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(54) Title: DUAL FUNCTION POLYMER MICELLES

(57) Abstract: The invention relates to micelles that are elaborated with functionality useful for imaging and/or selectively targeting tissue, e.g., in the delivery of hydrophobic agents.

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Dual Function Polymer Micelles

Field of Invention

This invention relates to functionalized micelles suitable for use as delivery vehicles.

Background of the Invention

An important objective in cancer therapy is to selectively deliver therapeutic agents to the tumor tissue. Low water solubility, rapid phagocytic and renal clearance, and systemic toxicity represent three major barriers that limit the therapeutic use of many hydrophobic anti-tumor agents such as doxorubicin (DOX) and paclitaxel. To overcome these limitations, various drug delivery systems, among which polymeric micelles have emerged as one important class, have been developed for delivering various drugs with varying degrees of *in vitro* and *in vivo* success. The hydrophobic core of the micelles is a carrier compartment that accommodates anti-tumor drugs, and the outside surface of the micelle consists of a brush-like protective corona that stabilizes the nanoparticles in aqueous solution.

Polymeric micelles in drug delivery applications are typically characterized by high drug-loading capacity, biodegradability, long blood circulation, and controllable drug release profiles. Polymeric micelles from amphiphilic block copolymers are supramolecular core-shell-type assemblies of tens of nanometers in diameter, which can mimic naturally occurring biological transport systems such as lipoproteins and viruses. Recently, polymeric micelles as carriers of hydrophobic drugs have drawn increasing interest, due to their various advantages in drug delivery applications. First, polymeric micelles are highly stable in aqueous solution because of their intrinsic low critical micelle concentration (cmc), which prevents the drug-entrapped micelles from dissociation upon dilution in the blood stream after intravenous injection. Furthermore, the nanoscale size of polymeric micelles can facilitate their extravasations at tumor sites while avoiding renal clearance and non-specific reticuloendothelial (RES) uptake. The micelle cores are usually constructed with biodegradable polymers such as aliphatic polyesters and polypeptide, and water-soluble poly(ethylene glycol) is most frequently used to build the micelle

corona because it can effectively stabilize the nanoparticles in blood compartments and reduce the uptake at the reticuloendothelial sites (e.g. liver and spleen). By encapsulating drugs within the micelles, solubility limits for hydrophobic drugs can be exceeded.

Antitumor drugs, such as doxorubicin (DOX) and paclitaxel, are widely used in cancer chemotherapy. Besides their low water solubility, major drawbacks of these drugs are the acute toxicity to normal tissue and inherent multi-drug resistance effect. To reduce the acute toxicity of the free drugs and improve their therapeutic efficacy, various liposome and polymeric micelle systems were designed as delivery vehicles. Hydrophobic drugs can be incorporated into the micelle inner core by both chemical conjugation and physical entrapment, depending on the chemical structure of drugs. For instances, paclitaxel was encapsulated into micelle cores usually by physical entrapment driven by hydrophobic interactions between the drug and the hydrophobic components of polymers. In contrast, doxorubicin can also be chemically bound to the core of polymeric micelles through amidation of doxorubicin amino groups, yielding high loading content. By this way, an efficient doxorubicin delivery system based on doxorubicin-conjugated poly(ethylene glycol)-poly(aspartic acid) block copolymer (PEG-PAsp-(DOX)) has been developed. The conjugation with DOX converted the hydrophilic poly(aspartic acid) into hydrophobic blocks that formed the hydrophobic micelle core and physically entrapped free DOX as well. Recently, DOX conjugation to the micelle cores through an acid-cleavable linkage, such as a hydrazone bond, was reported to be an effective way to enhance the bioavailability of the chemically bound DOX. The hydrazone linkage was cleaved in the endosomes/lysosomes (pH around 5) to yield free DOX molecules which then functioned as the physically entrapped DOX. Compared to the chemical conjugation strategy, physical entrapment of drugs in the micelle cores may be advantageous in terms of easy polymer preparation, simple micelle fabrication, and enhanced drug bioavailability. Although several micellar systems based on non-ionic amphiphilic block polymers such as PEO-PPO-PEO and PEG-b-PBLA have been reported, physically entrapped DOX delivery with polymeric micelles based on the well-known block copolymers of poly(ethylene glycol) and biodegradable polyesters is still very limited. Research on micelles has

been greatly advanced; however, the ability to achieve high targeting efficiency at the tumor site and associated cells remains a significant challenge for the development of micelle-mediated drug delivery systems.

Summary of the Invention

The invention relates in part to micelles that are elaborated with functionality that enables the micelles to selectively target tumor tissue in the delivery of hydrophobic agents. Thus, one aspect of the invention is the elaboration of the outer surface (corona) of the micelle with various protein sequences that selectively bind to certain types of tissue. These functionalized micelles may further comprise a hydrophobic chemotherapeutic agent in the core of the micellar structure, such as doxorubicin (DOX) or paclitaxel. Alternatively or additionally, these micelles may further comprise a hydrophobic MRI imaging agent in the core of the micellar structure, such as superparamagnetic iron oxide (SPIO).

Another aspect of the invention is the incorporation of radioactive moieties onto the surface of the micellar structure to facilitate single photon emission computed tomography (SPECT), which provides information about the availability of radioisotopes with high sensitivity and the capability to simultaneously monitor multiple probes with different emission wavelengths. This technique may be useful in measuring the *in vivo* pharmacokinetics and tumor targeting efficiency of drug loaded micelles.

Brief Description of the Figures

Figure 1 shows the preparation of cRGD-DOX-micelles.

Figure 2a shows the ^{1}H NMR of MAL-PEG-PCL in $CDCl_3$.

Figure 2b shows an expanded ^{1}H NMR of MAL-PEG-PCL in $CDCl_3$.

Figure 3 shows the synthesis of cRGD using solid phase synthesis.

Figure 4a shows the size characterization of 0% cRGD-DOX micelles by atomic force microscopy.

Figure 4b shows the size characteristics of 0% cRGD-DOX micelles by dynamic light scattering.

Figure 4c shows the size characteristics of 76% cRGD-DOX micelles by atomic force microscopy.

Figure 4d shows the size characteristics of 76% cRGD-DOX micelles by dynamic light scattering.

Figure 5a shows the percentage of micelle uptake in SLK tumor endothelial cells measured by flow cytometry as a function of cRGD density on the micelle surface.

Figure 5b shows confocal laser scanning microscopy images of SLK cells treated with 0% cRGD after incubation for 2 h.

Figure 5c shows confocal laser scanning microscopy images of SLK cells treated with 16% cRGD after incubation for 2 h.

Figure 6a shows a flow cytometry histogram of micelle uptake in SLK tumor endothelial cells as a function of a cRGD density of 0% on the micelle surface.

Figure 6b shows a flow cytometry histogram 76% cRGD-micelles in the presence of free RGS ligands (9mM) in solution.

Figure 6c shows a flow cytometry histogram of micelle uptake in SLK tumor endothelial cells as a function of a cRGD density of 76% on the micelle surface.

Figure 7 shows the preparation of DOTA-PEG-b-PCL micelles.

Figure 8a shows a transmission electron micrograph of a typical micelle containing numerous individual SPIO particles.

Figure 8b shows a T_2-weighted MRI image of a control mouse tumor.

Figure 8c shows a T_2-weighted MRI image of a tumor 72 hours after micelle administration.

Detailed Description of the Invention

One strategy to achieve cancer-targeted drug delivery is the utilization of unique molecular markers that are specifically overexpressed in the cancerous tissues. It is well known that tumor endothelial cells show increased expression of several cell surface molecules that potentiate cell invasion and proliferation during tumor vascular remodeling and angiogenesis. Thus, one aspect of the invention is

the attachment of moieties to the corona of a polymeric micellar structure that can effectively bind to such molecular markers.

In certain embodiments, the micelles are formed from amphiphilic block copolymers. In preferred embodiments, the polymer is selected from poly-γ-benzyl-L-glutamate-polyethylene oxide (PBLG-PEO), poly(ethylene oxide)-poly(proplylene oxide) (PEO-PPO-PEO), poly(ethylene glycol)-poly(β-benzyl-L-aspartate) (PEG-b-PBLA), and poly(ε-caprolactone)-poly(ethylene glycol) (PCL-PEG). In most preferred embodiments, the polymer is PCL-PEG.

Micelles may be prepared by adding the copolymer and optionally any additional hydrophobic agent(s) to a hydrophobic solvent in which they are soluble (an organic solvent), the resulting solution being added to water under conditions of vigorous agitation, for example, by ultrasonication, shaking, or other suitable agitation as is well understood in the art. The organic solvent may then be removed by slow evaporation to promote the formation of micelles. The residual organic solvent may then be completely removed to provide the desired micelles in an aqueous medium.

In preferred such embodiments, micelles may contain a hydrophobic agent in the core, wherein a "hydrophobic agent" is an agent that is poorly soluble in water, such as an agent that is more soluble in octanol than it is soluble in water. Preferably, the hydrophobic agent is a chemotherapeutic drug. In certain such embodiments, the chemotherapeutic drug is selected from aminoglutethimide, amsacrine, anastrozole, asparaginase, bcg, bicalutamide, bleomycin, buserelin, busulfan, campothecin, capecitabine, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, dienestrol, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, estradiol, estramustine, etoposide, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ironotecan, letrozole, leucovorin, leuprolide, levamisole, lomustine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone,

nilutamide, nocodazole, octreotide, oxaliplatin, paclitaxel, pamidronate, pentostatin, plicamycin, porfimer, procarbazine, raltitrexed, rituximab, streptozocin, suramin, tamoxifen, temozolomide, teniposide, testosterone, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, and vinorelbine. In more preferred such embodiments, the chemotherapeutic drug is selected from paclitaxel and doxorubicin.

In certain embodiments, the micelles are functionalized with a targeting moiety. The term "targeting moiety" refers to any molecular structure which assists the micelle in localizing to a particular target area, entering a target cell(s), and/or binding to a target receptor. For example, lipids (including cationic, neutral, and steroidal lipids, virosomes, and liposomes), antibodies, lectins, ligands, sugars, steroids, hormones, nutrients, and proteins can serve as targeting moieties. A number of suitable targeting moieties are disclosed in U.S. Patent Application 2003-0049203, which is incorporated herein in its entirety.

Since tumor endothelial cells show increased expression of several cell surface molecules, the attachment of moieties to the corona of a polymeric micellar structure that can effectively bind to such molecular markers may serve as a way to target such tumor tissue. One molecular marker known to be overexpressed in cancerous tissue is $\alpha_v\beta_3$ integrin, which plays a key role in endothelial cell survival during angiogenesis (Griffioen A.W., Molema G. (2000) *Pharmacol. Rev.* 52 237–268). The $\alpha_v\beta_3$ integrin was found to be unregulated in these cells and can induce cell internalization (Brooks P.C. et al. (1994) *Cell.* 79, 1157-1164). In preferred embodiments, an $\alpha_v\beta_3$ ligand is bound to the corona of micelles. In certain embodiments, c(Arg-Gly-Asp-D-Phe-Lys) (c-RGD) is bound to the corona of micelles.

Examples of other suitable targeting moieties include but are not limited to lipoproteins, glycoproteins, asialoglycoproteins, transferrin, toxins, carbohydrates, cell surface receptor ligands, antibodies, and homing peptides. Synthetic homing peptides with the desired levels of affinity and/or selectivity for specific organs or tissues may be employed as targeting moieties, for example as disclosed in U.S. patents 6,576,239, 6,306,365, 6,303,573, 6,296,832, 6,232,287, 6,180,084,

6,174,687, 6,068,829, and 5,622,699, U.S. patent applications 2001/0046498, 2002/0041898, 2003/0008819, and 2003/0077826, and PCT application PCT/GB02/04017 (WO 03/020751), all of which are incorporated herein by reference.

