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Polymeric Micelle Based Bioassay with Femtomolar Sensitivity

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Abstract

Target specific polymeric micelles loaded with fluorescence dye molecules in their hydrophobic cores were made from block co-polymer of poly(carpolactones)₂₃-b-poly(ethylene oxide)₄₅ (PCL23-b-PEO45). It was found that the micelles are stable against pH changes from 2 to 12 and temperature variation up to 65 °C. The dye molecules can be released to the solution upon exposing the micelles to organic solvents or ultrasound. A rapid and highly sensitive immunoassay based on the above micelles was developed, and the assay can detect specific target proteins in femtomolar range from complex biological samples such as serum mimics and cell lysate. For example, less than 0.15 unit/ml of ovarian cancer specific antigen 125 (CA125), equivalent to 7.5×10^{-15} M, can be reliably detected in solution. We also demonstrated the assay can detect a cell surface biomarker (SAAE-4) from a single human embryonic stem (hES) cell.

Introduction

Polymeric micelles have gained a lot of attention in the field of drug delivery due to their great stability and encapsulation capability [1]. Polymeric micelles are formed by self-assembling amphiphilic block-copolymers when they are dissolved in selective solvents [2]. Upon micellization, the hydrophobic blocks interact with each other and form the core of the micelle while the hydrophilic blocks stay in contact with water to form the corona [3]. The shape and the size of the polymeric micelles are influenced by: 1) the copolymer chains size and chemical nature 2) The type of the solvent used to dissolve the copolymer and 3) the critical micelle concentrations of the copolymer [4,5,6]. Since polymeric micelles can solubilize poorly water-soluble drugs and have a long circulation time in blood as compared to other types of delivery systems, these vesicles are promising candidates for the next generation of drug carriers with targeted and controllable drug release [7].

While the applications of polymeric micelles in drug delivery have been the subject of studies by others, we focused on the use of polymeric micelles as tracers and imaging probes for bioassay development and cellular imaging. Our strategy is very straightforward: since polymeric micelles have been proven to be stable and efficient carriers for hydrophobic drug molecules, we aimed to encapsulate hydrophobic fluorescent dye molecules within the antibody conjugated micelles, and use them as a probe to follow the binding event of antibody

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and antigen. After washing away unbound polymeric micelles, we can detect the antigen by first releasing the encapsulated dye molecules into the solution and subsequently measuring the fluorescence signal of the solution. This creates a highly sensitive bioassay due to the signal amplification process, in which one binding event translates into the fluorescence signal of hundred thousands of dye molecules released from a single polymeric micelle to the solution. While linking an antibody molecule with many dye molecules has been an effective way to increase an assay's sensitivity, it has typically been achieved by attaching the antibody to dendrimers or particles [8,9,10], on which the dye molecules were attached on the surface. In our case, the dye molecules are encapsulated inside the micelles. Due to self-quenching events, these dye loaded micelles have extremely low fluorescence. By removing free dye molecules in the solution through the dialysis or ultracentrifuge, we achieve a very low background before releasing the dye molecules. Thus, the assay exhibits great sensitivity due to the very high signal to noise ratio after the dye molecules are released.

To demonstrate this assay, we synthesized dye loaded polymeric micelles from block copolymer of poly(carpolactones)₂₂-b-poly(ethylene oxide)₄₅ and developed the corresponding bioassay to detect antigens in both serum mimics and cell lysate. Our results include the successful detection of cancer antigen 125 (CA125) [11] in the level of 1 unit/ml from a serum mimic sample and the detection of Stage Specific Embryonic Antigen 4 (SSEA-4) in cell lysate from a single human embryonic stem cell.

Experimental Section

Materials

Poly(carpolactones)₂₂-b-poly(ethylene oxide)₄₅ were obtained from Polymer Source Inc. Methoxypolyethylene glycol succinate N-hydroxysuccinimide ester, N,N-Dimethyl formamide (DMF 99.8% anhydrous) and Dimethyl Sulfoxide(DMSO) were purchased from Sigma Aldrich. Dialysis cassettes (MWCO-10K) were acquired from Pierce Biotechnology. Tosylactivated Dynabeads (M-280) and Streptavidin coated magnetic beads were purchased from Dynal Biotech. hES cell line H9 was obtained from Wicell Research Institute (Madison, WI).

