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Microfluidic Assembly of siRNA-Loaded Micelleplexes for Tumor Targeting in an Orthotopic Model of Ovarian Cancer

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Abstract

The use of cationic polymers to interact with negatively charged siRNA via charge complexation to form polyelectrolyte complexes has been widely studied ever since the 1998 report on RNA interference. These polyelectrolyte complex formulations aim to overcome the many pitfalls associated with the use of RNA interference as a potential cancer therapy. The triblock copolymer polyethylenimine-polycaprolactone-polyethylene glycol (PEI-PCL-PEG) contains the cation PEI and has been shown to be an efficient carrier capable of complexing with nucleic acids for gene delivery. This copolymer system also allows for targeting moieties to be linked to the micelleplex, thereby exploiting overexpressed receptors (such as the folate receptor) located within tumors. Additionally, we demonstrated recently that microfluidic mixing of PEI-PCL-PEG nanoparticles allows for the rapid, scaled-up production of micelleplexes while maintaining small and uniform particle distributions. The preparation of small and reproducible particles is imperative for clinical translation of nanomedicine and for tumor targeting via systemic administration. Furthermore, to enable tracing of its deposition in vivo after its administration, micelleplexes can be radiolabeled. To assess tumor targeting over time, the noninvasive imaging technique single-photon emission computed tomography (SPECT) offers the ability to examine the same subject at multiple time points and generate biodistribution profiles. Since the biodistribution and tumor targeting of the therapeutic load of micelleplexes is of foremost interest, we recently described an approach to modify siRNA with a DTPA (diethylenetriaminepentaacetic acid) chelator. Herein, we explain the details of encapsulating indium-labeled siRNA via microfluidic mixing in PEI-PCL-PEG nanoparticles with a folic acid targeting ligand for assessment of their in vivo tumor targeting in an orthotopic ovarian cancer model.

Keywords

siRNA delivery; SPECT imaging; Indium labeling; Microfluidic mixing; Triblock copolymer; Folate receptor targeting

1 Introduction

After the landmark 1998 study published in Nature by Mello et al. that provided the first demonstration of RNA interference (RNAi), the use of siRNA as a therapeutic molecule has attracted significant attention as an alternative cancer treatment. However due to its intrinsic properties, delivery of siRNA requires a carrier to ensure efficient therapeutic effects in vivo [1–4]. Therefore, the use of specialized delivery vehicles is imperative in order for therapeutic siRNA to be effective. Along with the benefit of shielding “naked” siRNA, the use of polymeric nanoparticles as a carrier also adds the ability to package multiple payloads, increases siRNA’s circulation time, and allows for tumor-specific targeting through surface functionalization [5]. Previously, the triblock copolymer polyethylenimine-polycaprolactone-polyethylene glycol (PEI-PCL-PEG) has been reported to spontaneously assemble with siRNA to form “micelleplexes” that are able to mediate RNAi in vivo and in vitro [6–8]. However, due to the chaotic nature of the self-assembly process, it is well documented that polyelectrolyte complexes have broad size distributions that limit their ability to be produced in large-scale batch reactors [9]. One strategy to address this limitation involves the use of microfluidic devices to increase colloidal stability by controlling the interaction of cationic polymer with anionic nucleic acid [10]. This strategy has proven useful in increasing siRNA complexation and uptake of the micelleplexes, which leads to a more efficient gene knockdown in vivo [11].

Therapeutic nanoparticle administration routes include intravenous, transdermal, pulmonary, and intraocular [12]. For therapeutic nanoparticles intended for cancer therapy, intravenous delivery is hypothesized to enable nanoparticles to accumulate preferentially in the tumor after extravasation from the bloodstream due to the enhanced permeability and retention (EPR) effect [13]. Intravenous administration, however, is not without its own hurdles to be overcome. Parameters such as the nanoparticle’s deposition, distribution, and elimination are key components to the success of any therapeutic.