Methods for identifying and using these and other tissue-homing peptides are known in the art, see for example W. Arap et al., *Science* **279**:377-380 (1998); R. Pasqualini, and E. Ruoslahti, *Nature* **380**:364-366 (1996); D. Rajotte et al., *J. Clin. Invest.* **102**:430-437 (1998); P. Laakkonen et al., *Nature Medicine* **8**(7):751-755 (2002); and K. Essler, E. Ruoslahti, *Proc. Natl. Acad. Sci. U.S.A.* **99**(4):2252-2257 (2002), all of which are hereby incorporated herein by reference in their entirety. Suitable tissue-specific homing peptides include, but are not limited to, the sequences in Table 1

Table 1

Brain:	
CLSSRLDAC	CVLRGGRC
CNSRLQLRC	CGVRLGC
CKDWGRIC	CLDWGRIC
CTRITESC	CETLPAC
CRTGTLFC	CGRSLDAC
CRHWFDVVC	CANAQSHC
CGNPSYRC	WRCVLREGPAGGCAWFNR HRL
YPCGGEAVAGVSSVRTMC SE	LNCDYQGTNPATSVSVPCTV
CNSRLHLRCCENWWGDVC	WRCVLREGPAGGCAWFNR HRL

Kidney:	
CLPVASC	CGAREMC
CKGRSSAC	CWARAQGC
CLGRSSVC	CTSPGGSC
CMGRWRLC	CVGECGGC
CVAWLNC	CRRFQDC
CLMGVHC	CKLLSGVC
CFVGHDLC	CRCLNVC
CKLMGEC	

Heart:	
GGGVFWQ	HGRVRPH
VVLVTSS	CLHRGNSC

CRSWNKADNRSC	
Gut:	
YAGFFLV	RSGARSS
CVESTVA	SRRQPLS
SKVWLLL	QVRRVPE
YSGKWGW	MVQSVG
LRAVGRA	MSPQLAT
GAVLPGE	WIEEAER
LVSEQLR	RGDRPPY
VRRGSPQ	RVRGPER
GISAVLS	GGRGSWE
GVSASDW	FRVRGSP
SRLSGGT	WELVARS
MRRDEQR	GCRCWA
LSPPYMW	LCTAMTE

Integrins:	
CRGDC	CRGDCL
CRGDCA	NGRAHA
DGRAHA	RCDVVV
SLIDIP	TIRSVD
KRGD	RRGD
RGDL	

RGD-binding determinants:	
CSFGRGDIRNC	CSFGRTDQRIC
CSFGKGDNRIC	CSFGRNDSRNC
CSFGRVDDRNC	CSFGRADRRNC
CSFGRSVDRNC	CSFGKRDMRNC
CSFGRWDARNC	CSFGRQDVRNC
CSFGRDDGRNC	

Angiogenic tumor endothelium:	
CDCRGDCFC	CNGRCVSGCAGRC

Ovary:	
EVRSRLS	RVGLVAR
AVKDYFR	GVRTSIW
RPVGMRK	RVRLVNL
FFAAVRS	KLVNSSW
LCERVWR	FGSQAFV
WLERPEY	GGDVMWR
VRARLMS	TLRESGP

Uterus:	

GLSGGRS	SWCEPGWCR

Prostate:	
EVQSAKW	KRVYVLG
GRLSVQV	WKPASLS
FAVRVVG	LVRPLEG
GFYRMLG	EGRPMVY
GSRSLGA	RVWQGDV
GDELLA	FVWLVGS
GSEPMFR	VSFLEYR
WHQPL	SMSIARL
RGRWLAL	QVEEFPC
LWLSGNW	GPMLSVM
WTFLERL	VLPGGQW
REVKES	RTPAAVM
GEWLGEC	PNPLMPL
SLWYLGA	YVGGWEL

Lung:	
CGFECVRQCPERC	CTLRDRNC
CIKGNVNC	CRHESSSC
CLYIDRRC	CYSLGADC
CSKLMMTC	CGFELETC
CNSDVDLC	CVGNLSMC
CEKKLLYC	CKGQRDFC
CTFRNASC	CNMGLTRC
CHEGYLTC	CGTFGARC
CIGEVEVC	CRISAHPC
CLRPYLNC	CSYPKILC
CMELSKQC	CSEPSGTC
CGNETLRC	CTLSNRFC
CMGSEYWC	CLFSDENC
CAHQHIQC	CKGQGDWC
CAQNMLCC	CWRGDRKIC
CLAKENVVC	CIFREANVC
CRTHGYQGC	CERVVGSSC
CKTNHMESC	CYEEKSQSC
CKDSAMTIC	CTRSTNTGC
CMSWDAVSC	CKWSRLHSC
CMSPQRSDC	CLHSPRSKC
CPQDIRRNC	CLYTKEQRC
CQTRNFAQC	CTGHLSTDC
CQDLNIMQC	TRRTNNPLT
CGYIDPNRISQC	CTVNEAYKTRMC
CRLRSYGTLSLC	CAGTCATGCNGVC
CADYDLALGLMC	CPKARPAPQYKC

CSSHQGGFQHGC	CQETRTEGRKKC
CRPWHNQAHTEC	CSFGTHDTEPHC
CSEAASRMIGVC	CWEEHPSIKWWC
CWDADQIEGIKC	CVDSQSMKGLVC
CRLQTMGQGQSC	CRPAQRDAGTSC
CGGRDRGTYGPC	CGEVASNERIQC
CNSKSSAELEKC	CVLNFKNQARDC
CRGKPLANFEDC	CEGHSMRGYGLC
CRDRGDRMKSLC	CDNTCTYGVDDC
CSAHSQEMNVNC	CGAACGVGCRGRC
CGFECVRQCPERC	CLVGCRLSCGGEC
CRSGCVEGCGGRC	CIARCGGACGRHC
CGGECGWECEVSC	CGVGCPGLCGGAC
CKWLCLLLCAVAC	CSEGCGPVCWPEC
CGAACGVGCGGRC	CSGSCRRGCGIDC
CGASCALGCRAYC	CDTSCENNCQGPC
CSRQCRGACGQPC	CYWWCDGVCALQC
CAGGCAVRCGGTC	CGGACGGVCTGGC
CGRPCVGECRMGC	CLVGCEVGCSPAC
CPRTCGAACASPC	CRGDCGIGCRRLC
CCFTNFDCYLGC	

Skin:	
CYADCEGTCGMVC	CWNICPGGCRALC
GPGCEEECQPAC	CKGTCVLGCSEEC
CSTLCGLRCMGTC	CMPRCGVNCKWAC
CVGACDLKCTGGC	CVALCREACGEGC
CSSGCSKNCLEMC	CGRPCRGGCAASC
CQGGCGVSCPIFC	CAVRCDGSCVPEC
CGFGCSGSCQMQC	CRVVCADGCRFIC
CTMGCTAGCAFAC	CEGKCGLTCECTC
CNQGCSGSCDVMC	CASGCSESCYVGC
CGGGCQWGCAGEC	CSVRCKSVCIGLC
CPSNCVALCTSGC	CVEGCSSGCGPGC
CRVVCADGCRLIC	CSTLCGLRCMGTC
CFTFCEYHCQLTC	

Retina:	
CRRIWYAVC	CSAYTTSPC
CSCFRDVCC	CTDKSWPC
CTDNRVGS	CTIADFPC
CTSDISWWDYKC	CTVDNELC
CVGDCIGSCWMFC	CVKFTYDC
CVSGHLNC	CYGESQQMC
CYTGETWTC	CAVSIPRC
CDCRGDCFC	CDSLCGGACAARC

CERSQSKGVHHC	CFKSTLLC
CFWHNRAC	CGDVCPSECPGWC
CGEFKVGC	CGLDCLGDCSGAC
CGPGYQAQCSLRC	CGSHCGQLCKSLC
CHMGCVSPCAYVC	CILSYDNPC
CISRPYFC	CKERLEYTRGVC
CKERPSNGLSAC	CKPFRTEC
CKSGCGVACRHMC	CLKPGGQEC
CMDSQSSC	CMNILSGC
CNIPVTTPIFGC	CNQRTNRESGNC
CNRKNSNEQRAC	CNRMEMPC
CQIRPIDKC	CAIDIGGAC
CGRFDTAPQRGC	CKRANRLSC
CLLNYTYC	CLNGLVSMC
CMSLGNNC	CNRNRMTPC
CQASASDHC	CQLINSSPC
CQRVNSVENASC	CRKEHYPC
CRRHMERC	CSGRPFKYC
CTHLVTLC	CTSSPAYNC
CVTSNLRVC	CWDSGSHIC
CERSHGRLC	CGNLLTRRC
CINCLSQC	CLRHDFYVC
CNSRSENC	CRYKGPSC
CSHHDTNC	CSRWYTTC
CYAGSPLC	CQTTSWNC
CQWSMNVC	CRARIRAEDISC
CRDVVSVIC	CRREYSAC

Pancreas:	
EICQLGSCT	WRCEGFNCQ
RKCLRPDCG	SWCEPGWCR
LACFVTGCL	GLCNGATCM
DMCWLIGCG	SGCRTMVCV
QRCPRSFCL	LSCAPVICG
RECTNEICY	NECLMISCR
SCVFCDWLS	WACEELSCF
QNCPVTRCV	CATLTNDEC
CDNREMSC	CFMDHSNC
CGEYGREC	CHMKRDRTC
CKKRLLNVC	CLDYHPKC
CMTGRVTC	CNKIVRRC
CPDLLVAC	CSDTQSIGC
CSKAYDLAC	CSKKGPSYC
CTLKHTAMC	CTQHIANC
CTTEIDYC	CVGRSGELC

Liver:	
ARRGWTL	SRRFVGG
QLTGGCL	ALERRSL
KAYFRWR	RWLAWTV
VGSFIYS	LSLLGIA
LSTVLWF	SLAMRDS
GRSSLAC	SELLGDA
CGGAGAR	WRQNMPL
DFLRCRV	QAGLRCH
RALYDAL	WVSVLGF
GMAVSSW	SWFFLVA
WQSVVRV	VKSVCRT
CGNGHSC	AEMEGRD
SLRPDNG	PAMGLIR

Lymph Node:	
WGCKLRFCS	MECIKYSCL
GICATVKCS	PRCQLWACT
TTCMSQLCL	SHCPMASLC
GCVRRLLCN	TSCRLFSCA
KYCTPVECL	RGCNGSRCS
MCPQRNCL	PECEGVSCI
AGCSVTVCG	IPCYWESCR
GSCSMFPCS	QDCVKRPCV
SECAYRACS	WSCARPLCG
SLCGSDGCR	RLCPSSPCT
MRCQFSGCT	RYCYPDGCL
STCGNWTCR	LPCTGASCP
CSCTGQLCR	LECRRWRCD
GLCQIDECR	TACKVAACH
DRCLDIWCL	XXXQGSPCL
PLCMATRCA	RDCSHRSCE
NPCLRAACI	PTCAYGWCA
LECVANLCT	RKCGEEVCT
EPCTWNACL	LVCPGTACV
LYCLDASCL	ERCPMAKCY
LVCQGSPCL	QQCQDPYCL
DXCXDIWCL	QPCRSMVCA
KTCVGVRV	WSCHEFNCR
LTCWDWSCR	SLCRLSTCS
KTCAGSSCI	VICTGRQCG
NPCFGLLV	SLCTAFNCH
RTCTPSRCM	QSCLWRICI
QYCWSKGCR	LGCFPSWCG
VTCSSEWCL	RLCSWGGCA
STCISVHCS	EVCLVLSCQ

IACDGYLCG	RDCVKNLCR
XGCYQKRCT	LGCFXSWCG
IRCWGGRCS	IPCSLLGCA
AGCVQSQCY	PRCWERVCS
KACFGADCX	TLCPLVACE
SACWLSNCA	SECYTGSCP
GLCQEHRCW	VECGFSAVF
EDCREWGCR	HWCRLLACR

