Fabrication of Dendrimer-Releasing Lipidic Nanoassembly for Cancer Drug Delivery

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Materials and Methods

Materials

1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG, or PEG), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (RhoDOPE) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2,1,3-benzoxadiazol-4-yl) (NBD-DOPE) were purchased from Avanti Polar Lipids Company (Alabaster, AL, USA). The generation 5 (G5) PAMAM dendrimer with ethylenediamine core and amino surface groups, sodium deoxycholate (SDC), sodium dodecyl sulfate (SDS), doxorubicin (DOX) hydrochloride salt, methotrexate (MTX), fluorescein isothiocyanate (FITC) (90+ %), bovine serum albumin (BSA) and McCoy 5A medium were purchased from Sigma-Aldrich (St Louis, MO, USA) and used as received. DRAQ5 TM was purchased from AXXORA LLC (San Diego, CA, USA). The generation 6 polyamino ester dendrimer (G6-PAE) with terminal methacrylates was synthesized using our sequential sticking method. The FITC-PAMAM conjugate was prepared according to our previous report. Gold nanoparticles (AuNPs) were synthesized via a reported method.

Fabrication of Nanoassemblies

PAE/DOPE Nanoassembly

The PAE/DOPE nanoassembly was prepared as follows. DOPE lipid in chloroform was dried by rotary evaporation to obtain a thin lipid film, and hydrated with 4 wt% ethanol aqueous solution. The resulting DOPE lipid solution was stirred at 40 °C to ensure the lipid to dissolve. The PAE dendrimer dissolved in water-miscible organic solvents (e.g., DMSO, DMF and THF) at a concentration of 10 mg/ml was dropped into the preheated DOPE solution with stirring. The
solution was stirred at room temperature for 6 h. The organic solvent was removed via dialysis (Spectra/Pro MWCO: 3500 Da) against pure water for 12 h. The PAE dendrimer concentration in the final solution was kept at 1.2 mg/ml.

During the nanoassembly fabrication, DOPE lipid was the only component which might be removed by dialysis (MWCO = 3500). Therefore, some DOPE lipid was labeled with a fluorescent probe Rhodamin B (\textsuperscript{RhoB}DOPE) and used to fabricate the nanoassembly. After dialysis of the formed nanoassembly solution, the dialysate had a hardly detectable amount of \textsuperscript{RhoB}DOPE, suggesting the actual composition of the nanoassembly was very close to the initial feeding ratio.

As a control, the blank DOPE liposome dispersion was obtained by directly dialysis of the preheated lipid 4 wt% ethanol aqueous solution against pure water for 12 h without the addition of PAE dendrimer.

\textit{PAMAM/DOPE/SDS/PEG Nanoassembly}

The PAMAM/DOPE/SDS/PEG nanoassembly was prepared as follows. PAMAM dendrimer in methanol was firstly dried by rotary evaporator and hydrated with pure water (2 mg/ml). DOPE and DSPE-PEG lipid (dissolved in chloroform, 25 mg/ml) were dried by rotary evaporator and hydrated with pure water to form liposome dispersions at different DOPE/PAMAM, DSPE-PEG/PAMAM molar ratios. SDS was added to the liposome dispersions at the SDS/PAMAM molar ratio of 128 and stirred for 5 minutes. After that, the PAMAM solution was titrated to the resulting DOPE/SDS/DSPE-PEG solution and kept stirring for 1 h. The final concentration in the solution was kept at 1 mg/ml based on PAMAM dendrimer. As a control, the blank liposome dispersion at the same condition was obtained.
Characterizations of Nanoassemblies

All sizes (in diameter) and \( \zeta \)-potentials were measured using dynamic light scattering (DLS) on a Nano-ZS Zetasizer (Malvern Instrument Ltd., UK). The \textit{in vitro} stability of all nanoassemblies was examined by monitoring its size in 10 wt% bovine serum albumin solution at 37 °C under gentle vortex. The nanostructure of PAE/DOPE nanoassembly was observed on a transmission electron microscope (HITACHI H-7000 TEM) and the sample was negatively stained with 2% photungstic acid. The nanostructure of PAM/DOPE/SDS/PEG nanoassembly was observed using a cryo-TEM method. Freeze dried samples were hydrated in 500 \( \mu \)L of distilled water to arrive at a sample concentration of 1 mg dendrimer/ml and filtered through 0.2 \( \mu \)m syringe filters. Cryo-TEM samples were vitrified by rapidly plunging into liquid ethane with the aid of a FEI Vitrobot operated at 22 °C and 100% relative humidity. The images were recorded on a FEI Technai G2 Spirit BioTWIN TEM scope using an accelerating voltage of 120 kV.