Instrumentation

A Dynapro laser light scattering instrument with Uniphase μ Blue laser at wavelength of 532 nm was used to determine the size distributions of the micelles at 25 °C. Labeled and unlabeled PCL₂₃-b-PEO₄₅ block copolymers micelle solutions were clarified by centrifugation (14,000 $\times$ g for 5 minutes) and used at a concentration of 0.1%

Transmission electron microscopy (TEM) was performed with a Tecnai F30 at 300KV (FEI Company).

For the polymer micelles fluorescence study, we used a PTI QuantaMaster™ Model QM-6/2005 spectrofluorometer to measure the dye emission during the micelle characterization and detection process.

Micelle Preparations

1) Preparation of polymeric micelles—20 mg of PEO₄₅-b-PCL₂₃ block copolymers were dissolved in 0.38 g of DMF to yield 0.4 g of solution; the solution was stirred for 3 h. Deionized water was added at a rate of 10 μ l every 30s for a total of 1.6 mL to induce micellization. The aqueous solution was dialyzed (10 kDa MWCO, *Pierce Biotech.*) against deionized water for 2 days. After dialysis, the polymer solution was diluted to 0.67% (w/w) by water for subsequent experiments.

2) Preparation of bioconjugated micelles

a)- Bioconjugation of Polyethylene glycol with anti-SSEA4 (anti-SSEA4-PEO):

Methoxypolyethylene glycol succinate N-hydroxysuccinimide ester (1 mg) was added to the solution of Anti-SSEA4 antibody (31 µg) in 0.1 M bicarbonate buffer (400 µl, pH ~8.3). The mixture was then gently stirred overnight in slow tilt rotation at 4°C. The resulting solution was diluted to 2 ml. The same procedure described above was used to prepare both anti-CD133-PEO and M11-PEO.

b)- Preparation of loaded bioconjugated micelles: 20 mg of PEO₄₅-*b*-PCL₂₃ block copolymers and 0.5 mg of dimethylamino stilbene acetyl ketone (DSAK) were dissolved in 0.5 ml of DMF. The mixture was stirred for 3 hours at room temperature. Subsequently, 2 mL anti-SSEA4-PEO aqueous solution was added at a rate of one drop every 10s to induce micellization. The resulting solution was then placed in a Dialysis cassette with a 10kDa cut-off and dialyzed against deionized water for 2 days at 4°C. After dialysis, the solution was diluted 5 times in PBS buffer. Based on the ELISA experiments using a HRP conjugated secondary antibody against the antibody-micelle complex and the supernatant of the reaction, we estimated the yield of the bioconjugation to be around 90%. Bioconjugated micelles with M11 and anti-CD133 were prepared similarly.

Polymeric micelle based bioassay

1) CA125 detection—50 µg of the Anti-CA125 antibody was added to tosylactivated magnetic beads (8×10^7 beads, 400 µl) in 0.1 M borate buffer (pH 9.5). The mixture was then incubated for 16-24 hours with slow tilt rotation at 4°C. Unbound antibody was removed using a magnetic separator. The beads were then washed twice with PBS (phosphate buffered saline, pH 7.4) containing 0.1% (w/v) BSA for 5 minutes each at 4°C, followed by blocking the remaining active tosyl- groups with 0.2 M Tris buffer (pH 8.5) containing 0.1% (w/v) BSA. The beads were finally washed with PBS (pH 7.4).

The freshly prepared antibody-coated beads were incubated with CA125 solution at different concentrations (30, 10, 1.1, 0.3 and 0.1 units per ml, respectively) for 2 hours. The unbound CA125 was then removed by washing four times in PBS followed by magnetic separation. Subsequently, 200 µl of polymeric micelles conjugated with M11 antibody was added to the beads. The mixture was gently stirred for 2 hours at 4°C. The unbound bioconjugated micelles were removed by magnetic separation. After washing the beads four times in PBS, the dyes were released from the polymeric micelles by the addition of THF (1 ml). The amount of dye released in each sample was quantitated by fluorimetry.