Adrenal Gland:	
WGCKLRFCS	MECIKYSCL
GICATVKCS	PRCQLWACT
TTCMSQLCL	SHCPMASLC
GCVRRLLCN	TSCRLFSCA
KYCTPVECL	RGCNGSRCS
MCPQRNCL	PECEGVSCI
AGCSVTVCG	IPCYWESCR
GSCSMFPCS	QDCVKRPCV
SECAYRACS	WSCARPLCG
SLCGSDGCR	RLCPSSPCT
MRCQFSGCT	RYCYPDGCL
STCGNWTCR	LPCTGASCP
CSCTGQLCR	LECRRWRCD
GLCQIDECR	TACKVAACH
DRCLDIWCL	XXXQGSPCL
PLCMATRCA	RDCSHRSCE
NPCLRAACI	PTCAYGWCA
LECVANLCT	RKCGEEVCT
EPCTWNACL	LVCPGTACV
LYCLDASCL	ERCPMAKCY
LVCQGSPCL	QQCQDPYCL
DXCXDIWCL	QPCRSMVCA
KTCVGVRV	WSCHEFNCR
LTCWDWSCR	SLCRLSTCS
KTCAGSSCI	VICTGRQCG
NPCFGLLV	SLCTAFNCH
RTCTPSRCM	QSCLWRICI
QYCWSKGCR	LGCFPSWCG
VTCSSEWCL	RLCSWGGCA
STCISVHCS	EVCLVLSCQ
IACDGYLCG	RDCVKNLCR
XGCYQKRCT	LGCFXSWCG
IRCWGGRCS	IPCSLLGCA
AGCVQSQCY	PRCWERVCS
KACFGADCX	TLCPLVACE
SACWLSNCA	SECYTGSCP

GLCQEHRCW	VECGFSAVF
EDCREWGCR	HWCRLLACR
LMLPRAD	

In addition, peptides that may be useful for targeting tumors *in vivo* include, but are not limited to, the peptide sequences shown in Table 2, which have been described as potential targeting peptides for tumor cells

Table 2

CGRECPRLCQSSC	CGEACGGQCALPC	PSCAYMCIT
SKVLYYNWE	CERACRNLCREGC	CKVCNGRCCG
CPTCNGRCVR	CRNCNGRCEG	CTECNGRCQL
CAVCNGRCGF	CWGCNGRCRM	CVPCNGRCHE
CVQCNGRCAL	CGRCNGRCLL	CVWCNGRCGL
CEGVNGRRLR	CGSLVRC	SKGLRHR
KMGPKVW	NPRWFWD	SGWCYRC
CWSGVDC	IVADYQR	LSMFTRP
CVMVRDGDC	CGVGSSC	CGEGHPC
CPEHRSLVC	CWRKFYC	CPRGSRC
CAQLLQVSC	CTDYVRC	TDCTPSRCT
CTAMRNTDC	VTCRSLMCQ	CISLDRSC
CYLVNVDC	RHCFSQWCS	EACEMAGCL
QWCSRRWCT	NACESAICG	FPCEGKKCL
AGCINGLCG	KGCGTRQCW	KRCSSSLCA
LDCLSELCS	IYCPGQECE	EDCTSRFCS
RWCREKSCW	CNKTDGDEGVTC	CPLCNGRCAL
CEQCNGRCGQ	CVTCNGRCRV	CETCNGRCVG
CSCCNGRCGD	CKSCNGRCLA	CRTCNGRCQV
CASNNGRVVL	CSKCNGRCGH	CGECNGRCVE
CEVCNGRCAL	HHTRFVS	WRVLAAF
SPGSWTW	IKARASP	LWAEMTG
SKSSGVS	VVDRFPD	IMYPGWL
CQLAAVC	CGLSDSC	CELSLISKC
CYVELHC	CYSYFLAC	CDDSWKC
CKALSQAC	VPCRFKQCW	CMEMGVKC
CGTRVDHC	CYLGVSNC	LVCLPPSCE
ISCAVDACL	RSCIKHQCP	GICKDLWCQ
NRCRGVSCT	FGCVMASCR	DTCRALRCN
YRCIARECE	QACPMLLCM	HTCLVALCA
RKCEVPGCQ	EICVDGLCV	RPCGDQACE
CEMCNGRCMG	CGVCNGRCGL	CVLCNGRCWS
CRTCNGRCLE	CRDLNGRKVM	CPLCNGRCAR
CQSCNGRCVR	CRCCNGRCSP	CWLCNGRCGR
CIRCNGRCSV	CLSCNGRCPS	GRSQMQI
VASVSVA	IFSGSRE	GRWYKWA
ALVGLMR	DTLRLRI	VWRTGHL

GLPVKWS	CVRIRPC	CVSGPRC
CYTADPC	CLVVHEAAC	CFWPNRC
CRLGIAC	CYPADPC	CGETMRC
SWCQFEKCL	CRESLKNC	CNNVGSYC
CAMVSMED	CIRSAVSC	FYCPGVGCR
PRCESQLCP	MFCRMRSCD	APCGLLACI
ADCRQKPCL	RSCAEPWCY	GRCVDGGCT
ICLLAHCA	AGCRVESC	RLCSLYGCV
LECVVDSCR	FRCLERVCT	CNGRCVSGCAGRC
IWSGYGVYW	WESLYFPRE	CGLMCQGACFDVC
CPRGCLAVCVSQC	RLCRIVVIRVCR	

Table 2 (continued)

YVPLPNVPQPGRRPFPTFPGQGPFNPKIKWPQGY
VFIDILDKVENAIHNAAQVGIGFAKPFEKLINPK
GNNRPVYIPQPRPPHPRI
GNNRPVYIPQPRPPHPRL
GNNRPIYIPQPRPPHPRL
RFRPPIRRPPIRPPFYPPFRPPIRPPIFPPIRPPFRPPLRFP
RRIRPRPPRLPRPRPRPLPFPRPGPRPIPRPLPFPRPGPRPIPRPLPFFRPG PRPIPRP
PRPIPRPLPFFRPGPRPIPR
WNPFKELERAGQRVRDAVISAAPAVATVGQAALARG
WNPFKELERAGQRVRDAIISAGPAVATVGQAAAIA
WNPFKELERAGQRVRDAIISAAPAVATVGQAAAIARG
WNPFKELERAGQRVRDAVISAAPAVATVGQAAAIARGG
GIGALSAKGALKGLAKGLAZHFAN
GIGASILSAGKSALKGLAKGLAEHFAN
GIGSAILSAGKSALKGLAKGLAEHFAN
IKITTMLAKLGKVLAHV
SKITDILAKLGKVLAIIV
RPDFCLEPPYTGPCKARII
RYFYNAKAGLCQTFVYG
GCRAKRINNFKSAEDCMRTCGGA
FLPLLAGLAANFLPKIFCKITRKC
GIMDTLKNLAKTAGKGALQSLLNKASCKLSGQC
KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK
KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL
GWILKKLGKRIERIGQHTRDATIQGLGIAQQAANVAATARG
WNPFKELEKVGQRVRDAVISAGPAVATVAQATALAK
SWLSKTAKKLENSAKKRISEGIAIAIQGGPR
ZFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS
FLPLILRKIVTAL
LRDLVCYCRSRGCKGRERMNGTCRKGHLLYTLCCR
LRDLVCYCRTRGCKRRERMNGTCRKGHLMYTLCCR
VVCACRRALCLPRERRAGFCRIRGRIHTPLCCRR

VVCACRRALCLPLERRAGFCRIRGRIHPLCCRR
RRCICTTRTCRFPYRRLGTCIFQNRVYTFCC
RRCICTTRTCRFPYRRLGTCLFQNRVYTFCC
ACYCRIPACIAGERRYGTCIYQGRLWAFCC
CYCRIPACIAGERRYGTCIYQGRLWAFCC
VVCACRRALCLPRERRAGFCRIRGRIHPLCCRR
VVCACRRALCLPLERRAGFCRIRGRIHPLCCRR
VTCYCRRTRCGFRERLSGACGYRGRIYRLCCR
VTCYCRSTRCGFRERLSGACGYRGRIYRLCCR
DFASCHTNGGICLPNRCPGHMIQIGICFRPRVKCCRSW
VRNHVTCRINRGFCVPIRCPGRTRQIGTCFGPRIKCCRSW
NPVSCVRNKGICVPIRCPGSMKQIGTCVGRAVKCCRKK
ATCDLLSGTGINHSACAAHCLLRGNRGGYCNGKAVCVCRN
GFGCPLDQMQCHRHCQTITGRSGGYCSGPLKLTCTCYR
GFGCPLNQGACHRHCRSIRRRGGYCAGFFKQTCTCYRN
ALWKTMLKKLGTMALHAGKAALGAADTISQTQ
GKPRPYSPRPTSHPRPIRV
GIFSKLGRKKIKNLLISGLKNVGKEVGMDVVRTGIDIAGCKIKGEC
ILPWKWPWWPWRR
FKCRRWQWRMKKLGAPSITCVRRAP
ITSISLCTPGCKTGALMGCNMKTATCHCSIHVSK
TAGPAIRASVKQCQKTLKATRLFTVSCKGKNGCK
MSKFDDFDLDVVKVSKQDSKITPQWKSESLCTPGCVTGALQTCFLQ TLTCNCKISK
KYYGNGVHCTKSGCSVN
WGEAFSAGVHRLANGGNGFW
GIGKFLHSAGKFGKAFVGEIMKS
GIGKFLHSAKKFGKAFVGEIMNS
GMASKAGAIAGKIAKVALKAL
GVLSNVIGYLKKLGTGALNAVLKG
GWASKIGQTLGKIAKVGLKELIQPK
INLKALAALAKKIL
GIGAVLKVLTTGLPALISWIKRKRQQ
ATCDLLSGTGINHSACAAHCLLRGNRGGYCNGKGVCVCRN
ATCDLLSGTGINHSACAAHCLLRGNRGGYCNRKGVCVRN
RRWCFRVCYRGFCYRKCR
RRWCFRVCYKGFCYRKCR
RGGRLCYCRRRFCVCVGR
RGGRLCYCRRRFCICV
RGGGLCYCRRRFCVCVGR
VTCDLLSFKGQVNDSACAANCLSLGKAGGHCEKGVCICRKTSFKDL WDKYF
GWLKKIGKKIERVGQHTRDATIQGLGIAQQAANVAATAR
GWLKKIGKKIERVGQHTRDATIQVIGVAQQAANVAATAR
SDEKASPDKHHRFSLSRYAKLANRLANPKLLETFLSKWIGDRGNRS V
KWCFRVCYRGICYRRCR

RWCFRVCYRGICYRKCR
KSCCKDTLARNCYNTCRFAGGSRPVCAGACRCKIIGPKCPSDYPK
GGKPDLRPCIIPPCHYIPRPKPR
VKDGYIVDDVNCTYFCGRNAYCNEECTKLKGESGYCQWASPYGNA CYCKLPDHVRTKGPGRCH

Incorporation of a targeting peptide or other targeting moiety into the outer shell may be accomplished by any of the methods known in the art of targeted drug delivery. Suitable methods include but are not limited to covalent attachment of a targeting moiety to one or more components of the outermost shell, either directly or via linkers and electrostatic binding of appropriately charged molecules. These and other methods are well known in the art; see for example A. Coombes et al., *Biomaterials* **18**:1153-1161, 1997.

Another aspect of the invention relates to the functionalization of the surface of the micelles with one or more imaging agents. An "imaging agent" is a moiety suitable for generating a detectable signal, e.g., using a technique such as positron emission tomography (PET), single photon emission tomography (SPECT), or magnetic resonance imaging (MRI), such as a radionuclides, unpaired spin atoms and free radicals (e.g., Fe, lanthanides, and Gd), and contrast agents (e.g., chelated (DTPA) manganese). A number of suitable imaging agents that can be employed in the micelles of the present invention are disclosed in U.S. Patent Application No. 2003-0049203, which is hereby incorporated herein by reference in its entirety.

