60H, -CH_3).

The amount of MTC-ethylCatechol monomer used to prepare polymer D is double compared to polymer C, but more DBU was used to achieve a complete polymerization during the synthesis of Polymer D depending on the exploration of the reaction conditions. Specifically, for the synthesis of Polymer C, the molar ratio of MPEG:MTC-ethylcatechol:DBU in the feed is 1:15:1.5; meanwhile, the molar in the feed for the synthesis of Polymer D is 1:30:4.5.

Preparation and characterisation of Magptx-loaded nanoparticles

The Magptx-loaded nanoparticles may be prepared by two possible methods – a sonication/dialysis method and a thin/film hydration method.

Sonication/dialysis method

Dimethyl sulfoxide (DMSO, 10 mL) was added to 10.0 mg of Magptx drug, and the mixture was sonicated for at least two days to fully dissolve the drug, forming a clear solution without any precipitation. 10.0 mg of the polymer was weighed in a separate glass vial, which was then sonicated to dissolve in 5 ml of Magptx-DMSO solution. Then, 10, 5, or 1.5 mL of de-ionized (DI) water was prepared in an ice bath for probe sonication, during which the polymer-Magptx-DMSO solution was added drop by drop. After complete addition, the mixture was left for an extra two minutes of sonication. The solution underwent two days of dialysis using a 3.5 kD dialysis membrane in 1 L of DI water, which was renewed twice a day. It was shown an increased in particle size and size distribution with increasing volume of DI water used.

To determine the concentration of the micelle (nanoparticles) solution, 100 µL of the solution was taken and diluted 10 times using DMSO. Two replicate of 100 µL DI water and 900 µL DMSO mixture were also prepared as baseline. The UV-vis absorbance was then measured using a UV-vis NIR spectrophotometer (Shimadzu, Japan) at the wavelength from 200 nm to 500 nm. The concentration of the nanoparticles solution could be calculated from the standard curve plotted at the particular wavelength of 331 nm. Magptx in DMSO was used as standard, Magptx solution with 200 µg/mL, then diluted sequentially using DMSO, the concentrations ranged from 0.625 to 10 µg/mL

Thin film/hydration method

Briefly, 10 mg of polymer B and 2 mg of the Magptx were dissolved in 5 mL of ethanol via ultrasonication, respectively. The two solutions were mixed and further sonicated for 2 hours. The ethanol was evaporated under reduced pressure using a rotatory evaporator at 35 °C and the trace amount of ethanol was removed via vacuum drying to form a thin film. This was followed by addition of 5 mL of saline to the thin film, and sonicated for 3 hours.

The size of the micelle was measured using Zetasizer (Malven Instruments Zetasizer Nano ZS, UK).

Drug loading

The UV-vis NIR spectrophotometer was used to determine the amount of Magptx drug encapsulated in the nanoparticle. The amount of Magptx encapsulated can be obtained by measuring the concentration of Magptx in the nanoparticle suspension. The following equation was used to calculate drug encapsulation efficiency, i.e. drug loading level shown in Table 2.

The loading level could be calculated using the equation below:

$$MPTX\ loading\ level\ (\%) = (Actual\ amount\ of\ Magptx\ encapsulated) / (final\ weight\ of\ the\ Magptx - loaded\ micelles) \times 100\%$$

In vitro cytotoxicity test using MTT assay

BT-474 cells were seeded on a 96-well plate at a cell density of 100k cells per ml of RPMI-1640 cell culture medium (with 10% fetal bovine serum and 1% antibiotic penicillin), which is equivalent to 10k cells per well. The seeded plate was placed in an incubator (37°C and 5% CO_2) for one day to let the cells attach to the bottom of the plate. The culture medium was removed, and 100 µl solutions of Magptx, or Magptx loaded nanoparticles, diluted to different concentrations with RPMI were added to each well. The plate was returned to the incubator for 72 h treatment, after which, the spent drug solution was removed from the wells. 10 µL of MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) solution was added to each well along with 90 µL of RPMI. It was left in the incubator for 4 hour for staining, during which the plate was wrapped with aluminium foil to prevent light exposure. The spent MTT solution was removed, and DMSO was used to refill the wells (150 µL per well). At the end of 10 minutes incubation, 100 µL of DMSO per well was transferred to a new 96-well plate. The absorbance was measured using a microplate reader (Safire 2, Tecan, Switzerland) at the wavelength of 570 nm (actual absorbance wavelength range 550 - 690 nm). The cell viability results were expressed as a percentage of the absorbance of the control cells, which was without any treatment.