2) The detection of hES cell-specific marker

a) hES Cell culture: hES cells were cultured using feeder-free culturing conditions [12]. In brief, hES cell colonies (NIH H9) at passages from 26 to 35 were dissociated by incubation with 1 mg/ml dispase (Invitrogen) for 3-5 mins at 37°C and seeded onto Matrigel (Becton-Dickenson labware, Bedford, MA) coated plates in MEF conditioned media supplemented with 4 ng/ml bFGF. Cells were passaged at least 3 times but no more than 10 times under feeder-free conditions before they were used for protein extraction.

b) Membrane protein preparation: hES cells grown under feeder free conditions were dissociated into single cells by incubation with Trypsin/EDTA (Invitrogen) for 8 mins at 37°C. Cells were washed 3 times with PBS, and resuspended in 1 ml of PBS containing 100 µg of sulfo-NHS-LC-biotin per 1 million cells (pH =8.5). The biotinylation was carried out on ice for a period of 30 minutes with occasional gentle mixing. The reaction was stopped by the addition of 300 µL of TBS (tris buffer saline) followed by two washes with TBS buffer. Surface biotinylated hES cells were resuspended in 2 ml membrane buffer containing protease

inhibitors and lysed via a nitrogen disruptor at 500 psi for 5 minutes. The clarified supernatant was used for the bioassay. Lysate from MEF was prepared in the same manner and used as controls.

c) The detection of hES cell-specific marker: Diluted stem cell lysates, equivalent to the amount from 1, 100, and 1000 cells, were incubated with 20 μ l of Streptavidin coated magnetic beads overnight at 4°C. The beads were then washed five times with PBS buffer, and incubated with 200 μ l of polymer micelles conjugated with *anti-SSEA4* or anti-CD133 antibodies for 2 hours at 4°C. After incubation, the beads were washed with PBS. The encapsulated dyes were released and the solution fluorescence was measured in the same manner as described above for CA125 detection.

hES Cell culture under the influence of SDAK (toxicity study)

hES cells were cultured under the feeder-free culturing condition using the above procedure with the exception that the media contains 0.1 mg/ml of SDAK or 0.5 mg/ml polymeric micelles encapsulated with SDAK. The SDAK media was replaced with normal MEF conditioned media supplemented with 4 ng/ml bFGF after one day and the morphology of the stem cell colony was characterized on the third day.

Results and Discussion

1. Fluorescent dye encapsulation and release from polymeric micelles

The preparation of PCL23-b-PEO45 micelles and dye encapsulation were carried out using a modified literature procedure [13]. In brief, the solution of PCL23-b-PEO45 in DMF at concentration of 11.4 mM was prepared and the micelle formation was achieved by adding deionized water (1:40) at a rate of 20 μ l/minute at room temperature. The polymeric micelles were purified by dialyzing against water. Polymeric micelles with dye encapsulated were prepared under the same conditions except the micelle was formed in the presence of 0.5 mg of dimethylamino stilbene acetyl ketone (DSAK). Figure 1 shows the size distribution profile of polymeric micelles with and without the dye inside their core, as measured by Dynamic Light Scattering (DLS). The native polymeric micelles have an average diameter of 23 nm, while the dye encapsulated polymeric micelles exhibit significantly increased diameters with an average value of 37 nm. The dye encapsulated polymeric micelles were also visualized under a Transmission Electron Microscope (TEM) by negatively staining with 1% uranyl acetate (Figure 1, inset). The size of the polymeric micelles obtained from TEM matches the values derived from the DLS studies.

The dye encapsulation was confirmed by the toxicity study of the dye encapsulated micelles with hES cells. SDAK molecules are very toxic to stem cells. When present in the media, 50 μ M of SDAK induced overall cell death in three days. However, upon the encapsulation of SDAK molecules within polymeric micelles, the polymeric micelles minimized the dye's toxicity against embryonic stem cells and cell colony growth remains normal for 3 days. This result suggests that the dye molecules are encapsulated and retained inside the micelles.