For example, SPECT can be used for molecular imaging studies to assess both drug distribution and physiological effects with high sensitivity of detection. Furthermore, the use of SPECT allows the simultaneous observation of multiple probes with different emission wavelengths. In certain embodiments, the radioactive moiety is selected from ^{225}Ac, ^{227}Ac, ^{241}Am, ^{72}As, ^{74}As, ^{211}At, ^{198}Au, ^{7}Be, ^{212}Bi, ^{213}Bi, ^{75}Br, ^{77}Br, ^{11}C, ^{14}C, ^{48}Ca, ^{109}Cd, ^{139}Ce, ^{141}Ce, ^{252}Cf, ^{55}Co, ^{57}Co, ^{60}Co, ^{51}Cr, ^{130}Cs, ^{131}Cs, ^{137}Cs, ^{61}Cu, ^{62}Cu, ^{165}Dy, ^{152}Eu, ^{155}Eu, ^{18}F, ^{55}Fe, ^{59}Fe, ^{64}Ga, ^{67}Ga, ^{68}Ga, ^{153}Gd, ^{68}Ge, ^{3}H, ^{122}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{132}I, ^{111}In, ^{115m}In, ^{191m}Ir, ^{192}Ir, ^{81m}Kr, ^{177}Lu, ^{51}Mn, ^{52}Mn, ^{99}Mo, ^{13}N, ^{95}Nb, ^{15}O, ^{191}Os, ^{194}Os, ^{32}P, ^{33}P, ^{203}Pb, ^{212}Pb, ^{103}Pd, ^{109}Pd, ^{238}Pu, ^{223}Ra, ^{226}Ra, ^{82}Rb, ^{186}Re, ^{188}Re, ^{105}Rh, ^{97}Ru, ^{103}Ru, ^{35}S, ^{72}Se, ^{75}Se, ^{28}Si, ^{145}Sm, ^{153}Sm, ^{117m}Sn, ^{85}Sr, ^{89}Sr, ^{90}Sr, ^{178}Ta, ^{179}Ta, ^{182}Ta, ^{149}Tb, ^{96}Tc, ^{99m}Tc, ^{228}Th, ^{229}Th, ^{201}Tl, ^{170}Tm, ^{171}Tm, ^{188}W, ^{127}Xe, ^{88}Y, ^{90}Y, ^{91}Y, ^{169}Yb, ^{62}Zn, ^{65}Zn, ^{95}Zr,

and ^{99m}Tc-labeled Annexin V^{28}. Additionally, ^{99m}Tc-labeled Annexin V^{28} is an available apoptosis probe available when using SPECT, wherein Annexin V is a human protein known to bind phosphatidylserine on the exterior of apoptotic cells.

In certain embodiments, the imaging moiety may be incorporated onto the surface of the micellar structure by fabricating a micelle with a chelating group on the surface that is capable of chelating an imaging moiety. This can be accomplished by, for example, synthesizing block copolymers of PEG and PCL, some of which contain a terminal methyl group (MPEG-PCL), and others in which the PEG chain is covalently linked to the chelating group.

In certain embodiments, the chelating group is selected from 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), diethylenetriaminepentaacetic acid bis (methylamide) (DTPA-BMA), N, N'-bis(2-pyridylmethyl)diethylenetriamine-N,N',N''-triacetic acid (DTPA-BP), and trans-1,2-diaminocyclohexane-N,N',N'',N'''-tetraacetic acid (CDTA). In preferred embodiments, the chelating group is DOTA.

Examples of radioactive imaging moieties include, but are not limited to, ^{225}Ac, ^{227}Ac, ^{241}Am, ^{72}As, ^{74}As, ^{211}At, ^{198}Au, ^{7}Be, ^{212}Bi, ^{213}Bi, ^{75}Br, ^{77}Br, ^{11}C, ^{14}C, ^{48}Ca, ^{109}Cd, ^{139}Ce, ^{141}Ce, ^{252}Cf, ^{55}Co, ^{57}Co, ^{60}Co, ^{51}Cr, ^{130}Cs, ^{131}Cs, ^{137}Cs, ^{61}Cu, ^{62}Cu, ^{165}Dy, ^{152}Eu, ^{155}Eu, ^{18}F, ^{55}Fe, ^{59}Fe, ^{64}Ga, ^{67}Ga, ^{68}Ga, ^{153}Gd, ^{68}Ge, ^{3}H, ^{122}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{132}I, ^{111}In, ^{115m}In, ^{191m}Ir, ^{192}Ir, ^{81m}Kr, ^{177}Lu, ^{51}Mn, ^{52}Mn, ^{99}Mo, ^{13}N, ^{95}Nb, ^{15}O, ^{191}Os, ^{194}Os, ^{32}P, ^{33}P, ^{203}Pb, ^{212}Pb, ^{103}Pd, ^{109}Pd, ^{238}Pu, ^{223}Ra, ^{226}Ra, ^{82}Rb, ^{186}Re, ^{188}Re, ^{105}Rh, ^{97}Ru, ^{103}Ru, ^{35}S, ^{72}Se, ^{75}Se, ^{28}Si, ^{145}Sm, ^{153}Sm, ^{117m}Sn, ^{85}Sr, ^{89}Sr, ^{90}Sr, ^{178}Ta, ^{179}Ta, ^{182}Ta, ^{149}Tb, ^{96}Tc, ^{99m}Tc, ^{228}Th, ^{229}Th, ^{201}Tl, ^{170}Tm, ^{171}Tm, ^{188}W, ^{127}Xe, ^{88}Y, ^{90}Y, ^{91}Y, ^{169}Yb, ^{62}Zn, ^{65}Zn, ^{95}Zr, and ^{99m}Tc-labeled Annexin V^{28}. In preferred embodiments, the radioactive moiety is capable of being chelated by a chelating agent and is selected from ^{225}Ac, ^{227}Ac, ^{241}Am, ^{198}Au, ^{7}Be, ^{212}Bi, ^{213}Bi, ^{48}Ca, ^{109}Cd, ^{139}Ce, ^{141}Ce, ^{252}Cf, ^{55}Co, ^{57}Co, ^{60}Co, ^{51}Cr, ^{130}Cs, ^{131}Cs, ^{137}Cs, ^{61}Cu, ^{62}Cu, ^{165}Dy, ^{152}Eu, ^{155}Eu, ^{18}F, ^{55}Fe, ^{59}Fe, ^{64}Ga, ^{67}Ga, ^{68}Ga, ^{153}Gd, ^{68}Ge, ^{111}In, ^{115m}In, ^{191m}Ir, ^{192}Ir, ^{177}Lu, ^{51}Mn, ^{52}Mn, ^{99}Mo, ^{95}Nb, ^{194}Os, ^{203}Pb, ^{212}Pb, ^{103}Pd, ^{109}Pd, ^{238}Pu, ^{223}Ra, ^{226}Ra, ^{82}Rb, ^{186}Re, ^{188}Re, ^{105}Rh, ^{97}Ru, ^{103}Ru, ^{145}Sm, ^{153}Sm, ^{117m}Sn, ^{85}Sr, ^{89}Sr, ^{90}Sr, ^{178}Ta, ^{179}Ta, ^{182}Ta, ^{149}Tb, ^{96}Tc, ^{99m}Tc, ^{228}Th, ^{229}Th, ^{201}Tl, ^{170}Tm, ^{171}Tm, ^{188}W, ^{88}Y, ^{90}Y, ^{91}Y,

^{169}Yb, ^{62}Zn, ^{65}Zn, ^{99m}Tc-labeled Annexin V^{28}, and ^{95}Zr. In certain embodiments, the radioactive moiety is ^{111}In, ^{99m}Tc-labeled Annexin V^{28}, or ^{99m}Tc. In certain such embodiments, the radioactive moiety is ^{111}In.

Yet another aspect of the invention relates to micelles containing within the hydrophobic core, a magnetic resonance imaging (MRI) contrast imaging agent either alone or in combination with another hydrophobic agent and/or functionalization of the corona of the micelle. Examples of such contrast agents, include, but are not limited to, gadopentetate dimeglumine, gadoteridol, gadoterate meglumine, mangafodipir trisodium, gadodiamide, gadoversetamide, and superparamagnetic iron oxide. Superparamagnetic iron oxide (SPIO) nanoparticles are a class of MRI contrast agents that provide extremely strong enhancement of proton relaxation. In contrast to low molecular weight "T1" paramagnetic metal chelates such as Gd-DTPA, SPIO nanoparticles are classified as T2 negative contrast agents, with MR sensitivity approximately 1000 times higher than T1 agents. SPIO agents are composed of iron oxide nanocrystals which create a large, dipolar magnetic field gradient that creates a relaxation effect on nearby water molecules. According to their sizes and applications, SPIO nanoparticles have been classified into four different categories: large, standard, ultrasmall, and monocrystalline agents. Large SPIO agents are mainly used for gastrointestinal lumen imaging, while standard SPIO agents are used for liver and spleen imaging. When the SPIO nanoparticles are in the range of 20-40 nm (ultrasmall category), they can be injected to visualize lymph node metastases. The smallest monocrystalline SPIO agents are used for tumor-specific imaging when attached to monoclonal antibodies, growth factors, and antigens.

In preferred embodiments, the MRI contrast imaging agent is superparamagnetic iron oxide (SPIO). Figure 8A shows a transmission electron micrograph of a typical micelle containing numerous individual SPIO particles, wherein the white circle denotes the approximate boundary of the micelle core.

Another aspect of the invention relates to method for the treatment of cancer, comprising administering micelles of the present invention, wherein the micellar structure comprises an encapsulated chemotherapeutic agent. In certain such

embodiments, the coronas of the micelles are functionalized with peptides that are capable of targeting tumor cells. Additionally, or alternatively, the corona of the micelle is functionalized with a peptide that is capable of targeting a specific tissue in the body, preferably a peptide selected from either Table 1 or 2.

In certain embodiments, the coronas of the micelles are functionalized with chelating agents that are capable of chelating a radioactive moiety. In preferred such embodiments, the micelles further comprise a radioactive moiety that is capable of being chelated by the chelating agent. A further aspect of the invention relates to a method for monitoring the delivery of a hydrophobic agent, comprising administering micelles, wherein the micellar structure comprises an encapsulated hydrophobic agent, and the hydrophobic agent is an MRI contrast agent. In preferred embodiments, the micellar structure further comprises a functionalized corona and/or an additional hydrophobic agent. In more preferred embodiments, the additional hydrophobic agent is a chemotherapeutic agent.

Another aspect of the invention is a pharmaceutical composition, comprising micelles as described herein and a pharmaceutically acceptable carrier.

The phrase "pharmaceutically acceptable" is employed herein to refer to those ligands, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject chemical from one organ or portion of the body, to another organ or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose, and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; (4)

powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil, and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol, and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations. In certain embodiments, pharmaceutical compositions of the present invention are non-pyrogenic, i.e., do not induce significant temperature elevations when administered to a patient.

As used herein, the term "treating" or "treatment" includes reversing, reducing, or arresting the symptoms, clinical signs, and underlying pathology of a condition in manner to improve or stabilize a subject's condition.

The present invention may be further appreciated upon consideration of the following illustrative and non-limiting examples.

Examples

Synthesis of diblock copolymers of PCL and MPEG

Detailed synthetic method for this class of block copolymers was described in a recent publication (X. Shuai et al., Macromolecules (2003) 36 5751-5759. Briefly, the diblock copolymers (MPEG-b-PCL), with yields >95%, were synthesized by ring-opening polymerization of ε-caprolactone at 115 °C using MPEG as a macro-initiator and $Sn(Oct)_2$ as a catalyst. The degree of polymerization of the PCL block was calculated by comparing integrals of characteristic peaks of the PCL block at ~2.25 ppm and PEG block at 3.35ppm in the ^1H NMR spectrum.

Characterization of copolymers

Fourier transform infrared (FTIR) spectral studies were carried out with a BIO-RAD FTS-575C FTIR spectrometer in the range between 4000 and 750 cm^{-1}, with a resolution of 2 cm^{-1}. Powdery samples were compressed into KBr pellets for the FTIR measurements. ^1H NMR spectra were recorded on a Varian 600-MHz

NMR spectrometer in deuterated water (D_2O) or chloroform ($CDCl_3$) at room temperature.