Example 4

Figures 4 to 6 demonstrate that Magptx nanoparticles B1 showed enhanced antitumor efficacy as compared to free Magptx drug in the presence of magnet field. All formulations did not cause significant body weight loss after treatment. Notably, the percentage of cancer stem cells from mice treated with Magptx nanoparticles B1 significantly decreased the population of cancer stem cells in the tumors in the presence of magnetic field as compared to the control (N+ vs. M+, $p < 0.05$).

In vivo toxicity studies

LD_{50} was determined using female Balb/c mice (8 weeks old, 18-22 g). The Up-and-Down-Procedure described in OECD Guidelines for the Testing of Chemicals (OECD 425) was used to determine LD_{50}. Magptx were prepared at various concentrations in mixed solvents (0.9% saline: ethanol: cremophor = 99:0.5:0.5) with continuous sonication for 6 hours, Magptx were administered to mice via tail vein injection at various doses (i.e., 25, 50, 100, 125, 150, 175 and 200 mg/kg, 0.2 mL). Mortality was monitored for 14 days post treatment, and the maximum likelihood method was used to estimate LD_{50}.

In vivo antitumor activity

Female BALB/c nude mice at 6 weeks of age (body weight: 20-22 g) were provided by the Singapore Biological Research Center (BRC). All animal studies were adhered to protocols approved by the Institutional Animal Care and Use Committee (IACUC), BRC, Agency for Science, Technology and Research (A*STAR), Singapore. BT-474 cells (density of 5×10^5 per mouse) were suspended in 200 µL of a mixture solution of Matrigel and PBS (volume ratio: 50/50) and subcutaneously injected into the right flank of the mouse. Estradiol pellet (0.72 mg/pellet, 60 days release) was subcutaneously administered into each mouse one day before the inoculation. When the tumor volume reached approximately 100 mm^3 on the 14th day (recorded as day 0), the mice were randomly divided into 5 groups (8-10 mice per group): Group 1 for 0.9 % saline as control without Magnetic field, Group 2 for 0.9 % saline as control with magnetic field, Group 3 for Magptx free drug without Magnetic field,, Group 4 for Magptx free drug with Magnetic field, Group 5 for Magptx nanoparticles B1 without magnetic field, and Group 6 for Magptx nanoparticles B1 with magnetic field. The dose of Magptx in all formulations was 40 mg/kg. All

formulations were freshly prepared prior to the day of injection, and were given to the mice via tail vein injection at day 0, 4, 8 and 12. At the predetermined time points, mice were weighted and tumor size was measured with a Vernier caliper. The tumor volume was calculated using the following formula:

$$Tumor\ volume = \frac{1}{2}\ (Length \times Width^2)$$

It is noted that free Magptx were prepared at a concentration of 4000 µg/mL in mixed solvents (0.9% saline: ethanol: cremophor = 99:0.5:0.5) with continuous sonication for 6 hours, Magptx nanoparticles B1 were prepared using solvent evaporation/hydration method, Briefly, 10 mg of polymer B and 4 mg of the magptx were dissolved in 5 mL of ethanol via sonication, respectively. The two solutions were mixed and further sonicated for 2 hours. Ethanol was then evaporated under reduced pressure using a rotatory evaporator at 35 °C and the trace amount of ethanol was removed via vacuum dry. This was followed by addition of 1 mL 0.9% saline and sonicated for 3 hours. The magnetic field was introduced using a homemade jacket made of rubber gloves with a Neodymium Magnet (190mT) placed on the tumor site.