The fluorescence of SDAK is self-quenched when the dye molecules are encapsulated within the micelles, whereas the fluorescence signal is dramatically enhanced when the molecules are released to the solution. To use this signal amplification process for a novel bioassay, several approaches for releasing the dye, including pH variation, temperature changes, and exposure of the micelles to organic solvents or ultrasound, were probed. It was found (supplementary data) that dye encapsulated micelles remain stable against pH variation (from pH 2 to 12) and temperature changes from 20 to 65 °C. No fluorescence increases (i.e. dye leakage) could be observed under the above conditions. However, when the dye encapsulated micelles were

treated with organic solvents such as THF or acetonitrile, the fluorescence intensity of the bulk solution increases dramatically (Figure 2). This indicated substantial release of dye molecules into the solution. Similar observations were obtained when the micelle solution was exposed to probe sonification at a frequency of 20 KHz at 0 °C for 5 minutes (supplementary data).

The approximate micelle aggregation number (N_{agg}) can be calculated from the ratio of the average molecular weight of the micelle to the molecular weight of the block copolymer [14, 15,16]. Dynamic Light Scattering experiments indicated that the average molecular weight of the micelle is $\sim 4.6 \times 10^4$ Dalton. We thus estimated an aggregation number of 10. Accordingly, the maximum number of micelles formed in a solution of 1 mL was found to be $\sim 3 \times 10^{10}$. By correlating the dye concentration with its fluorescence intensity experimentally (supplementary data), we quantified the total dye molecule number as 3.3×10^{15} after they were released to the solution. Since we washed off any uncapsulated dye in the solution before the disruption of micelles, we can consequently estimate that one micelle with an average diameter ($R_h = 37$ nm, $N_{agg} \sim 10$) can load at least one hundred and ten thousand (1.1×10^5) dye molecules. The release of these dye molecules to the solution provides a significant signal amplification process for monitoring the antigen-antibody binding event, which can be harnessed to develop a novel, sensitive bioassay.

2. Polymeric micelle based bioassay for CA125 detection

Figure 3A depicts the new bioassay using target specific, dye encapsulated polymeric micelles. In brief, the assay uses magnetic beads with a capturing reagent to capture the target molecules from the sample (pre-prepared solution, serum mimic or cell lysate, etc.). After washing off non-specifically bound molecules, antibody labeled micelles are incubated with the beads, followed by washing off unbound micelles. The presence of target molecules was detected by first adding organic solvent to the solution and then measuring the fluorescence enhancement in solution.

The first target molecule we tested with the new assay was cancer antigen 125 (CA-125). CA125 is a secreted protein that is found at elevated levels in the serum of ovarian cancer patients. Clinically, a CA125 test of great than 30 unit/ml (1 unit is roughly equivalent to 1 ng) is an indication of possible ovarian carcinoma. Currently, two types of antibodies have been developed against CA125: antibody OC125 that recognizes epitope A of CA125 and antibody M11 that recognizes epitope B of the CA125 [17]. In our assay, we used OC125 antibody as the capture reagent to capture the CA125 in the solution to the beads and M11 antibody conjugated with polymeric micelles as the secondary antibody. The preparation of M11 antibody conjugated micelles was carried out by conjugating poly(ethylene oxide) with the M11 antibody to form PEG-M11 complex first, followed by mixing PEG-M11 with dye and block copolymer during the micellization process to form M11 antibody conjugated micelles. Commercial CA125 antigen was used to prepare positive control samples containing CA125 with concentrations ranging from 10 unit/ml to 0.1 unit/ml. Figure 3B shows the fluorescence intensity at 540 nm from DSAK released from the micelle after binding to CA125 as a function of the concentration of CA 125. When only 0.15 unit/ml of CA 125 is presented in the solution, the assay still exhibits detecting signal (6000) 3 times stronger than the background and control experiments signals (ranging from 800 to 2000 counts per second, Figure 3C). The fact that the polymeric micelle assay could easily detect CA125 at the level of 0.15 unit/ml suggests that it has a sensitivity ~ 20 fold higher than the reliable detection limitation of current commercial ELISA CA125 kit specified by the manufacturer (2 units/ml). Considering the molecular weight of CA125, which is ~ 2 million Dalton [18], the assay can reliably detect 7.5×10^{-15} M of CA125 without any optimization. A series of control experiments (Figure 3C) were also conducted. Virtually no fluorescence signals were observed in magnetic beads with bioconjugated micelle samples, indicating no nonspecific interactions with polymeric micelles.

Furthermore, when the CA125 antigen was replaced with BSA (1mg/ml), again we did not observe any significant fluorescence signals.