Gel permeation chromatography (GPC) was employed to determine the molecular weight and the molecular weight distribution. GPC analysis was carried out using a PLgel 5μm Mixed-D 300×7.5mm column (Polymer Laboratories) with THF as an eluent (1 mL/min) and polystyrene standards for column calibration. 20 μL samples were injected. The eluent was analyzed with a Perkin-Elmer Series 200 differential refractive index (RI) detector.

Preparation of DOX-loaded micelles

Polymeric micelles containing DOX were prepared as following: MPEG-b-PCL copolymer (10 mg) and doxorubicin (2 mg) were dissolved in THF (2 mL) in a glass vial. Afterwards, the solution was added dropwise to pure water (20 mL) under vigorous ultrasonic agitation using a Type 60 Sonic Dismembrator (Fisher Scientific) at a power level of 10. The beaker was then open to air overnight, allowing slow evaporation of THF and formation of micelles. The residual THF was completely removed by vacuum distillation with a rotary evaporator. The micelle solution concentrated to 5 mL was filtered with a syringe filter (pore size: 0.45 μm) to eliminate the polymer and DOX aggregates, and then filtered through a MILLIPORE Centrifugal Filter Device (Mw cut-off: 100,000 Da) to remove free DOX dissolved in the micelle solution.

The micelles thus obtained were characterized with photon correlation spectroscopy. Measurements were performed at 25 °C on a 90 Plus Particle Size Analyzer from Brookhaven Instruments Corporation. Scattered light was detected at 90° angle and collected on an autocorrelator. For each sample, data obtained from five measurements were averaged to yield the size and size distribution.

Determination of DOX-loading content (DLC)

The DOX-loading content (DLC) was defined as the weight percentage of DOX in the micelle. DLC was quantified by determining the absorbance at 485 nm using a Perkin-Elmer Lambda 20 UV-Vis spectrophotometer. First, the micelle solutions were frozen and lyophilized to yield the solid micelle samples. Then the

dried samples were redissolved in a mixture of chloroform and DMSO (1:1, v/v) for the UV-Vis measurement. DOX solutions of various concentrations were prepared, and the absorbance at 485 nm was measured to generate a calibration curve for the DLC calculations from various micelles.

In vitro release of DOX from polymer micelles

Freeze-dried micelle samples (15 mg each) were re-suspended in PBS or acetate buffered solutions and transferred into dialysis tubing (Mw cut-off: 50,000 Da, supplied by Spectrum Laboratories Inc., USA). The tubing was placed into 50 mL PBS or acetate buffered solutions. Release study was performed at 37 °C in a New Brunswick Scientific C24 Incubator Shaker. At selected time intervals, buffered solution outside the dialysis bag was removed for UV-Vis analysis and replaced with fresh buffer solution. DOX concentration was calculated based on the absorbance intensity at 485 nm.

Hemolysis study

Blood was freshly obtained from a male beagle dog and collected in heparin-coated tubes. Blood was washed three times with PBS and collected by centrifugation at 2,800 rpm for 5 min. Micelle solutions were prepared at different concentrations in the PBS buffer, and 100 μL of the erythrocyte suspension were added to 900 μl of micelle solutions. The samples were incubated for 60 min at 37 °C in a New Brunswick Scientific C24 Incubator Shaker. The release of hemoglobin was measured by UV-Vis analysis of the supernatant at 540 nm after centrifugation at 12,000 g for 60 min. The complete hemolysis was achieved by incubating the same amount of erythrocytes with 0.2% Triton X-100, and all hemolysis data points are presented as the percentage of the complete hemolysis.

Confocal laser scanning microscopy (CLSM)

Free DOX and DOX-containing micelles were incubated in MCF-7 cell culture for 2 and 24 hours before confocal laser scanning microscopy (CLSM) examination. To identify the micelle location, cell nuclei were stained with Hoechst 33342 (Molecular Probes, Inc.) and culture media were replaced with PBS during microscopy. Samples were examined by CLSM using a Zeiss LSM 510 (Zurich,

Switzerland) with a confocal plane of 300 nm. Hoechst 33342 and DOX were excited at 352 and 485 nm with emissions at 455 and 595 nm, respectively.

In vitro cytotoxicity study against MCF-7 breast tumor cells

Human MCF-7 breast cancer cells were seeded onto 48-well plates with a seeding density of 7,000 cells/well. Cells were maintained in Roswell Park Memorial Institute (RPMI-1640, Sigma) media supplemented with 5% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 5,000 units/ml penicillin, 5 mg/ml streptomycin, 0.1 mg/ml gentamicin sulfate and Amphotericin-B, 25 mM KCl, 25 mM D-glucose, and incubated at 37 °C in a humidified atmosphere with 5% CO_2. Cells were incubated for one day, and then micelle or free DOX solutions were added. After one or five days, cells were harvested and DNA levels were analyzed through a DNA assay with a Perkin-Elmer HTS 7000 Bioassay Reader. The cytotoxicity of DOX and DOX-containing micelles in aqueous solution was analyzed and compared.

Synthesis of maleimide-terminated block copolymer (MAL-PEG-PCL)

In contrast to the reported ε-caprolactone polymerization procedure with stannous (II) octoate as a catalyst (X. Shuai, T. Merdan, A. K. Schaper, F. Xi, T. Kissel, *Bioconjug. Chem.* (2004) 15 441-448), synthesis of MAL-PEG-PCL, as shown in Figure 1, must be conducted at a lower temperature due to the thermal susceptibility of the maleimide end groups. Reaction at 68 °C led to desired molecular weights of PCL segments (e.g., 2.4 kD), while greatly reducing the thermal decomposition of maleimide to a negligible level. DOX-loaded, MAL-PEG-PCL micelles were prepared by a solvent-evaporation method. Different amounts of methoxy-terminated MPEG-PCL copolymer were also introduced to control maleimide density at the micelle surface, which subsequently controls the cRGD density (5, 16, and 76% of all PEG chains).

Figure 2a shows the ^{1}H NMR spectrum of MAL-PEG-PCL copolymer in $CDCl_3$. Resonances of the PEG methylene protons (mainly at 3.64 ppm) and PCL protons (1.38, 1.65, 2.31 and 4.06 ppm) were observed. A small triplet shown at 4.2 ppm was attributed to proton resonance of the methyleneoxyl group linking PCL and PEG blocks. The integration intensity of maleimide vinyl protons at 6.74 ppm

confirms that the maleimide group in MAL-PEG-PCL copolymers remained intact as in the MAL-PEG-OH. These data strongly demonstrated that the desired block copolymers were successfully synthesized. The number-averaged molecular weight of PCL blocks was calculated to be 2.4 kD using the integral intensity of PCL proton at 2.31 ppm *versus* that of the PEG proton at 3.64 ppm. To ensure the localization of cRGD on the surface of micelles, we used a post-micellar modification strategy to prepare cRGD-functionalized micelles as shown in Figure 1. The NMR spectrum of the freeze-dried micelles in D_2O strongly suggests the core-shell structure of DOX-loaded micelles as shown in Figure 2b. The micelle corona shells consisting of PEG blocks were well solvated in D_2O and showed clear ^1H NMR signals. In contrast, DOX was loaded inside the solid PCL cores of micelles, and thus resonance peaks of both PCL blocks and DOX molecules were significantly reduced due to their insufficient chain mobility in D_2O. Moreover, successful conjugation of cRGDfK onto the well-solvated PEG corona shells was verified by the resonance peaks of phenyl protons of cRGDfK at 7.4 ppm and complete disappearance of maleimide peak at 6.74 ppm (Fig. 1B).

Synthesis of cRGD using solid phase peptide synthesis chemistry

The synthetic scheme for cRGD as shown in Figure 3 is revised based on a reported procedure by Schatzlein *et al. Bioorg. Med. Chem. Lett.* (2002) 12 547–549. NovaSyn TGT alcohol resin (1.25 mmol) (Novabiochem, CA) was converted to its active chloride form with acetyl chloride (1 ml/g resin) (62.5 mmol) in toluene for 3 h at 60 °C. The resin was then washed with dry toluene and dichloromethane (DCM). The synthesis of linear peptide started with the attachment of aspartic acid, by mixing the chlorinated resin with a solution of Fmoc-Asp-OAll (2.5 eq.) and *N*, *N*-diisopropylethylamine (DIPEA) (10 eq.) in dry DCM at rt for 1.5-2.5 h. The solution of DCM, methanol and DIPEA was added to cap the unreacted sites of resin. After 30 min the resin was washed with dimethylformamide (DMF). The Fmoc protecting group was removed with a solution of piperidine–DMF (1:4) at rt for 4 min, 2 times. The rest of amino acids were added consecutively (Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Lys(Dde)-OH and Fmoc-D-Phe-OH) using standard Fmoc strategy. The amino acid (2 eq.) was added first followed by *N,N,N',N'*-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium

hexafluorophosphate (HATU) (2 eq.) and DIPEA (4 eq.) and allowed to react for 1.5 h at rt. The C-terminal allyl ester group of the aspartic acid was removed after addition of the last amino acid with palladium tetrakis(triphenylphosphine) ($Pd(PPh_3)_4$) (3 eq.) in a solution of chloroform, acetic acid and *N*-methylmorpholine for 2 h at rt. The mixture was washed with DIPEA in DMF followed by 0.5%w/w diethyldithiocarbamic acid sodium salt in DMF. The head-to-tail cyclization was preformed by removal of the *N*-terminal Fmoc group before addition of HATU (2 eq.) and DIPEA (4 eq.) in DMF at rt for 16 h. The protecting group of the amino side-chain of lysine (4, 4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl, (Dde)) was removed by hydrazine monohydrate–DMF (2:98) for 3 min at rt, 3 time. The thiol addition was preformed by swelling resin with DIPEA (15 eq.) in DMF, followed by addition of *S*-acetylthioglycolic acid *N*-hydroxysuccinimide ester (SATA) solution (2 eq.) in DMF (15 mg/mL) for 2 h at rt. The resin was then washed with DMF followed by DCM. Next, the protecting group of arginine (pentamethyl-dihydrobenzofuran-5-sulfonyl, (Pfb)) was removed using TFA–DCM (1:1) for 2 h before washing with trifluoroacetic acid–DCM (1:9). The solutions were concentrated and precipitated with cold ether several times. The precipitate was then redissolved in aqueous buffer, purified using reverse phase HPLC and lyophilized to give cRGD peptide (90 mg, 10% yield based on initial loading of resin).

Preparation of cRGD-DOX-Micelles.

20 mg of MAL-PEG-PCL and 2 mg of doxorubicin were dissolved in 0.5 mL THF in a glass vial. Next, the mixture was slowly added into 10 mL of an aqueous solution of 0.05 M HEPES and 0.01 M EDTA under sonication (60 Sonic Dismembrator, Fisher Scientific). The mixture was vigorously stirred under argon for 3 h to remove THF. Then different amounts of c(RGDf(*ε*-*S*-acetylthioacetyl)K and 0.05 M hydroxyamine in HEPES/EDTA aqueous solution were added into solutions of micelles with 5, 16, and 76% maleimide density. The conjugation was allowed to occur for 4 h followed by filtration through a Millipore centrifugal filter (pore size 0.45 μm) to remove DOX aggregates in micelle solution. Then the cRGD-micelles were dialyzed with Spectra/Por dialysis membrane (molecular weight cutoff = 50,000 Da) until free cRGD was completely removed. Micelles

were then characterized by dynamic light scattering and atomic force microscopy. Micelle solutions were then lyophilized to obtain the powdery form. ^{1}H NMR was used to confirm the formation of core-shell structure and conjugation of cRGD to micelles. The strong resonance of methylene proton in PEG was detected where as all of caprolactone proton resonance were hardly observed demonstrating the core-shell structure of these micelles. The successful conjugation of cRGD onto the surface of micelles was verified by the appearance of phenyl protons of cRGD at 7.4 ppm.