Analysis of cancer stem cells in tumor after treatment

At the end of the treatment, the tumors were collected from all groups and washed 3 times in PBS. After tumors were cut into 1 mm3 of small pieces, enzymatic digestion was performed by using collagenase III at 37°C for 2h. To obtain single cells, cell suspension was filtered through a 40 µm of cell strainer. Cells were washed 3 times with PBS, resuspended in RPMI-1640 medium with 2% FBS was used to dilute Hoechst 33342 dye to a concentration of 5 µl/ml with or without 100mM verapamil-HCL, which was then used to re-suspend the cell for 90 min at 37°C for staining purpose (1 ml per tube). Hoechst 33342 is a permeable DNA dye that can bind to A-T base pairs, but it cannot enter SP cells without the existence of verapamil, a calcium-channel blocker. The medium was then removed by centrifugation at 1000 rpm for 5 min, the cells were re-suspended in cold PBS with propidium iodide at a concentration of 1µg/ml (500 µl per tube) to exclude dead cells. The prepared samples were then analyzed using FACSDiva (Becton Dickinson, San

Jose, CA, U.S.A.) by using a dual-wavelength analysis (blue, 420-470 nm; red, 660-680 nm).

Statistics

The results are expressed as mean ± standard deviation. Standard deviation is indicated by the error bars. Student's t test was used to determine significance among the small groups. A value of $p < 0.05$ was considered to be statistically significant.

Results and discussion

LD50 of free Magptx were estimated to be 175 mg/Kg. In vivo antitumor activity and the effects of magnet-guided delivery of free Magptx and Magptx loaded nanoparticles were evaluated by i.v. injection in nude mice bearing BT-474 tumor, and the results were summarized in Figure 5. Treatment of free Magptx and Magptx nanoparticles B1 (40 mg/Kg) in the presence of magnetic field suppressed tumor growth significantly as compared to the control in the absence of magnetic field (M+ or N+ vs. C-, $p < 0.05$). Magptx nanoparticles B1 demonstrated enhanced antitumor efficacy in the presence of magnet field as compared to that in the absence of magnetic field (N+ vs N-, $p < 0.05$). Notably, Magptx nanoparticles B1 suppressed tumor growth more effectively as compared to free Magptx (N+ vs. M+, $p < 0.05$).

To evaluate the general toxicity of free Magptx and Magptx nanoparticles B1 formulations, the body weight of mice in each group was monitored over the treatment period (Figure 6). There are no significant body weight changes for all groups before and after treatment ($p > 0.05$).

To investigate the effect of free Magptx and Magptx nanoparticles B1 on cancer stem cells in vivo, the population of cancer stem cells recovered from the tumors at the end of the treatments was analyzed. As illustrated in Figure 7, the percentage of cancer stem cells from mice treated with Magptx nanoparticles B1 significantly decreased the population of cancer stem cells in the tumors in the presence of magnetic field as compared to the control ($p < 0.05$).

In conclusion, amphiphilic PEG/catechol-containing polycarbonate diblock copolymers have been synthesized, and Magptx was successfully encapsulated into nanosized particles at high loading content. In addition, Magptx-loaded nanoparticles A and B1 demonstrated strong inhibitory effect against BT-474 cells (higher than free Magptx). More importantly, Magptx-loaded nanoparticles exhibited enhanced in vitro cytotoxicity against BT-474 cells in the presence of magnetic field. When injected into BT-474 tumor-bearing nude mice, Magptx-loaded nanoparticles B1 exhibited enhanced antitumor efficacy in the presence of magnetic field. Notably, Magptx-loaded nanoparticles B1 demonstrated greater anti-tumor efficacy than free Magptx. Magptx-loaded nanoparticles B1 decreased the population of cancer stem cells in the tumors in the presence of magnetic field.