With over 85% of all ovarian tumors overexpressing folate receptor alpha (FR α), the receptor has become an attractive candidate for targeted therapy of ovarian cancer [14]. To date, numerous groups have exploited folate receptors to deliver a targeted payload to the cancer cells of interest by attaching folic acid to the backbone of the drug itself or onto the delivery vehicle [15–17]. Subsequently, this approach has been applied in nanomedicine as a way to selectively deliver nanoformulated payloads to target cells, thereby reducing its uptake in healthy cells and decreasing off target toxicity. Additionally, attaching folic acid onto nanoparticles serves as active targeting of the carrier to the tumor site, therefore increasing the overall amount of payload reaching the intended target.

To assess the accumulation of nanoparticles in the organ or tissue of interest, SPECT imaging can be used. SPECT imaging is often employed to detect radioactive species within the body [18]. Furthermore, siRNA has the ability to be radioactively labeled, and this radioactive tag can be used as a tracer to track where the siRNA travels throughout the bloodstream and its deposition inside the body. Specifically, SPECT imaging can illustrate whether siRNA and polymeric carrier disassemble in circulation before reaching the tumor site, allows monitoring of biodistribution, and finally its route of elimination [19, 20].

Therefore, this approach provides useful information about the parameters that are crucial for intravenous delivery. Due to the need to overcome these hurdles for a successful treatment, siRNA imaging techniques are needed.

This chapter outlines the technique to label siRNA with a DTPA chelator and its subsequent chelation of ¹¹¹Indium. We further describe the microfluidic mixing of the radioactively labeled siRNA with folic acid conjugated triblock copolymer PEI-PCL-PEG to be used as a tracer in an orthotopic model of ovarian cancer following intravenous and intraperitoneal injection of the resulting micelleplexes via SPECT imaging.

2 Materials

2.1 siRNA Formulation

Due to their increased stability, high activity, and ability to be covalently modified, 2'-*O*-methylated 25/27mer DsiRNA14 [21] targeting EGFP was used and is recommended for use. For coupling of DTPA, amine-labeled siRNA is recommended. Here, we used a duplex with an amino-hexyl modification at the 5-prime of the antisense strand:

siEGFP: sense: 5'-pACCCUGAAGUUCAUCUGCACCACdCdG Antisense: 3' - mAmCmUGmGGmACmUUmCmAGmUAmGA mCGUGGUGGC-C₆H₁₂NH₂

whereby p denotes a phosphate residue, capital letters are ribonucleotides, d denotes 2'-deoxyribonucleotides, and m denotes 2'-*O*-methylribonucleotides.

2.2 Covalent Modification of siRNA with p-SCN-Bn- DTPA

1. siEGFP—see above.
2. p-SCN-Bn-DTPA (Macrocyclics, Plano, TX).
3. 0.1 M NaHCO₃ in DEPC water—filtered through a 0.22 µm filter before use.
4. 2 M NaOAc in DEPC water—filtered through a 0.22 µm filter before use.
5. Dried DMSO (about 3 mL).
6. 0.22 µm filter.
7. 2 mL centrifuge tube.
8. Metal spatula wrapped in parafilm—used to weigh out p-SCN- Bn-DTPA (see Note 1).
9. Aluminum foil.
10. Vortex mixer.

2.3 Precipitation of the siRNA-DTPA Complex

1. 2 M NaOAc in RNase-free water—filtered through a 0.22 µm filter before use.
2. Absolute ethanol—filtered through a 0.22 µm filter before use.
3. 15 mL conical tube.

2.4 Isolation of the siRNA-DTPA Complex

1. Ultracentrifuge (*see* Note 2).
2. Lysis buffer from Absolutely RNA miRNA Kit.
3. 2 M NaOAc in RNase-free water—filtered through a 0.22 μ m filter before use.
4. Absolute ethanol—filtered through a 0.22 μ m filter before use.
5. RNeasy Midi Kit (10) columns.

2.5. siRNA-DTPA Purification

1. Centrifuge.
2. Low-salt buffer from the Absolutely RNA miRNA Kit.
3. RNase-free water.
4. Sterile 2 mL collection centrifuge tubes.

2.6 siRNA Concentration Measurement

1. Spectrophotometer (e.g., Nanodrop™ 2000c).
2. RNase-free water.
3. 0.5 mL centrifuge tubes.
4. Parafilm.
5. Dry heat bath set to 94 °C.
6. Timer.