Since clinical detection of CA125 will be performed on a serum sample, we tested the performance of the polymeric micelle assay in goat serum. To do this, we mixed goat serum with CA125 with concentrations ranging from 30 unit/ml to 0.1 unit/ml. Pure goat serum without CA125 antigen was also used as a negative control sample. As shown in Figure 3D, 1 unit/ml of CA125 can be reliably detected in goat serum. It is interesting to see that native goat serum sample showed a fluorescence peak at 520nm, likely due to the presence of fluorescent species in the goat serum. Overall, this assay allowed us to detect CA125 at femtomolar levels.

3 Human embryonic stem cell biomarker detection from a single cell

The ability of quantifying the level of biomarker from a single stem cell is critical to evaluate its differentiation or proliferation states. Traditional immunofluorescence detection may not be sensitive enough to achieve this goal. Accordingly, we applied the above bioassay to determine the level of surface biomarker, SSEA-4, in the human embryonic stem cells. Cell surface proteins were first biotinylated by sulfo-NHS-LC-biotin reagent *in situ* using living stem cells. After washing twice with TBS buffer to remove the excess of sulfo-NHS-LC-biotin, biotinylated stem cells (1.0×10^6 cells) were re-suspended in 2 mL membrane buffer containing protease inhibitors and lysed via nitrogen disruptor at 500 psi for a period of 5 minutes. Lysate samples from the biotinylated stem cells were diluted 10^3 , 10^4 and 10^6 fold, which corresponds to cell lysate from 1000, 100 and one cell, and subjected to analysis using the new developed bioassay.

As depicted in Figure 4A, we used avidin (immobilized on the bead surface) as the capture reagent to capture the biotinylated surface proteins, and the SSEA-4 antigen present in different samples was detected using SSEA-4 antibody conjugated polymeric micelles. After releasing the dye from the micelles, the abundance of SSEA-4 among the surface proteins can be evaluated by measuring the emission intensity of the solution. Figure 4B demonstrated the fluorescence intensity at 540 nm from DSAK released from the micelle after binding to SSEA-4 as a function of stem cell quantity. The assay can easily detect SSEA-4 biomarker from cell lysis of a single stem cell with a strong signal to noise ratio (7:1, Figure 4C). Also a series of control experiments were performed using the same procedures and reagents to probe the SSEA-4 level in biotinylated mouse embryonic fibroblast cells lysate (~200,000 cells) and unbiotinylated human embryonic stem cells lysis (~200,000 cells). Also illustrated in Figure 4C, negligible fluorescence signals can be observed from both solutions indicating that not only is no SSEA-4 expressed on the mouse embryonic fibroblast cells, but there are no non-specific interactions between the unbiotinylated proteins and the streptavidin coated beads, confirming the high fidelity of this novel bioassay.

To evaluate the assay performance, the level of SSEA-4 in human embryonic stem cells was also characterized by a conventional ELISA using a HRP conjugated SSEA-4 antibody. While the ELISA can reliably detect SSEA-4 from cell lysis of 1000 or more cells (see supplementary section), optical density signals for the cell lysis sample from less than 1000 cells was buried by the background noises. The results demonstrate that the polymeric micelle based bioassay shows a better performance by at least a factor of 100 compared to the conventional ELISA for stem cell biomarker detection.

At the same time, we also conducted a negative control experiment using human embryonic stem cells. It has been reported that surface biomarker CD133 [19,20] is not present in embryonic stem cells. We tried to confirm this observation using our newly developed assay. Accordingly, dye loaded polymer micelles were bioconjugated with CD133 antibody. After mixing biotinylated human embryonic stem cells lysate (200,000 cells) with streptavidin coated

beads, CD133 antibody conjugated polymeric micelles were introduced, incubated and washed, and mixed with organic solvent. Only background levels of fluorescence were observed, indicating that the CD 133 antigen is not present on the surface of human embryonic stem cells within our detection limit. A conventional ELISA using a HRP conjugated CD133 antibody was also performed and the outcome confirms the absence of this antigen from the human embryonic stem cell (no positive control was used).