Atomic force microscopy (AFM)

Two group of micelles were used for AFM study. The first group was DOX-micelles without cRGD ligand and the second group was DOX-micelles with 76% cRGD density. The micelle suspension (2 μL) was placed on the mica surface, and allowed to dry at room temperature overnight before imaging with an atomic force microscope (Multimode, Digital Instruments, Santa Barbara, CA) operated in tapping mode using a silicon cantilever (Pointprobe, Nanoworld, Switzerland). The constant force mode was used with a scan frequency of 2 Hz. Both non-functionalized and 76% cRGD containing micelles show discrete and round-shaped nanoparticles. These results were shown in Figures 4a and 4c. Micelles with 76% cRGD attachment (43.2 ± 3.9 nm, n=29) showed a mean size slightly larger than that of RGD-free micelles (37.5 ± 2.6 nm, n=29).

Dynamic light scattering (DLS)

DLS was performed on a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corporation). Scattered light was detected at 90° at room temperature and collected on an autocorrelator. The data for each sample was obtained in five measurements and the average number was used. The same two groups of micelles as in AFM studies were used for DLS characterization. The sizes of these micelles are 20.9 ± 1.7 and 24.4 ± 2.7 nm for cRGD-free and 76% cRGD micelles, respectively. The results were shown in Figure 4b and 4d.

Flow cytometry analysis

SLK cells were seeded at 125,000 cells /well in 6-well plates in 2 mL DMEM with 10% FBS. After 24 h, 1 mg of micelles (from 3.3 mg/ml micelle

suspension) for each micelle formulation with different cRGD density (0, 5, 16, 76% cRGD) was added into each well and incubated at 37 °C for 2 h. Then, cells were washed, trypsinized and neutralized. After centrifugation at 1200 rpm for 5 min, cells were re-suspended in 1 mL PBS, followed by filtration and analysis using flow cytometry. Cell uptake was found to increase up to 30-fold with 76% cRGD-DOX-micelles compared to those not attached with cRGD (0% cRGD). In the control experiment, SLK cells were first incubated with a free blocking peptide, Ala-Ala-Arg-Gly-Asp-Tyr (AARGDY), and then co-incubated with 76% cRGD-functionalized micelles. Almost 100% inhibition by AARGDY at 9 mM concentration was observed as demonstrated by the flow cytometry histograms as shown in Figure 6.

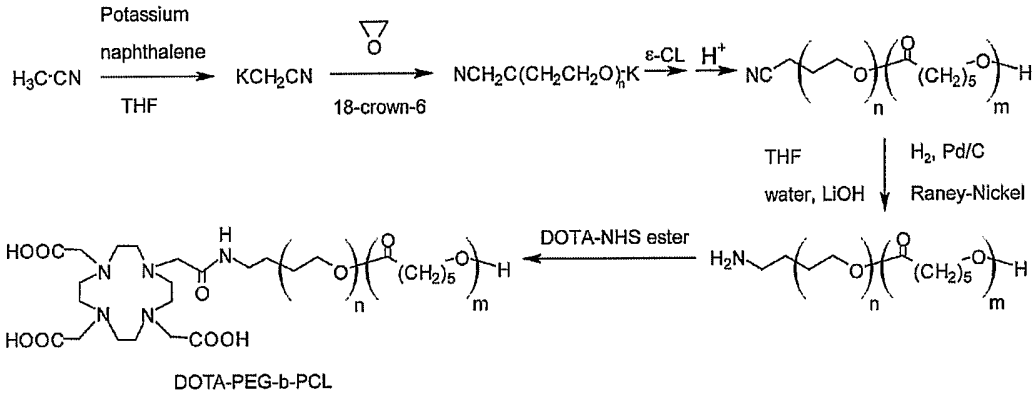
Confocal laser scanning microscopy (CLSM)

DOX-micelles with 0 and 16% cRGD density (0.5 mg/well) were incubated with SLK cells (6000 cells/well) culture wells for 2 hrs. Before the CLSM examination, cell nuclei were stained with Hoechst 33342 (Molecular Probes, Inc.). Cells were examined by a Zeiss LSM 510 microscope (Zurich, Switzerland, laser: Ar 351-364 nm, Ar 458-488 nm) with a confocal plane of 300 nm. Doxorubicin and Hoechst 33342 were excited at 485 and 352 nm, respectively. The emission wavelength of doxorubicin and Hoechst 33342 are 595 and 455 nm, respectively. A significantly increased amount of micelle uptake was observed in micelles with 16% cRGD surface density as shown in Figure 5c compared to those without cRGD as shown in Figure 5b. This result is consistent with those from flow cytometry studies as shown in Figure 5a.

Synthesis of DOTA-PEG-PCL

The MPEG-PCL and amino group terminated PEG-PCL polymer (H_2N-PEG-PCL) are synthesized following reported procedures (Shuai, X.T. et al., Macromolecules, 2003. 36 5751-5759; Deng, M.X. et al., Biomaterials, (2004) 25 3553-3558). Briefly, synthesis of H_2N-PEG-PCL is accomplished by the metalation of acetonitrile with potassium naphthalide to initiate living anionic ring-opening-polymerization with ethylene oxide first and ε-caprolactone (ε-CL) second. Hydrogenation of the CN-PEG-PCL copolymer using Pd/C as catalyst provides a

diblock copolymer with a functionalizable amino group (H_2N-PEG-PCL). To synthesize the DOTA containing PEG-PCL polymer (DOTA-PEG-PCL), DOTA-NHS ester (Macrocyclics, Inc., Dallas, TX) is added to a H_2N-PEG-PCL solution in organic solvent. The subsequent copolymer is purified by precipitation in diethyl ether and hexane. Both polymers can be manufactured with various PEG and PCL chain lengths, which can be used to modify both the micelle size and doxorubicin loading within the micelles.



Scheme 1

After syntheses of both block copolymers, polymer micelles that incorporate different amounts of chelating agent are fabricated. The amount of chelating agent can be controlled by varying the weight ratio of DOTA-PEG-PCL to MPEG-PCL used. Micelles with 1, 3, and 5% (w/w) DOTA-PEG-PCL are fabricated using a phase inversion technique. A schematic illustrating this process is shown in Figure 7. Micelle size is characterized through the use of dynamic light scattering and TEM, while drug content and release from the micelles is measured by UV-Vis spectroscopy.

Incorporation of radioactive moieties

Immediately prior to each imaging experiment, ^{111}In is added to each micelle formulation to allow binding of the metal ion to the DOTA chelating group. Excess free ^{111}In ions are removed through a gel filtration process before imaging, and each micelle sample is serially diluted to generate vials containing a logarithmic range of known concentrations. Multiple vials containing concentrations of each of the three formulations are then imaged via both SPECT and computed tomography (CT), and

the resulting imaging activity of each vial is plotted against micelle concentration for that formulation. In this manner, the lower detection limit as well as a calibration value which can be used to convert SPECT activity to micelle concentration is determined for each of the three formulations.

Following *in vitro* calibration, each of the three micelle formulations are injected into the tail vein of two mice with breast tumors. An additional two sets of mice, one with drug-containing, unlabeled micelles and another with drug-free labeled micelles, are injected and imaged as controls.

Additionally, a separate SPECT imaging probe, ^{99m}Tc-labeled Annexin V can be introduced to simultaneously measure cell apoptosis. One unique property of SPECT imaging is its ability to discern activity from multiple probes that emit gamma photons of different energy, in this case 245 keV for ^{111}In and 140 keV for ^{99m}Tc. Prior to use in animals, an *in vitro* calibration similar to that described above is performed. In this study, vials containing known concentrations of both ^{111}In-labeled micelles and ^{99m}Tc-labeled Annexin V are prepared and used to generate limit of detection values for the simultaneous detection of both labels. Animal groups will be the same as those used for ^{111}In imaging with the addition of a group to receive systemic free doxorubicin to ensure that the tumors in this model are susceptible to doxorubicin treatment. The ^{99m}Tc-labeled Annexin V is administered via the tail vein prior to each imaging session, and SPECT imaging is used to generate both micelle concentration and apoptosis density measurements. The results from this section are confirmed via histological measurements taken after the animals are sacrificed. Tumor volume is measured by gross tissue measurement, doxorubicin concentration in tissue is obtained by fluorescence microscopy, apoptosis is validated using TUNEL stained sections, and micelle concentrations are confirmed by scintillation counting of extracted tissues.

In vivo efficacy data to optimize the design of drug-loaded micelles

Different micelle formulations are compared based on their treatment efficacy as measured by total micelle accumulation, apoptotic response, and tumor volume change, all of which are measured non-invasively. Five different micelle formulations are used to investigate the effects of two different micelle properties:

size and PEG chain length. These two parameters affect the blood circulations times as well as the extravasation efficiency across the tumor endothelium. Micelle size will be investigated using three micelle formulations with the same PEG length (MW 5kD) and different PCL lengths (2, 5, and 10 kD). Alternatively, PEG chain length effects are elucidated by using micelle formulations with a fixed PCL length (5 kD) and varying PEG size (2, 5, and 10 kD), for a total of five experimental groups. Control mice are injected with labeled micelles containing no doxorubicin. Each group contains approximately 6 subjects. Once optimized, the micelles are compared directly with systemically administered, dose-matched doxorubicin on the same measures.

Additionally, the data from micelle comparison is used to develop a model to correlate treatment efficacy with micelle accumulation and apoptosis as measured by SPECT. Relationships between micelle accumulation, tumor apoptosis, and tumor volume change over the one week period of monitoring are investigated in order to create a method of predicting the tumor volume change of the tumors based on early (<24 hours) measurements of micelle accumulation and tumor apoptosis.

Micelles containing SPIO particles

In Figure 8a, hydrophobic SPIO particles of approximately 4 nm in diameter were incorporated into the hydrophobic micellar core. *In vitro* imaging of particle solutions was performed on a Siemens Sonata 1.5T clinical MRI scanner using a fast low angle shoot (FLASH) pulse sequence, and the T_2 contrast effect of the micelles was quantified. Particle T_2 relaxivity values ranged from 76 – 102 $(mM\ Fe*s)^{-1}$, suggesting that the particles had largely retained their contrast despite micellar incorporation. For *in vivo* studies, micelle solutions were injected into the tail vein of breast tumor bearing mice (the bi-transgenic mice bearing both the MMTV-c-neu and LHCTP transgenes proposed for use in this study) at and imaged with T_2-weighted sequences at 1 hour and 72 hours after micelle injection. By 72 hours, the peripheral region of the tumor had darkened noticeably, indicating accumulation of SPIO-containing micelles in the region as shown in Figures 8b and 8c. MRI has some intrinsic difficulties, however, such as relatively low sensitivity compared to nuclear medicine and problems distinguishing contrast effects from the anatomical

background, that promote the development of other imaging modalities for quantitative micelle tracking.

Equivalence

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the compounds and methods of use thereof described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

All of the above-cited references and publications are hereby incorporated by reference.

Claims:

1. A micellar structure comprising an amphiphilic block copolymer, a hydrophobic agent encapsulated within the micellar structure, and a functionalized corona.

2. A micellar structure of claim 1, wherein the amphiphilic block copolymer is selected from PBLG-PEO, PEO-PPO-PEO, PEG-b-PBLA, and PCL-PEG.

3. A micellar structure of claim 2, wherein the amphiphilic copolymer is PCL-PEG.

4. A micellar structure of any one of claims 1 to 3, wherein the hydrophobic agent is a chemotherapeutic agent.

5. A micellar structure of claim 4, wherein the chemotherapeutic agent is selected from aminoglutethimide, amsacrine, anastrozole, asparaginase, bcg, bicalutamide, bleomycin, buserelin, busulfan, campothecin, capecitabine, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, dienestrol, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, estradiol, estramustine, etoposide, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ironotecan, letrozole, leucovorin, leuprolide, levamisole, lomustine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oxaliplatin, paclitaxel, pamidronate, pentostatin, plicamycin, porfimer, procarbazine, raltitrexed, rituximab, streptozocin, suramin, tamoxifen, temozolomide, teniposide, testosterone, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, and vinorelbine.