Other embodiments will be apparent to those skilled in the art from consideration of the specification and practice of the invention. Furthermore, certain terminology has been used for the purposes of descriptive clarity, and not to limit the disclosed embodiments of the invention. The embodiments and features described above should be considered exemplary, with the invention being defined by the appended claims.

Whilst there has been described in the foregoing description preferred embodiments of the present invention, it will be understood by those skilled in the technology concerned that many variations or modifications in details of design or construction may be made without departing from the present invention.

Claims

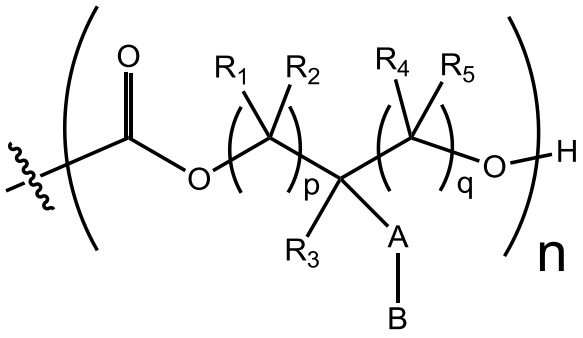
1. A nanoparticle comprising:

(a) a therapeutic agent; and

(b) a diblock polymer, the polymer comprising:

(i) a first block polymer comprising of a polyether repeating unit; and

(ii) a second block polymer comprising of a repeating unit of a general Formula I



(I),

wherein

(a) R_1, R_2, R_3, R_4, and R_5 is each independently selected from the group comprising: hydrogen, halogen, alkyl group comprising 1 to 20 carbon atoms, and aryl group comprising 6 to 20 carbon atoms,

(b) p and q is each independently an integer from 0 to 6, wherein p and q cannot both be 0,

(c) A is a linker, and

(d) B is an aromatic moiety with two adjacent hydroxyl groups.

2. The nanoparticle according to claim 1, wherein the diblock polymer has a number average molecular weight (M_n) of between 3 kDa to 40 kDa.

3. The nanoparticle according to any one of claims 1 or 2, wherein the first block polymer is a mono endcapped polyethylene glycol the endcapped polyethylene

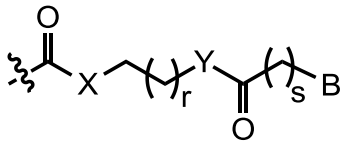
glycol is an alkyl group capped polyethylene glycol, wherein the alkyl group comprises 1 to 6 carbon atoms.

4. The nanoparticle according to any preceding claim, wherein the first block polymer has a number average molecular weight (M_n) of between 2 kDa to 10 kDa.

5. The nanoparticle according to any preceding claim, wherein a mean number of repeating units in the second block polymer is between 5 to 50.

6. The nanoparticle according to any preceding claim, wherein the linker is any one selected from the group comprising: alkyl group comprising 1 to 20 carbon atoms, aryl group comprising 1 to 20 carbon atoms, a ketone, an ester, an amide, a urea, a carbomate, and a carbonate.

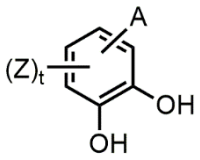
7. The nanoparticle according to claim 6, wherein the linking group has a general Formula (II)

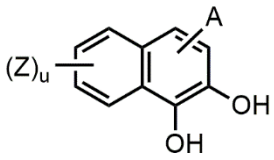


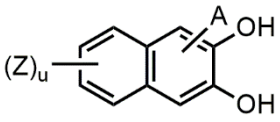
(II)

wherein r is an integer from 1 to 6, and s is an integer from 0 to 5, X and Y is each independently selected from the group comprising: O, NH, or NR_6; and
wherein R_6 is an alkyl group comprising 1 to 6 carbon atoms.