FITC Labeling the PAE Dendrimer

The G6-PAE dendrimer was synthesized from G5.5-PAE dendrimer and 2-methacyryloyloxyethyl acrylate monomer according to our previous report. Cysteamine (13.9 mg, 181 mmol) was added to the PAE dendrimer (0.5 g, 5.9 \( \mu \)mol) DMSO solution (8 ml) and stirred at room temperature for 30 min. FITC (84.3 mg, 216 mmol) was added to the above dendrimer solution and stirred overnight at room temperature in the dark. The excess FITC was removed by dialysis against methanol for 12 h and the methanol was evaporated using rotary
evaporator to give the FITC-conjugated PAE dendrimer. The number of FITC conjugated to each dendrimer molecule was measured to be 7.9 based on a standard curve of FITC.

**FITC PAE Fluorescence Quenching Experiment**

FITC PAE/DOPE nanoassembly was fabricated from FITC PAE using the procedure described above. FITC PAE/DOPE solution (1.2 mg dendrimer/ml, 2 ml) in the cuvette was gradually added with AuNPs solution (1 mg/ml) using a microsyringe with stirring. After 30 seconds, the fluorescence emission spectra of the solution were recorded using a SpectraMax M2 microplate reader (Molecular Devices, Inc.). The blank FITC PAE solution as a control was prepared by dissolving it in THF and then diluting the solution with H$_2$O to obtain a FITC PAE solution (H$_2$O: THF= 2:1, v/v) at 1.2 mg/ml. The fluorescence spectra of the solution after adding different amounts of AuNPs were recorded similarly.

The percentage of dendrimer encapsulated in PAE/DOPE nanoassembly was estimated as Equation (1):

$$\text{The percentage of dendrimer encapsulated in PAE/DOPE} = \left(\frac{\text{RFU of FITC (60 } \mu \text{A AuNPs)}}{\text{RFU of FITC (before quenching)}}\right) \times 100\%$$

Equation (1)

**Drug Loading and Release**

*(PAE/DOX)/DOPE Nanoassembly*

PAE dendrimer (6 mg) and DOX hydrochloride salt (0.9 mg for loading efficiency or 1.8 mg for loading content) were dissolved in DMSO (10 mg/ml) with 3 drops of triethylamine and the solution was stirred overnight. The PAE/DOX was used to fabricate the nanoassembly as described above and obtained 5 ml of (PAE/DOX)/DOPE nanoassembly solution. The free DOX and DMSO were removed by dialysis (MWCO: 3500 Da) against pure water (1 L) for 12 h. To
determine the DOX loading capacity, 1 ml of the (PAE/DOX)/DOPE nanoassembly solution was dried and the residue was dissolved in 1 ml of DMSO. The concentration of the DOX was determined by its UV absorbance at 488 nm.

The DOX-loaded liposome control was prepared as follows: DOX hydrochloride salt (0.32 mg for loading efficiency or 0.64 mg for loading content) was stirred in DOPE liposome dispersion (0.64 mg/ml, 5 ml) for 3 h and then 3 drops of triethylamine were added to the mixture and stirred for another 3 h at room temperature.

The drug loading efficiency and loading content were calculated according to the following equations:

\[
\text{Drug Loading Efficiency} (\%) = \frac{\text{Loaded Drug}}{\text{Initial Input Drug}} \times 100\% \quad \text{Equation (2)}
\]

\[
\text{Drug Loading Content} (\%) = \frac{\text{Loaded Drug}}{\text{Initial Input Drug} + \text{total weight of dendrimer and lipid}} \times 100\% \quad \text{Equation (3)}
\]

To measure the DOX release profile, 4 ml of the (PAE/DOX)/DOPE solution (0.1 mg/ml DOX equivalent) was loaded into a dialysis bag (MWCO: 3500 Da). The dialysis bag was immersed in 100 ml of pH 7.4 PBS buffer at 37°C with gentle stirring. At timed intervals, 10 ml of the PBS buffer solution was sampled, and then 10 ml of fresh PBS buffer was refilled. The DOX concentration in the PBS was determined by its UV absorbance at 488 nm according to a calibration curve. The experiments were triplicate.