2.7 DTPA Concentration Measurement

1. Stock solution of the yttrium(III)-arsenazo III complex containing 5 μ M arsenazo(III) and 1.6 μ M yttrium(III) chloride (Acros Organics, New Jersey, US) in a 0.15 M NaOAc buffer at pH 4.
2. Stock solution of 0.123 mM DTPA dissolved in DI-H₂O with three molar equivalents of NaOH (*see* Note 3).
3. UV-Vis spectrophotometer.
4. UV-Vis disposable cuvette.

2.8 Indium Labeling

1. Radioactive Indium(III) chloride.
2. GE Healthcare Disposable PD-10 Desalting Columns.
3. RNase-free water.
4. Scintillation vials (make and model to fit gamma counter).
5. Gamma counter (e.g., Packard Tricarb 2910TR).

6. Spectrophotometer (e.g., Nanodrop™ 2000c).

2.9 Establishment of Orthotopic Model of Ovarian Cancer

1. Syringes, 1 mL.
2. 25-gauge sterile needle.
3. Sterile PBS buffer.
4. Mice (e.g., 6-week-old female nu/nu nude mice).
5. Folic acid-deficient diet.
6. Human ovarian cancer cell line (e.g., SKOV-3/LUC cells).

2.10 Monitoring Tumor Progression via Bioluminescence Imaging

1. Stock solution of D-luciferin in sterile PBS (e.g., 15 mg/mL).
2. Syringes, 1 mL.
3. 25-gauge sterile needle.
4. Bioluminescence imager (e.g., In-Vivo Extreme II).
5. Anesthetic agent (e.g., 3% isoflurane).

2.11 Microfluidic Preparation of Indium-Labeled siRNA Micelleplexes

1. 70% isopropyl alcohol.
2. 5% glucose solution—filtered through a 0.22 µm filter before use.
3. Polyethylenimine-*graft*-polycaprolactone-*block*-polyethylene glycol (PEI-g-PCL-b-PEG) triblock copolymer dissolved in water and sterile filtered to yield desired polymer concentration based upon the PEI 25 kDa content (*see* Note 4).
4. Luer Lock disposable syringes.
5. Syringe pump (e.g., KD Scientific 220 syringe pump).
6. Fluorinated ethylene propylene tubing (1/16" OD × 0.25 mm ID).
7. Dolomite micromixer chip with hydrophobic coating.
8. Sterile collection tubes.

2.12 SPECT/CT Animal Imaging

1. Sterile syringes.
2. Sterile indium-labeled siRNA micelleplexes.
3. Mouse anesthesia.
4. SPECT imaging device and mouse cradle.

2.13 Pharmacokinetics

1. Microhematocrit blood collection tubes.
2. Gauze sponges.
3. General anesthetic agents and equipment.
4. Scintillation vials (make and model to fit gamma counter).
5. Gamma counter (e.g., Packard Tricarb 2910TR).

2.14 Biodistribution of Micelleplexes

1. Surgery table and surgical instruments.
2. Scintillation vials (make and model to fit gamma counter).
3. Gamma counter (e.g., Packard Tricarb 2910TR).

3 Methods

3.1. React siRNA with p-SCN-Bn-DTPA

1. Weigh out 5.11 mg of double-stranded siRNA in a 2 mL centrifuge tube, and dissolve it in 100 μ L of RNase-free water (*see* Note 5).
2. To the 2 mL tube, add 100 μ L of filtered 0.1 M NaHCO₃.
3. Next, weigh out 9.76 mg of p-SCN-Bn-DTPA, and dissolve it in 540 μ L of dry DMSO (*see* Notes 1 and 6).
4. Add the 540 μ L of the DTPA to siRNA mixture. The new total volume should be 740 μ L (*see* Note 7).
5. Vortex the solution thoroughly, and incubate for 6 h. Agitate the solution every 30 min.

3.2 Precipitation of the siRNA-DTPA Complex

1. Add 74 μ L of filtered 2 M Na-acetate to the mixture (10% of the total amount of mixture).
2. Transfer the mixture to a 15 mL conical tube.
3. Add filtered absolute ethanol so that the final concentration is 80% v/v.
4. Freeze solution overnight at -80°C .