8. The nanoparticle according to any preceding claim, wherein the aromatic moiety is selected from the group comprising:



,


, and


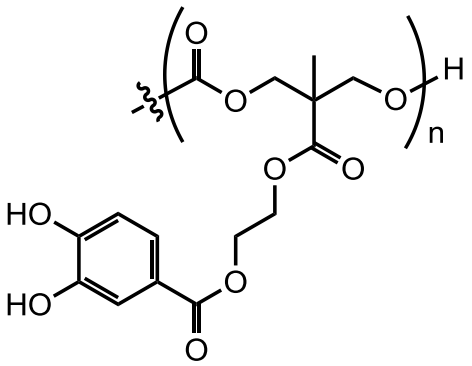
wherein Z is a hydrogen, an electron donating group, or an electron withdrawing group; and

wherein t is an integer from 0 to 3, u is an integer from 0 to 5.

9. The nanoparticle according to claim 8, wherein the electron donating group is selected from the group comprising: OR_7, NR_8R_9, and R_{10}, wherein R_7, R_8, R_9, and R_{10} are each independently selected from the group comprising: hydrogen, an alkyl comprising 1 to 20 carbon atoms, and aryl group comprising 1 to 20 carbon atoms.

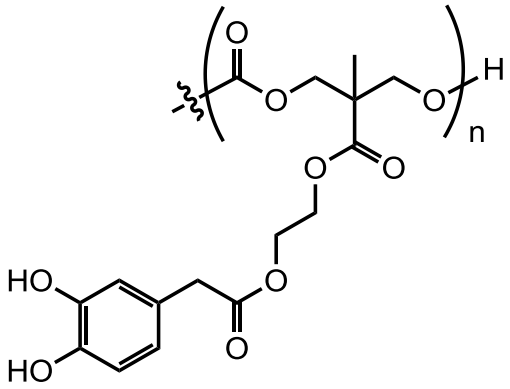
10. The nanoparticle according to claim 10, wherein the electron withdrawing group is selected from the group comprising: halogen, trihalogencarbon, carbonyl, nitrile, sulfonyl, and nitro.

11. The nanoparticle according to any preceding claims, wherein the repeating unit of the second polymer block is Polymer B having a general formula (III)



(III).

12. The nanoparticle according to any one of claims 1 to 10, wherein the repeating unit of the second polymer block is Polymer C or D having a general Formula (IV)

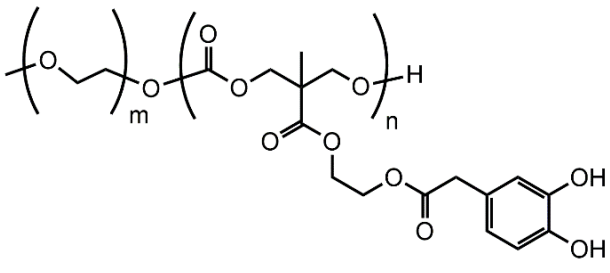


(IV).

13. The nanoparticle according to claim 12, wherein the mean number of repeating units (n) in the second block polymer is 10, or 20.

14. The nanoparticle according to any one of claims 11 to 13, wherein the nanoparticle has an average particle size of between 20 to 400 nm.

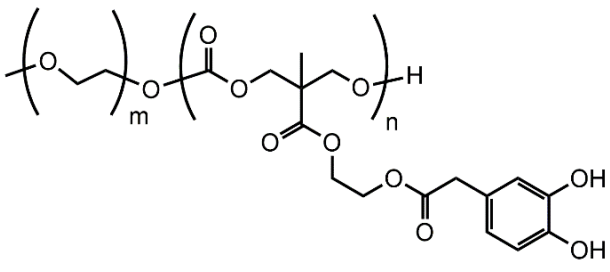
15. The nanoparticle according to claim 12, wherein Polymer C has a general Formula (VI)



(VI),

the mean number of repeating units (m) in the first block polymer is 113, and the mean number of repeating units (n) in the second block polymer is 10.