(PAMAM/MTX)/DOPE/SDS/PEG Nanoassembly

MTX was dissolved in 0.01 N NaOH at the high concentration of 10 mg/ml and dispersed into the PAMAM aqueous solution (obtained as described above, 2 mg/ml) at certain
concentrations. The solution pH was adjusted to 7.4 and kept stirring for 6 h. SDS was added to the DOPE/PEG solutions (obtained as described above) at the SDS/PAMAM molar ratio of 25 and stirred for 5 min. After that, the PAMAM/MTX solution was titrated into the DOPE/SDS/PEG solution and kept stirring for 6 h. The extra MTX was removed via dialysis (Spectra/Pro MWCO: 3500 Da) against pure water for 12 h. The final concentration in the solution was kept at 1 mg/ml based on PAMAM dendrimer. To determine the MTX loading capacity, 0.2 ml of the (PAMAM/MTX)/DOPE/SDS/PEG solution was taken to a well of 96-well plate and the concentration of the MTX was determined by its UV absorbance at 370 nm using a SpectraMax M2 microplate reader (Molecular Devices, Inc.). The MTX-loaded PAMAM dendrimer and liposome control were prepared at the same conditions.

To measure the MTX release profile, 5 ml of the (PAMAM/MTX)/DOPE/SDS/PEG solution (0.26 mg/ml MTX equivalent) was loaded into a dialysis bag (MWCO: 3500 Da). The dialysis bag was immersed in 100 ml of pH 7.4 PBS buffer at 37°C with gentle vortex. At timed intervals, 0.2 ml of the PBS buffer solution was sampled. The MTX concentration in the PBS buffer was determined by its UV absorbance at 370 nm according to a calibration curve. The experiments were triplicate.

**Cellular Internalization**

**Cellular Uptake of PAE/DOPE**

A confocal scanning laser microscope (Zeiss 710 Confocal Microscope) was used to observe the cellular uptake of the FITC-labeled PAE dendrimer and RHoB-labeled DOPE nanoassembly (FITC-PAE/RHoB-DOPE, 10 mol% DOPE was replaced by RHoB-DOPE) as well as (PAE/DOX)/DOPE. SKOV-3 ovarian cancer cells (American Type Culture Collection,
Rockville, MD) were plated into glass-bottom petri dishes (MatTek, Ashland, MA, no. P35G-1.0-14-C) at 250,000 cells per dish in 2 ml of medium supplemented with 10% fetal bovine serum (FBS). Cells were incubated for 24 h at 37 °C and 5% CO₂ before use. The cells were treated with \textsuperscript{FITC}PAE/\textsuperscript{RHoB}DOPE (1.2 mg dendrimer/ml) or (PAE/DOX)/DOPE (0.8 µg/ml DOX-equivalent) medium solution for timed intervals at 37 °C and 5% CO₂ except for observation. \textsuperscript{FITC}PAE and DOX were excited with a 488 nm laser and the emission wavelength ranged 493-548 nm and 535-560 nm were observed, respectively; \textsuperscript{RHoB}DOPE was excited with a 561 nm laser and the emission wavelength ranged 562-660 nm was observed; DRAQ5 TM was excited with a 633 nm laser and the emission wavelength ranged 661-710 nm was observed.