Conclusion

We synthesized and characterized polymeric micelles encapsulating a hydrophobic fluorescent dye. The studies showed that the polymeric micelles are stable against pH, and have a phase transition temperature around 65 °C. The polymeric micelles can encapsulate around 1.1×10^5 dye molecules and the dye molecules can be released by ultrasound or organic solvent treatment. We further developed a biodetection assay based on the polymeric micelles with femtomolar sensitivity and capable of detecting a stem cell biomarker from a single cell. We expect to further improve the assay sensitivity by 1) developing new hydrophobic dye with enhanced fluorescence features, 2) developing new class of amphiphilic polymers with larger loading capacity and 3) scaling down the handling volumes by using microfluidic system. Future work, including use of ultrasound as a gentler dye-release mechanism for samples of proteins or cells is currently in progress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Hamley IW. Nanoshells and nanotubes from block copolymers. *Soft matter* 2005;1:36–43.
2. Meier, Michael AR.; Aerts, Sebastianus NH.; Staal, Bastiaan BP.; Rasa, Mircea; Schubert, Ulrich S. PEO-b-PCL block copolymers: Synthesis, detailed characterization, and selected micellar drug encapsulation behavior. *Macromolecular Rapid Communications* 2002;24:1918–1924.
3. Loh, Watson. *Encyclopedia of Surface and Colloid Science*. Marcel Dekker, Inc.; 2002.
4. Savic, Radoslav; Luo, Laibin; Eisenberg, Adi; Maysinger, Dusica. Micellar Nanocontainers Distribute to Defined Cytoplasmic Organelles. *Science* 2003;300:615–618. [PubMed: 12714738]
5. Sidorov SN, Bronstein LM, Kabachii YA, Valetsky PM, Lim Soo P, Maysinger D, Eisenberg A. Influence of Metallation on the Morphologies of Poly (ethylene oxide)-block-Poly (4-vinylpyridine) Block Copolymer Micelles. *Langmuir* 2004;20:3543–3550. [PubMed: 15875382]
6. Nasongkla N, Shuai X, Ai H, Weinberg BD, Pink J, Boothman DA, Gao J. cRGD-functionalized polymer micelles for targeted doxorubicin delivery. *Angew Chem Int Ed Engl* 2004;43(46):6323–6327. [PubMed: 15558662]
7. Lavasanifar A, Samuel J, Kwon GS. Poly (ethylene oxide)-*block*-poly (L-amino acid) micelles for drug delivery. *Advanced Drug Delivery Reviews* 2002;54:169–190. [PubMed: 11897144]
8. Choi, Youngseon; Thomas, Thommey; Kotlyar, Alina; Islam, Mohammad T.; Baker, James R, Jr. Synthesis and functional evaluation of DNA-assembled polyamidoamine dendrimer clusters for cancer cell-specific targeting. *Chemistry & Biology* 2005;12:35–43. [PubMed: 15664513]