16. The nanoparticle according to claim 12, wherein Polymer D has a general Formula (VII)



(VII),

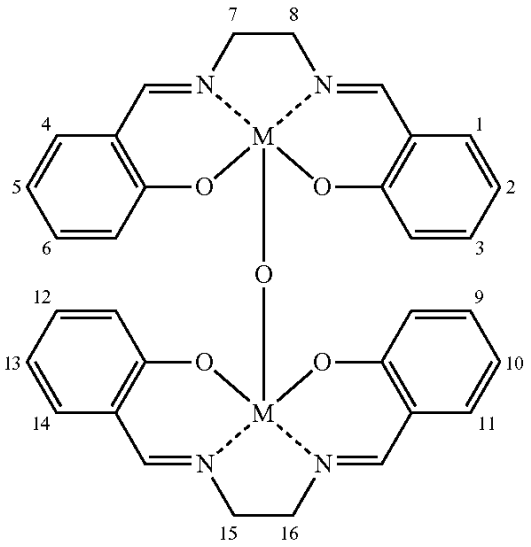
the mean number of repeating units (m) in the first block polymer is 113, and the mean number of repeating units (n) in the second block polymer is 20.

17. The nanoparticle according to any one of the preceding claims, wherein the first polymer block is a methoxy polyethylene glycol.

18. The nanoparticle according to any one of the preceding claims, wherein the therapeutic agent is selected from the group comprising: paclitaxel, docetaxel, and cabazitaxel.

19. The nanoparticle according to claim 18, wherein the therapeutic agent is attached to a magnetic moiety.

20. The claim according to claim 19, wherein the magnetic moiety has a general Formula (V):



(V),

wherein M is selected from Fe, Cr, Mn, Co, Ni, Mo, Ru, Rh, Pd, W, Re, Os, Ir, Pt, Nd, Sm, Eu, and Gd,

wherein the therapeutic agents are bonded to locations 5 and 10, or locations 2 and 13, and the therapeutic agents are independently selected from any one of the group comprising:

paclitaxel, docetaxel, cabazitaxel.

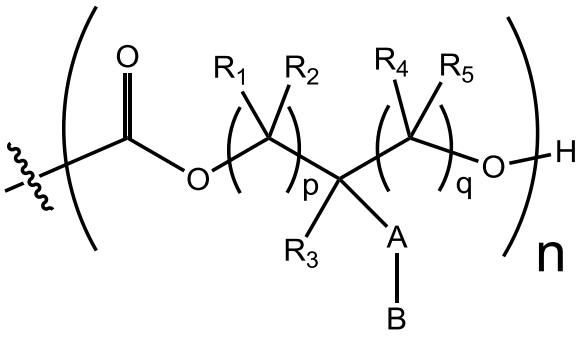
21. The nanoparticle according to any one of the preceding claims, comprising 1 to 20 weight of the therapeutic agent; and 80 to 99 weight percent of the diblock polymer.

22. The nanoparticle according to any one of the preceding claims, wherein the diblock polymer encapsulates the therapeutic agent.

23. A diblock polymer comprising:

(a) a first block polymer comprising of a polyether repeating unit; and

a second block polymer comprising of a repeating unit of a general formula I



(I),

wherein

(a) R_1, R_2, R_3, R_4, and R_5 is each independently selected from the group comprising: hydrogen, halogen, alkyl group comprising 1 to 20 carbon atoms, and aryl group comprising 6 to 20 carbon atoms,

(b) p and q is each independently an integer from 0 to 6, wherein p and q cannot both be 0,

(c) A is a linker, and

(d) B is an aromatic moiety with two adjacent hydroxyl groups.

24. The diblock polymer according to claim 23, wherein the diblock polymer has a number average molecular of between 3 kDa to 40 kDa.

25. The diblock polymer according to any one of claims 23 or 24, wherein the first block polymer is an endcapped polyethylene glycol, the endcapped polyethylene glycol is

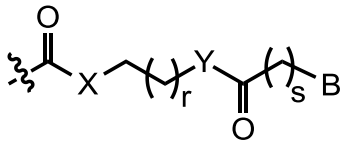
an alkyl group capped polyethylene glycol, wherein the alkyl group comprises 1 to 6 carbon atoms.