The cellular uptake mechanism was studied using flow cytometry (Guava easycyte 8HT, Millipore Inc.). Chlorpromazine (an inhibitor of clathrin-dependent endocytosis) \textsuperscript{6}, filipin (an inhibitor of caveolae-dependent endocytosis) \textsuperscript{7} and wortmannin (an inhibitor of phosphatidylinositol 3-kinasespathway) \textsuperscript{8} were dissolved in Mccoy 5A medium at the concentrations of 50, 0.015 and 5 µM, respectively. SKOV-3 cells were seeded in 6-well plates at a density of 250,000 cells per well in 2 ml of McCoy 5A medium and incubated at 37 °C and 5% CO₂ for 24 h. The cells in each well were washed twice with PBS and then added with 2 ml of McCoy 5A medium containing the inhibitor at the concentration indicated above, in order to keep cells possessing a healthy morphology \textsuperscript{9}, and incubated for 30 min at 37 °C and 5% CO₂. After that, the medium containing the inhibitor was removed and the cells were incubated with \textsuperscript{FITC}PAE/\textsuperscript{RHoB}DOPE medium solution (1.2 mg/ml dendrimer equivalent) for 6 h, and then washed three times with cold PBS and harvested by trypsin treatment. The harvested cells were suspended in 1 ml of PBS and then centrifuged at 1000 rpm for 5 min. The supernatants were discarded and the cell pellets were washed with 1 ml of PBS to remove the background
fluorescence. After two cycles of washing and centrifugation, cells were re-suspended and
diluted to a final volume of 1 ml in PBS. Control experiments were carried out at the same time.
Intracellular fluorescence of $\text{FITC}_{\text{PAE}}$ and $\text{RHoB}_{\text{DOPE}}$ was analyzed immediately using flow
cytometry. Data were analyzed with guavaSoft 2.2 to obtain the FITC-positive and RHoB-
positive cell percentage.

**Fusion Assay of PAE/DOPE**

The fusion occurred between PAE/DOPE and cell membrane was determined using
fluorescence resonance energy transfer (FRET) approach $^{10, 11}$. DOPE/$\text{RHoB}_{\text{DOPE}}$/NBD/DOPE at a
molar ratio of 94/1/5 was used to formulate the lipid dual-labeled PAE/$\text{RHoB}/\text{NBD}_{\text{DOPE}}$. SKOV-3
cells ($5 \times 10^5$) were plated in 25 cm$^2$- flask containing 5 ml of McCoy 5A medium with 10% FBS
24 h prior to the treatment. The cells were washed three times with PBS-CM (PBS containing
0.36 mM calcium and 0.42 mM magnesium) and were incubated with the 5 ml of
PAE/$\text{RHoB}/\text{NBD}_{\text{DOPE}}$ PBS-CM solution (1.2 mg dendrimer/ml) at 37 °C and 5% CO$_2$ for 6 h.
After the incubation, the cells were washed three times with PBS-CM and once with PBS, and
were then exposed to PBS containing 1mM EDTA for 5 min to dislodge the cells. The harvested
cells were twice washed with PBS and re-suspended in 2 ml of PBS-CM in cutteve. The
fluorescence spectrum of obtained treated-cell suspension was recorded.

To determine and quantitatively estimate the fusion fraction, the fluorescence spectra of
the PAE/$\text{RHoB}/\text{NBD}_{\text{DOPE}}$ PBS-CM solution, solubilized solution (PAE/$\text{RHoB}/\text{NBD}_{\text{DOPE}}$ PBS-CM
solution was solubilized via adding Triton X-100 at the concentration of 0.24 vol%) and treated-
cell suspension (the cells were treated with the nanoassembly solution at 1.2 mg dendrimer/ml
for 6 h and then isolated and resuspended in fresh PBS-CM solution) obtained as described
above were recorded. All of the spectra were excited at 450 nm. Based on the spectra, the ratio of $R_{\text{HoB}}$DOPE fluorescence intensity at 585 nm to $\text{NBD}$DOPE fluorescence intensity at 525 nm for the PAE/$R_{\text{HoB}}$/NBD$\text{DOPE}$ taken up by cells can be expressed as $R$ in the Equation (4)\textsuperscript{11}.

\[
R = \frac{I_{585}}{I_{525}} = \frac{I_{\text{St}}^{R_{\text{HoB}}} \times F + I_{\text{St}}^{R_{\text{HoB}}} \times (1 - F)}{I_{\text{So}}^{\text{NBD}} \times F + I_{\text{St}}^{\text{NBD}} \times (1 - F)}
\]

\text{Equation (4)}

where $I_{\text{St}}^{R_{\text{HoB}}}$, $I_{\text{St}}^{R_{\text{HoB}}}$, $I_{\text{St}}^{\text{NBD}}$, and $I_{\text{So}}^{\text{NBD}}$ are the fluorescence intensities of and $\text{NBD}$DOPE of the starting PAE/$R_{\text{HoB}}$/NBD$\text{DOPE}$ PBS-CM solution (st) and solubilized solution (so), respectively. $F$ is the fraction of PAE/$R_{\text{HoB}}$/NBD$\text{DOPE}$ fused with cell membrane, and its value can be calculated from Equations (5):

\[
F = \frac{I_{\text{St}}^{R_{\text{HoB}}} - RI_{\text{St}}^{\text{NBD}}}{R(I_{\text{So}}^{\text{NBD}} - I_{\text{St}}^{\text{NBD}}) - I_{\text{So}}^{R_{\text{HoB}}} + I_{\text{St}}^{R_{\text{HoB}}}}
\]