6. A micellar structure of claim 5, wherein the chemotherapeutic agent is selected from paclitaxel and doxorubicin.

7. A micellar structure of any one of claims 1 to 6, wherein the functionalized corona comprises a targeting moiety.

8. A micellar structure of claim 7, wherein the targeting moiety is a peptide sequence.

9. A micellar structure of claim 8, wherein the peptide sequence is a $\alpha_v\beta_3$ ligand.

10. A micellar structure of claim 9, wherein the $\alpha_v\beta_3$ ligand is cRGD.

11. A micellar structure of any one of claims 1 to 7, wherein the functionalized corona comprises an imaging agent.

12. A micellar structure of claim 11, wherein the imaging agent comprises a chelating agent.

13. A micellar structure of claim 12, wherein the chelating agent is selected from DOTA, DTPA-BMA, DTPA-BP, and CDTA.

14. A micellar structure of claim 12 or 13, further comprising a radioactive moiety that is chelated by the chelating agent.

15. A micellar structure of claim 14, wherein the radioactive moiety is selected from ^{225}Ac, ^{227}Ac, ^{241}Am, ^{198}Au, ^{7}Be, ^{212}Bi, ^{213}Bi, ^{48}Ca, ^{109}Cd, ^{139}Ce, ^{141}Ce, ^{252}Cf, ^{55}Co, ^{57}Co, ^{60}Co, ^{51}Cr, ^{130}Cs, ^{131}Cs, ^{137}Cs, ^{61}Cu, ^{62}Cu, ^{165}Dy, ^{152}Eu, ^{155}Eu, ^{18}F, ^{55}Fe, ^{59}Fe, ^{64}Ga, ^{67}Ga, ^{68}Ga, ^{153}Gd, ^{68}Ge, ^{111}In, ^{115m}In, ^{191m}Ir, ^{192}Ir, ^{177}Lu, ^{51}Mn, ^{52}Mn, ^{99}Mo, ^{95}Nb, ^{194}Os, ^{203}Pb, ^{212}Pb, ^{103}Pd, ^{109}Pd, ^{238}Pu, ^{223}Ra, ^{226}Ra, ^{82}Rb, ^{186}Re, ^{188}Re, ^{105}Rh, ^{97}Ru, ^{103}Ru, ^{145}Sm, ^{153}Sm, ^{117m}Sn, ^{85}Sr, ^{89}Sr, ^{90}Sr, ^{178}Ta, ^{179}Ta, ^{182}Ta, ^{149}Tb,

^{96}Tc, ^{99m}Tc, ^{228}Th, ^{229}Th, ^{201}Tl, ^{170}Tm, ^{171}Tm, ^{188}W, ^{88}Y, ^{90}Y, ^{91}Y, ^{169}Yb, ^{62}Zn, ^{65}Zn, ^{99m}Tc-labeled Annexin V^{28}, and ^{95}Zr.

16. A micellar structure of claim 15, wherein the radioactive moiety is selected from ^{99m}Tc-labeled Annexin V^{28}, and ^{111}In.

17. A micellar structure of claim 1, wherein the imaging agent comprises an MRI contrast agent.

18. A micellar structure of claim 17, wherein the MRI contrast agent is selected from gadopentetate dimeglumine, gadoteridol, gadoterate meglumine, mangafodipir trisodium, gadodiamide, gadoversetamide, and superparamagnetic iron oxide.

19. A micellar structure of claim 18, wherein the MRI contrast agent is superparamagnetic iron oxide.

20. A micellar structure comprising an amphiphilic block copolymer and an imaging agent encapsulated within the micellar structure.

21. A micellar structure of claim 20, wherein the amphiphilic block copolymer is selected from PBLG-PEO, PEO-PPO-PEO, PEG-b-PBLA, and PCL-PEG.

22. A micellar structure of claim 20 or 21, wherein the imaging agent is an MRI contrast agent.

23. A micellar structure of claim 22, wherein the MRI contrast agent is selected from gadopentetate dimeglumine, gadoteridol, gadoterate meglumine, mangafodipir trisodium, gadodiamide, gadoversetamide, and superparamagnetic iron oxide.

24. A micellar structure of claim 23, wherein the MRI contrast agent is superparamagnetic iron oxide.

25. A method for administering a hydrophobic agent to a patient, comprising administering to the patient micellar structures of any one of claims 1 to 19.

26. A method for monitoring the delivery of a hydrophobic agent to a patient, comprising administering micellar structures of any one of claims 11 to 19 and imaging the imaging agent in the patient.

27. A method for treating cancer, comprising administering to a patient micellar structures of claim 4, 5, or 6.

28. A pharmaceutical composition comprising micelles of any one of claims 1 to 24 and a pharmaceutically acceptable carrier.

29. A method for the preparation a micellar structure, comprising

(i) adding an amphiphilic block copolymer and an imaging agent to a hydrophobic solvent;

(ii) adding the resulting mixture to water under conditions of vigorous agitation; and

(iii) removing the hydrophobic solvent by slow evaporation, thereby preparing a micellar structure comprising an imaging agent encapsulated in the micellar structure.

30. A method for the preparation a micellar structure, comprising

(i) adding an amphiphilic block copolymer to a hydrophobic solvent;

(ii) adding the resulting mixture to water under conditions of vigorous agitation;

(iii) removing the hydrophobic solvent by slow evaporation, thereby preparing a micellar structure having a corona; and

(iv) coupling a targeting moiety and/or an imaging moiety to the corona of the micellar structure.