26. The diblock polymer according to any one of claims 23 to 25, wherein the first block polymer has a number average molecular weight (M_n) of between 2 kDa to 10 kDa.

27. The diblock polymer according to any one of claims 23 to 26, wherein a mean number of repeating units in the second block polymer is between 5 to 50.

28. The diblock polymer according to any one of claims 23 to 27, wherein the linker is any one selected from the group comprising: alkyl group comprising 1 to 20 carbon atoms, aryl group comprising 1 to 20 carbon atoms, a ketone, an ester, an amide, a urea, a carbomate, and a carbonate.

29. The diblock polymer according to claim 28, wherein the linking group is of a general Formula (II)

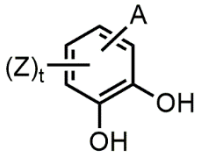


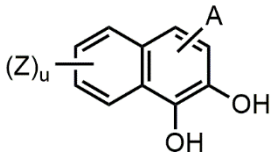
(II)

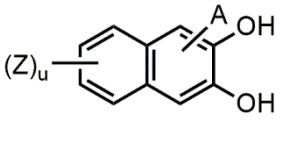
wherein r is an integer from 1 to 6, and s is an integer from 0 to 5, X and Y is each independently selected from the group comprising: O, NH, or NR_6,

wherein R_6 is an alkyl group comprising 1 to 6 carbon atoms.

30. The diblock polymer according to any one of claims 23 to 29, wherein the aromatic moiety is selected from the group comprising:



,


, and


,

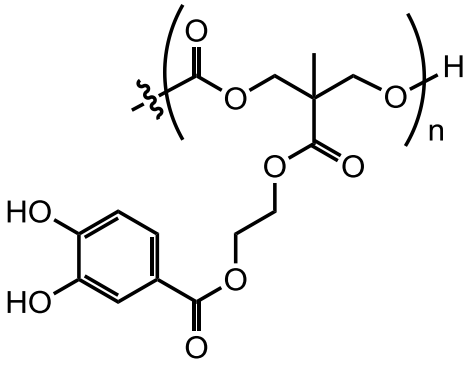
wherein Z is a hydrogen, an electron donating group, or an electron withdrawing group,

wherein t is an integer from 0 to 3, and u is an integer from 0 to 5.

31. The diblock polymer according to claim 30, wherein the electron donating group is selected from the group comprising: OR_7, NR_8R_9, and R_{10}, wherein R_7, R_8, R_9, and R_{10} are each independently selected from the group comprising: hydrogen, an alkyl comprising 1 to 20 carbon atoms, and aryl group comprising 1 to 20 carbon atoms.

32. The diblock polymer according to claim 30, wherein the electron withdrawing group is selected from the group comprising: a halogen, a trihalogencarbon, a carbonyl, a nitrile, a sulfonyl, and a nitro.

33. The diblock polymer according to any one of claims 23 to 32, wherein the repeating unit of the second polymer block is Polymer B having a general Formula (III)



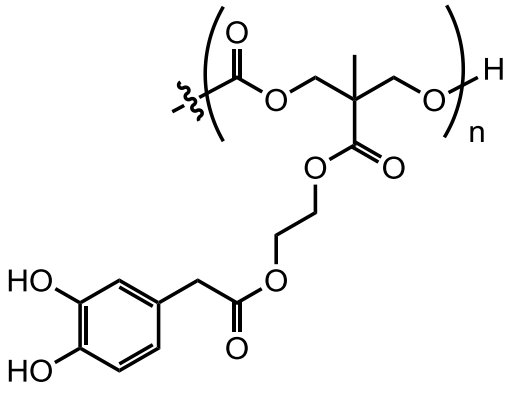
(III).

34. The diblock polymer according to claim 33, wherein the first polymer block is a methoxy polyethylene glycol with a number average molecular weight of 10 kDa.

35. The diblock polymer according to any one of claims 33 or 34, wherein the mean number of repeating units in the second block polymer is 15.

36. The diblock polymer according to any one of claim 33 to 35, wherein the diblock polymer has a number average molecular weight of 15 kDa.