\text{Equation (5)}

**Extracellular Dendrimer Release of PAE/DOPE**

SKOV-3 cells ($2.5 \times 10^5$) were plated in 6-well plates containing 3 ml of McCoy 5A medium with 10% FBS 24 h prior to the experiment. The cells were washed three times with PBS, and then added with 2 ml of $\text{FITC}$PAE/DOPE PBS-CM solution (1.2 mg dendrimer /ml) and incubated at 37 °C for timed intervals. After the incubation, the $\text{FITC}$PAE/DOPE PBS-CM solution over the cells was collected to a cuvette. The fluorescent spectra of the solution before and after adding the AuNP suspension (1 mg/ml) were recorded.

**Cellular Uptake of (PAMAM/MTX)/DOPE/SDS/PEG**

A confocal scanning laser microscope was used to observe the cellular uptake of ($\text{FITC}$PAMAM/MTX)/$R_{\text{HoB}}$DOPE/SDS/PEG, in which PAMAM dendrimers were labeled by
FITC and DOPE lipids were labeled by RHoB (10 mol% DOPE was replaced by RHoB-DOPE, 5 mg/ml). SKOV-3 ovarian cancer cells (American Type Culture Collection, Rockville, MD) and MDA-MB-468 breast cancer cells (American Type Culture Collection, Rockville, MD) were plated into glass-bottom petri dishes (MatTek, Ashland, MA, no. P35G-1.0-14-C) at 250,000 cells per dish in 3 ml of medium supplemented with 10% FBS. Cells were incubated for 24 h at 37 °C and 5% CO₂ before use. The cells were treated with (FITC-PAMAM/MTX)/RHoB-DOPE/SDS/PEG (10 µg/ml MTX-equivalent) medium solution for timed intervals at 37 °C and 5% CO₂ except for observation. FITC-PAMAM was excited with a 488 nm laser and the emission wavelength ranged 493-548 nm was observed; RHoB-DOPE was excited with a 561 nm laser and the emission wavelength ranged 562-660 nm was observed, respectively; MTX was excited with a 358 nm laser and the emission wavelength ranged 440-480 nm was observed.

The cellular uptake was quantitatively studied using flow cytometry. SKOV-3 and MDA-MB-468 breast cancer cells were seeded in 6-well plates at a density of 250,000 cells per well in 3 ml of medium supplemented with 10% FBS and incubated at 37 °C and 5% CO₂ for 24 h before use. The cells in each well were washed twice with PBS and then added with 3 ml of (FITC-PAMAM/MTX)/RHoB-DOPE/SDS/PEG medium solution (10 µg/ml MTX-equivalent) for different time intervals, and then washed three times with cold PBS and harvested by trypsin treatment. The harvested cells were suspended in 1 ml of PBS and then centrifuged at 1000 rpm for 5 min. The supernatants were discarded and the cell pellets were washed with 1 ml of PBS to remove the background fluorescence. After two cycles of washing and centrifugation, cells were re-suspended and diluted to a final volume of 1 ml in PBS. Control experiments were carried out.
at the same time. Intracellular fluorescence of \textit{FITC}PAMAM and \textit{RHoB}DOPE was analyzed immediately with guavaSoft 2.2 to obtain the fluorescent-positive cell percentage.