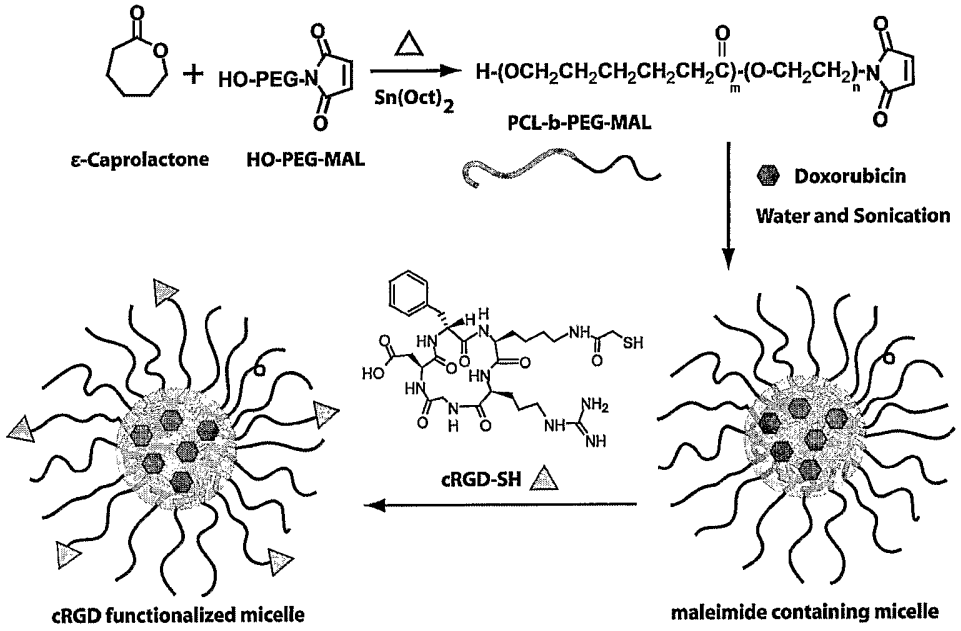


Figure 1

Preparation of cRGD-DOX-Micelles

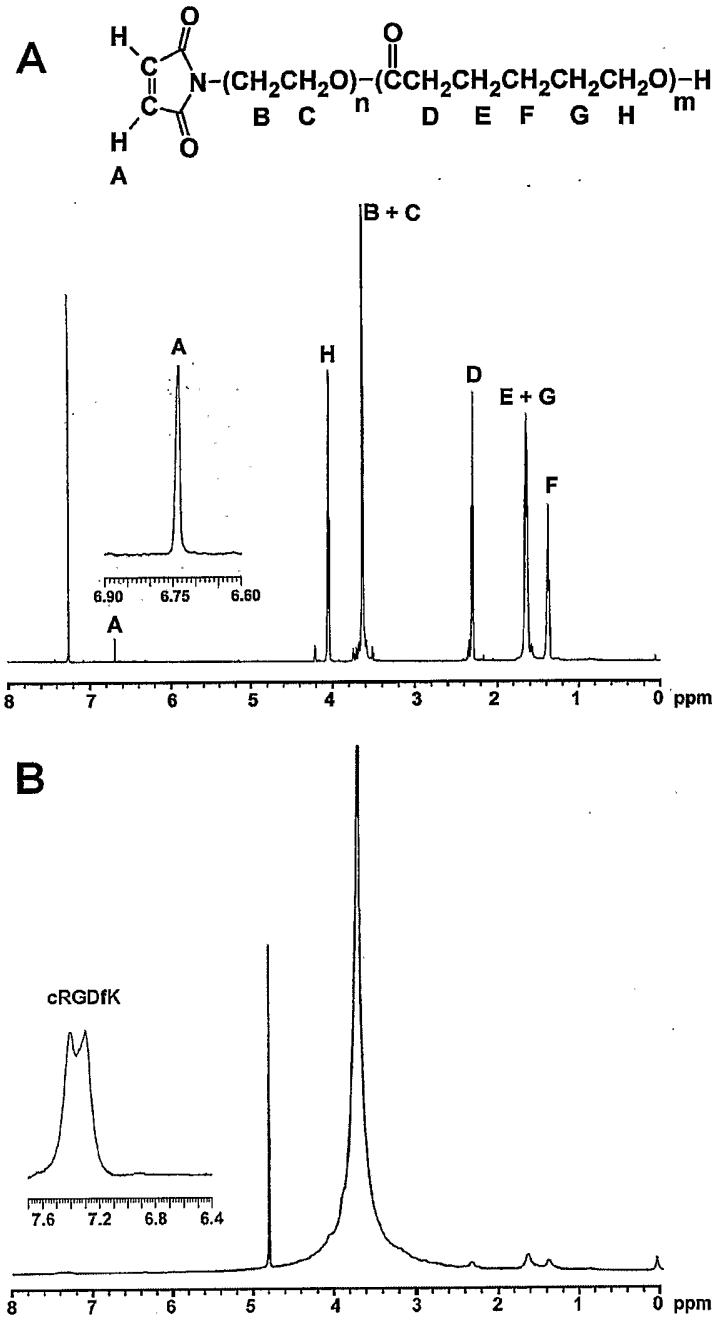


Figure 2

^{1}H NMR of MAL-PEG-PCL in $CDCl_3$

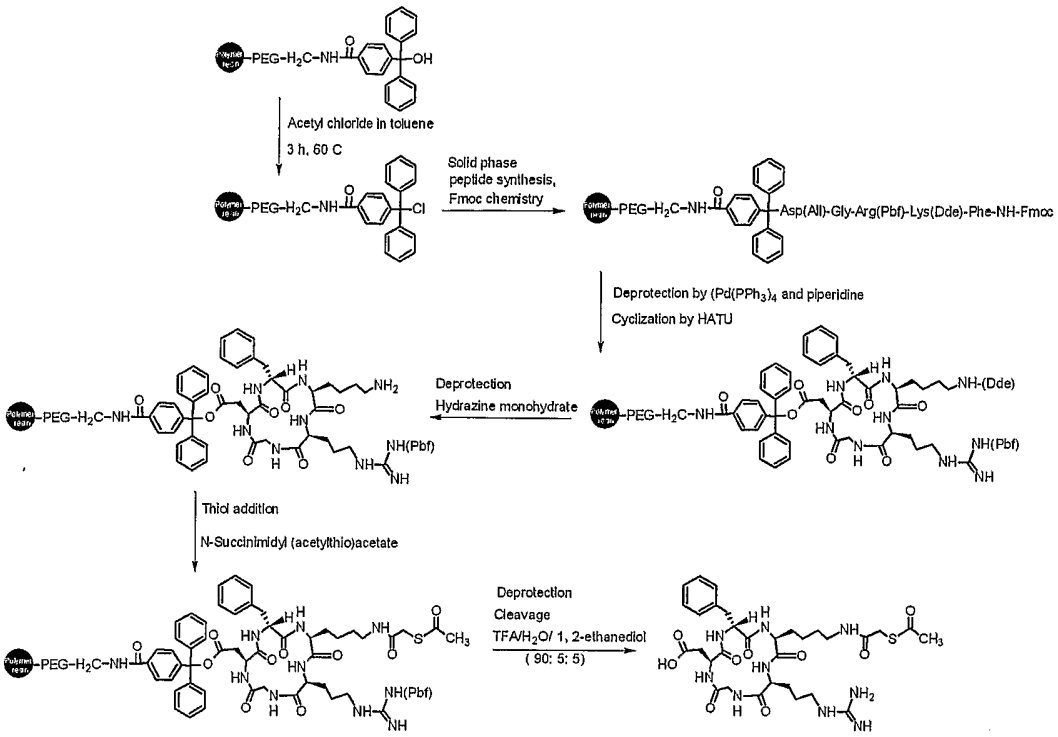


Figure 3

Synthesis of cRGD Using Solid Phase Peptide Synthesis Chemistry

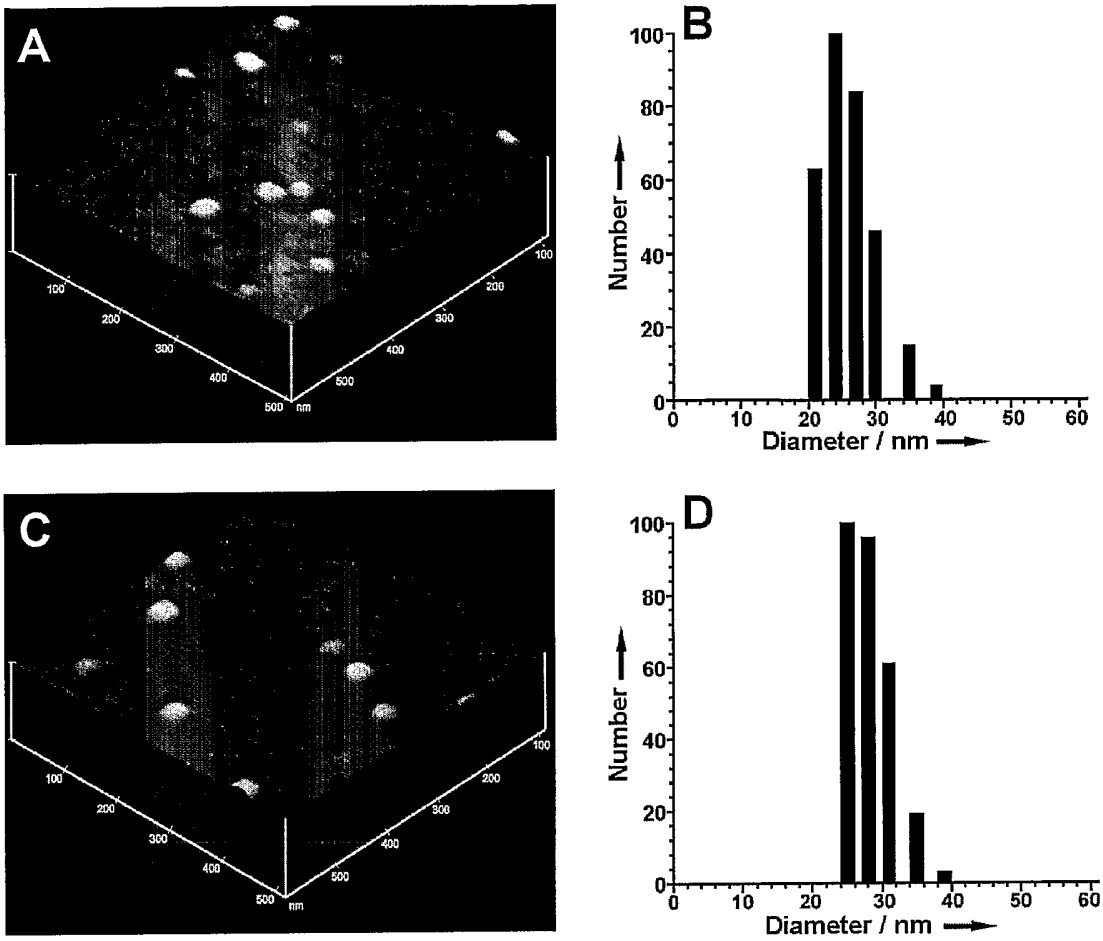


Figure 4

Size characterization of 0% (A, B) and 76% (C, D) cRGD-DOX micelles by atomic force microscopy (A, C) and dynamic light scattering (B, D)

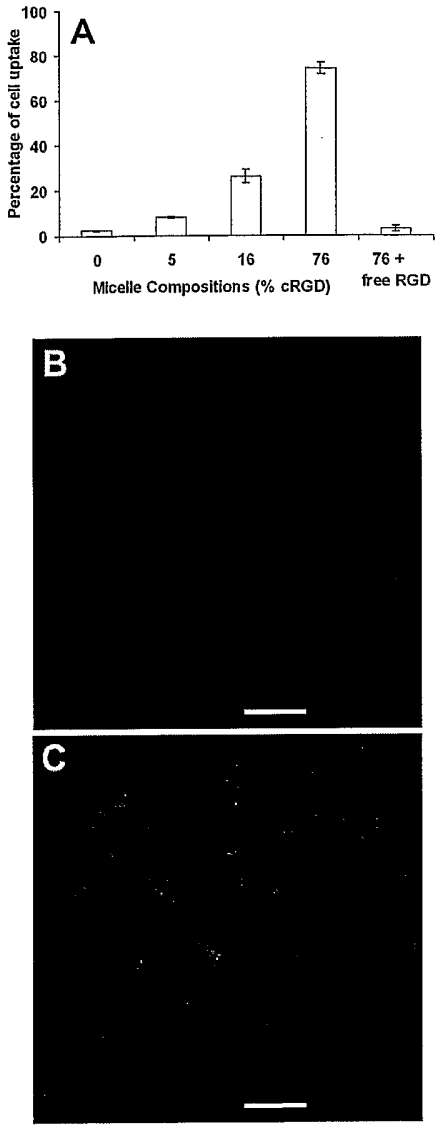


Figure 5

(**A**) Percentage of micelle uptake in SLK tumor endothelial cells measured by flow cytometry as a function of cRGD density (0-76%) on the micelle surface. The last panel shows that the cell uptake of 76% cRGD-micelles is inhibited by the presence of free RGD ligands (9 mM) in solution. (B, C) Confocal laser scanning microscopy images of SLK cells treated with 0% (**B**) and 16% (**C**) cRGD-micelles after incubation for 2 h. Cell nuclei were stained blue by Hoechst 33342 (λ_{ex} = 352 nm, λ_{em} = 455 nm) and overlaid with DOX fluorescent images (λ_{ex} = 485 nm, λ_{em} = 595 nm). The scale bars are 20 μm in both images

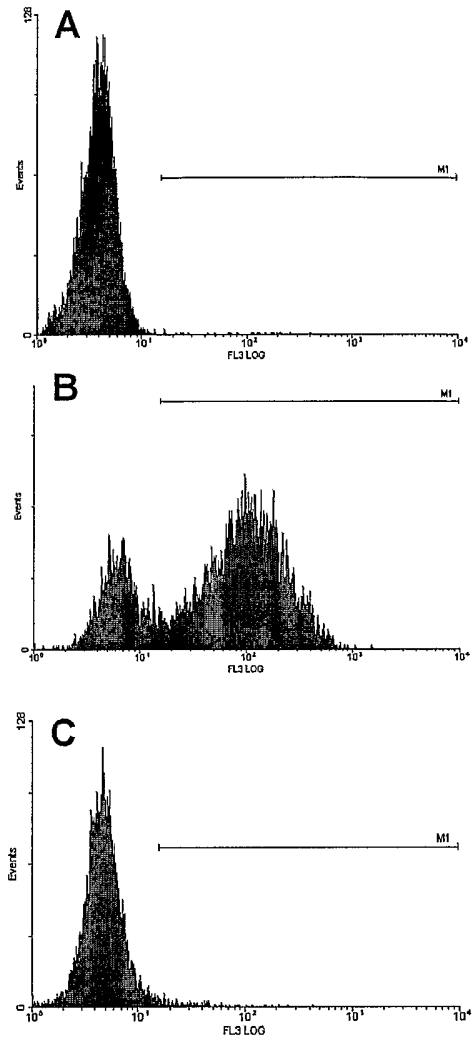


Figure 6

Flow cytometry histogram of micelle uptake in SLK tumor endothelial cells as a function of cRGD density (**A**) 0% (**B**) 76% on the micelle surface. (**C**) Cell uptake of 76% cRGD-micelles is inhibited by the presence of free RGD ligands (9 mM) in solution

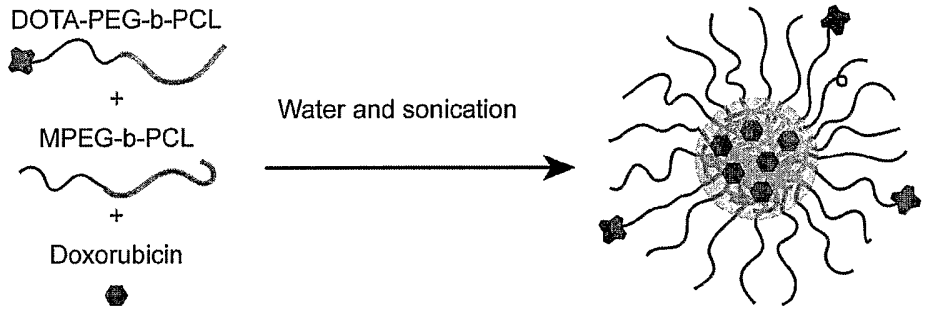


Figure 7

Preparation of DOTA-PEG-b-PCL Micelles

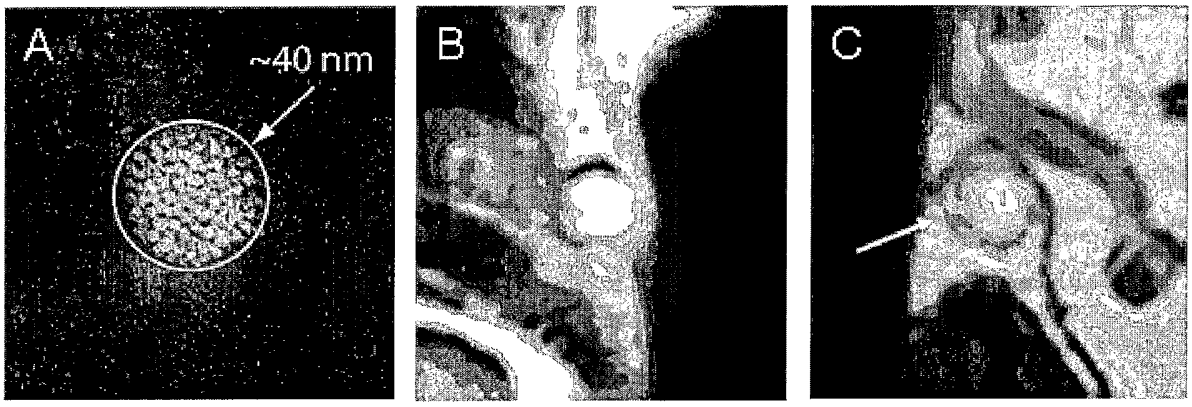


Figure 8

(**A**) Transmission electron micrograph (TEM) of a typical micelle containing numerous individual SPIO particles. The white circle denotes the approximate boundary of the micelle core. (**B**) T_2-weighted MRI image of a control mouse tumor. (**C**) T_2-weighted MRI image of a tumor 72 hours after micelle administration. The white arrow denotes the darkened rim of the tumor likely due to micelle accumulation

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/19308

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 49/00
US CL : 424/9.3
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/9.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US 2005/0025819 A1 (ONYUKSEL et al) 03 February 2005 (03.02.2005), see entire document, especially, abstract; page 6, [0040]; page 7, [0041], [0042], [0045]; and page 8, [0049])	1-30

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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