9. DeCory, Thomas R.; Durst, Richard A.; Zimmerman, Scott J.; Garringer, Linda A.; Paluca, Gary; DeCory, Heleen H.; Montagna, Richard A. Development of an immunomagnetic bead-immunoliposome fluorescence assay for rapid detection of *Escherichia coli* O157:H7 in aqueous samples and comparison of the assay with a standard microbiological method. *Applied And Environmental Microbiology* 2005;71:1856–1864. [PubMed: 15812012]
10. Quinti, Luisa; Weissleder, Ralph; Tung, Ching-Hsuan. A. Fluorescent Nanosensor for Apoptotic Cells. *Nano Lett* 2006;6:488–490. [PubMed: 16522048]
11. Saksela F. Prognostic markers ovarian cancer. *Intl J Gynecol Pathol* 1993;12:115–114.
12. Thomson, James A.; Itskovitz-Eldor, Joseph; Shapiro, Sander S.; Waknitz, Michelle A.; Swiergiel, Jennifer J.; Marshall, Vivienne S.; Jones, Jeffrey M. Embryonic Stem Cell Lines Derived from Human Blastocysts. *Science* 1998;282:1145–1147. [PubMed: 9804556]
13. Laibin L, Tam J, Maysinger D, Eisenberg A. Cellular Internalization of Poly (ethylene oxide)-b-poly (caprolactone) diblock copolymer Micelles. *Bioconjugate Chem* 2002;13:1259–1265.
14. Riley T, Stolnik S, Heald CR, Xiong CD, Garnett MC, Illum L, Davis SS. Physicochemical Evaluation of Nanoparticles Assembled from Poly(lactic acid)-Poly(ethylene glycol) (PLA-PEG) Block Copolymers as Drug Delivery Vehicles. *Langmuir* 2001;17:3168–3174.
15. Carstens, Myrra G.; van Nostrum, Cornelius F.; Ramzi, Aissa; Meeldijk, Johannes D.; Verrijck, Ruud; de Leede, leo L.; Crommelin, Daan JA.; Hennink, Wim E. PEG-oligolactates with monodisperse hydrophobic blocks: Preparation, characterization and behavior in water. *Langmuir* 2005;21:11446–11454. [PubMed: 16285824]
16. Nolan SL, Phillips RJ, Cotts PM, Dunga SR. Light Scattering Study on the Effect of Polymer Composition on the Structural Properties of PEO-PPO-PEO Micelles. *J Colloid Interface Sci* 1997;191:291–302. [PubMed: 9268511]
17. Rump, Armin; Morikawa, Yoshihiro; Tanaka, Minoru; Minami, Sawako; Umesaki, Naohiko; Takeuchi, Masaki; Miyajima, Atsushi. Binding of Ovarian Cancer Antigen CA125/MUC16 to Mesothelin Mediates Cell Adhesion. *The Journal Of biological Chemistry* 2004;279:9190–9198. [PubMed: 14676194]
18. Nustad K, Onsrud M, Jansson B, Warren D. CA125: epitopes and molecular size. *International Journal Of Biological Markers* 1998;13:196–199. [PubMed: 10228900]
19. Gehling UM, Ergun S, Schumacher U, Wagener C, Pantel K, Otte M, Schuch G, Schafhausen P, Mende T, Kilic N, Kluge K, Schafer B, Hossfeld DK, Fiedler W. In vitro differentiation of endothelial cells from AC133-positive progenitor cells. *Blood* 2000;95(10):3106–3112. [PubMed: 10807776]
20. Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J, Buck DW. AC133, a Novel Marker for Human Hematopoietic Stem and Progenitor Cells. *Blood* 1997;90(10):5002–5012. [PubMed: 9389720]

List of abbreviation

ELISA

Enzyme-linked immunosorbent assay

CA125

Abbreviation for cancer antigen 125

OC125 and M11

both recognize antigenic determinants on the cancer antigen 125 (CA125)

SSEA-4

Embryonic Stem Cell Marker

hES

Human Embryonic Stem

DLS

Dynamic Light Scattering

PCL-b-PEOpoly(ethylene oxide)-poly(ϵ -caprolactone)**DSAK**

Dimethylamino Stilbene Acetyl Ketone

TEM

Transmission Electron Microscopy

Figure 1. Dynamic Light Scattering (DLS) **A)** free polymer micelles **B)** polymer micelles loaded with the dye. Inset: Transmission electron micrograph (TEM) of micelles of PEO45-*b*-PCL23, bar scale 50nm.

Figure 2.

a) Emission spectrum of polymeric micelles loaded with DSAK. **b)** Emission spectrum of DSAK loaded micelles after treating with THF organic solvent.

(A)

(B) **(C)**

(D)

Figure 3.

A) Depiction of polymeric micelle assay for the detection of CA125; **B)** Fluorescence intensities from THF treated polymeric micelles as function of CA-125 concentrations; **C)** Fluorescence spectra of polymeric micelles in the presence of different concentrations of CA-125 after treating with THF as well as with different control conditions. *a* 1.1 unit/ml, *b* 0.15 unit/ml, *c* CA125 was replaced with BSA (1mg/ml), *d* magnetic beads mixed with polymeric micelles without any CA 125; **D)** The detection of the CA125 in mimic serum using polymeric micelles. *a*: goat serum with CA125 at 1unit/ml. Red line: *b* goat serum without CA125.

(C)

Figure 4.

A) Depiction of polymeric micelle assay for the detection of SSEA-4; **B)** Fluorescence intensities from THF treated polymeric micelles as function of stem cell numbers; **C)** Fluorescence spectra of polymeric micelles after treating with THF. **a:** against SSEA-4 from one embryonic stem cell; **b:** against cell lysate from unbiotinylated stem cells and **c:** against MEF medium solution.