The cellular uptake mechanism was studied using flow cytometry. Chlorpromazine, filipin and wortmannin were dissolved in McCoy 5A medium at the concentrations of 50, 0.015 and 5 µM, respectively. SKOV-3 cells were seeded in 6-well plates at a density of 250,000 cells per well in 2 ml of McCoy 5A medium and incubated at 37 °C and 5% CO\textsubscript{2} for 24 h. The cells in each well were washed twice with PBS and then added with 2 ml of McCoy 5A medium containing the inhibitor at the concentration indicated above and incubated for 30 min at 37 °C and 5% CO\textsubscript{2}. After that, the medium containing the inhibitor was removed and the cells were incubated with \textit{(FITC}PAMAM/MTX)/\textit{RHoB}DOPE/SDS/PEG medium solution (10 µg/ml MTX-equivalent) for 2 h, and then washed three times with cold PBS and harvested by trypsin treatment. The harvested cells were suspended in 1 ml of PBS and then centrifuged at 1000 rpm for 5 min. The supernatants were discarded and the cell pellets were washed with 1 ml of PBS to remove the background fluorescence. After two cycles of washing and centrifugation, cells were re-suspended and diluted to a final volume of 1 ml in PBS. Control experiments were carried out at the same time. Intracellular fluorescence of \textit{FITC}PAE and \textit{RHoB}DOPE was analyzed immediately using flow cytometry. Data were analyzed with guavaSoft 2.2 to obtain the FITC-positive and RHoB-positive cell percentage.

\textbf{In Vitro Cytotoxicity}

The cytotoxicity assay was carried out using the (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) (MTT) cell proliferation kit (ATCC, Manassas, VA), according to the modified manufacturer’s protocol. SKOV-3 and MDA-MB-468 cells were seeded in 96-well
plates at a density of 12,000 or 6000 cells per well and cultured in 200 μl of McCoy 5A medium for 24 h before the treatment. For the PAE dendrimer system, the original medium was removed and replaced with free DOX, PAE/DOPE and (PAE/DOX)/DOPE at different doses and incubated for 24 h or 72 h. For the PAMAM dendrimer system, the original medium was removed and replaced with free MTX, blank PAMAM dendrimer and (PAMAM/MTX)/DOPE/SDS/PEG at different doses and incubated for 24 h. The medium in each well was then replaced with fresh medium and the cells were further incubated for 24 h. MTT reagent (10 μl) was then added to each well and incubated for 6 h at 37 °C or until purple crystals were visible. The absorbance intensity at 570 nm was recorded and the cytotoxicity was expressed as the percentage of the control.

**Figures**

**Fig. S1** The fluorescence spectrum of FITC PAE dendrimer (a) and the standard curve of FITC in DMSO (b). The excitation and emission wavelength of FITC was 480 and 530 nm, respectively.
Fig. S2 The size (a) and ζ-potential (b) of (PAMAM/MTX\textsubscript{12})/DOPE\textsubscript{20}/SDS\textsubscript{25}/PEG\textsubscript{5}.

Fig. S3 MTX loading capacity of the nanoassembly compared with the blank PAMAM dendrimer and liposome carrier. The concentration was 1.0 mg/ml based on the dendrimer-equivalent for all solutions.
**Fig. S4** MTX release profile (a) and the plot of MTX release versus square root of time (t^{1/2}) (b) of (PAMAM/MTX)/DOPE/SDS/PEG nanoassembly, compared to free MTX diffusion, blank PAMAM dendrimer and liposome carrier. The MTX-equivalent dose of starting was 0.26 mg/ml.

**Fig. S5** Fluorescence spectrum of FITC-PAMAM (a) and the standard curve of FITC in methanol (b). The excitation and emission wavelength of FITC was 480 and 530 nm, respectively.
Fig. S6 Cellular uptake of \((\text{FITC-PAMAM/MTX})/\text{RhoB-DOPE/SDS/PEG}\) nanoassembly as observed by confocal laser scanning fluorescence microscopy. MDA-MB-468 cells were incubated with the nanoassembly (10 μg/ml MTX-equivalent) for timed intervals. \text{FITC-PAMAM} is shown in green, \text{RhoB-DOPE} is shown in red, and MTX is shown in blue.
**Fig. S7** Cellular uptake of (**FITC**PAMAM/MTX)/**RhoB**DOPE/SDS/PEG analyzed using flow cytometry. The MTX-equivalent dose was 10 µg/ml. Intracellular fluorescence of **FITC**PAMAM and **RhoB**DOPE was analyzed to obtain the fluorescent-positive cell percentage.
Fig. S8  *In vitro* cytotoxicity MTT assay of free MTX, PAMAM dendrimer, and (PAMAM/MTX)/DOPE/SDS/PEG to MDA-MB-468 cells (24 h) as a function of the MTX or PAMAM dendrimer dose.

References