37. The diblock polymer according to any one of claim 23 to 32, wherein the repeating unit of the second polymer block is Polymer C or D having a general Formula (IV)



(IV).

38. The diblock polymer according to claim 37, wherein the first polymer block is a methoxy polyethylene glycol with a number average molecular weight of 5 kDa.

39. The diblock polymer according to any one of claim 37 or 38, wherein the mean number of repeating units in the second block polymer is 10.

40. The diblock polymer according to any one of claim 37 to 39, wherein the block copolymer has a number average molecular weight of 8.5 kDa.

41. The diblock polymer according to claim 37, wherein the first polymer block is a methoxy polyethylene glycol with a number average molecular weight of 5 kDa.

42. The diblock polymer according to any one of claims 37 or 41, wherein the mean number of repeating units in the second block polymer is 20.

43. The diblock polymer according to any one of claim 23 to 42, further comprising a therapeutic agent.

44. The diblock polymer according to claim 43, wherein the therapeutic agent is selected from the group comprising: paclitaxel, docetaxel, and cabazitaxel.

45. The diblock polymer according to claim 44, wherein the therapeutic agent is attached to a magnetic moiety.

46. The diblock polymer according to any one of claims 43 to 45, wherein the therapeutic agent is encapsulated, surface conjugated or adsorbed on the diblock. polymer.

47. A method to prepare a therapeutic nanoparticle, the method comprising:

(a) dissolving a therapeutic agent and a diblock polymer according to any one of claims 23 to 46 in a water miscible solvent to form a solution; and

(b) forming the therapeutic agent with the diblock polymer to form the therapeutic nanoparticle.

48. The method according to claim 47, wherein dissolving the therapeutic agent and block copolymer is by sonication.

49. The method according to any one of claims 47 or 48, wherein the therapeutic agent is attached to a magnetic moiety.

50. The method according to any one of claims 47 to 49, wherein the therapeutic agent is selected from the group comprising: paclitaxel, docetaxel, and cabazitaxel.

51. The method according to any one of claims 47 to 50, wherein forming the therapeutic agent with the diblock polymer comprises adding the polymer/therapeutic agent solution to water under sonication.

52. The method according to any one of claims 47 to 50, further comprising dialysing the solution with a dialysis membrane to remove the solvent.

53. The method according to claim 51, wherein the ratio of the volume of the solution to water is 1: 0.3 to 2.

54. The method according to any one of claim 51, wherein adding water to the solution is performed at a temperature of between 0 °C to 10 °C.

55. The method according to any one of claims 47 to 54, wherein the water miscible solvent is dimethyl sulfoxide.

56. The method according to any one of claims 47 to 55, wherein forming the therapeutic agent with the diblock polymer comprises removing the water miscible solvent under low pressure conditions to form a thin film, adding water or saline solution to the thin film, and sonicating the mixture.

57. The method according to claim 56, wherein sonicating the mixture is performed for 3 hours.

58. The method according to any one of claims 56 or 57, wherein the water miscible solvent is an alcohol.

59. The method according to claim 58, wherein the alcohol is any one selected from the group comprising ethanol, methanol and isopropyl alcohol.

60. A drug delivery system comprising the diblock polymer according to any one of claims 23 to 46.

61. The system according to claim 60, further comprising one or more therapeutic agents.

62. The system according to claim 61, wherein the therapeutic agent is selected from the group comprising: paclitaxel, docetaxel, and cabazitaxel.

63. The system according to claim 62, wherein the therapeutic agent is attached to a magnetic moiety.

64. The nanoparticle according to any one of claims 1 to 22 or the diblock polymer 23 to 46 for use in medicine.

65. A method for treating disease, the method comprising administering the nanoparticle according to any one of claims 1 to 23 to a subject in need thereof.

66. Use of a nanoparticle according to any one of claims 1 to 23 in the manufacture of a medicament for the treatment of a disease.

67. The method according to claim 65 and the use according to claim 66, wherein the disease is breast cancer.