3.3 Isolation of the siRNA-DTPA Complex

1. Centrifuge the sample for 30 min at $12,000 \times g$ in an ultracentrifuge (*see* Note 2).
2. Discard the supernatant.
3. Add 2.5 mL of lysis buffer from “Absolutely RNA miRNA Kit.”
4. Vortex the solution.

5. Add 250 μ L of filtered 2 M Na-Acetate.
6. Add 7.25 mL of filtered absolute ethanol for a total of 10 mL (*see* Note 8).
7. Vortex the solution and equally distribute the 10 mL onto five RNeasy Midi Kit Qiagen Columns (*see* Note 9).

3.4 siRNA-DTPA Purification

1. Centrifuge the columns at $4500 \times g$ for 5 min, discard the flow through.
2. To each column, add 200 μ L of the low-salt buffer from the “Absolutely RNA miRNA Kit.”
3. Centrifuge the solution at $4500 \times g$ for 2 min, discard the flow through.
4. Repeat **steps 2 and 3**.
5. To dry the column, spin them down at $5000 \times g$ for 5 min.
6. Transfer the columns to a new collection tube, and add 200 μ L of 60 °C hot RNase-free water.
7. Centrifuge the solution at $5000 \times g$ for 5 min to collect the purified siRNA-DTPA.
8. Add 100 μ L of 60 °C hot RNase-free water and centrifuge at $5000 \times g$ for 5 min.
9. Combine the flow through from all of the columns into one sterile 2 mL tube.

3.5 siRNA Concentration Measurement

1. Measure the siRNA concentration on a spectrophotometer (e.g., Nanodrop 2000c). Use RNase-free water as your blank.
2. Under the hood, dilute the siRNA to a desired concentration, and aliquot into 0.5 mL sterile tubes (*see* Note 10).
3. Sterilize siRNA-DTPA with a 0.22 μ m syringe filter.
4. Parafilm each tube, and anneal the siRNA at 94 °C for exactly 2 min.
5. Let the samples cool down to room temperature.
6. Freeze the samples, and keep frozen until needed.

3.6 DTPA Concentration Measurement

1. Using a UV-Vis spectrophotometer, create the standard curve for DTPA concentrations.
2. Pipette 3 mL of the Y(III)-arsenazo III complex stock solution into a cuvette and read (652 nm) this as the blank.
3. Add 5 μ L of the stock DTPA solution to the cuvette, gently mix, and read the solution again (*see* Note 11).
4. Add another 5 μ L of the stock DTPA solution, read, and repeat.

5. Do this until you have generated enough points for your standard curve (each new data point will have an additional 5 μ L added into the cuvette).
6. Once all the standards have been made and read on the spectrophotometer, discard the solution inside the cuvette, and put 3 mL of fresh Y(III)-arsenazo III complex stock solution into the cuvette (*see* Note 12).
7. Add 5 μ L of your siRNA-DTPA sample into the cuvette and take the measurement (652 nm).
8. Plot the standard curve for the DTPA concentrations versus absorbance and insert a linear line of best fit (Fig. 1).
9. Using the equation yielded from the line of best fit, plot the absorbance value obtained from your sample measurement, and plug that into the *Y*-value of the equation in order to solve for *X*.
10. The *X*-value obtained will be the concentration of DTPA in your sample.
11. Now that the DTPA and siRNA concentrations have been found for the siRNA-DTPA mixture, calculate the molar amounts of the siRNA and DTPA within your sample. Use this to determine the molar equivalency of the siRNA and DTPA (*see* Note 13).

3.7 Indium Labeling and Purification

1. React radioactive $^{111}\text{InCl}_3$ with DTPA-siRNA. In the example shown below, 116.9 MBq $^{111}\text{InCl}_3$ were reacted with 15 nmol DTPA- siRNA. Incubate for 2 min at 94 $^{\circ}\text{C}$, and allow solution to cool down to room temperature.
2. Equilibrate a PD-10 column with RNase-free water by washing it with 25 mL.
3. Prepare 24 scintillation vials in a rack and label them from 1 to 24.
4. Place vial 1 underneath the PD-10 column, and start adding the siRNA-indium mixture to the column slowly.
5. Collect 13 drops in the first vial as fraction 1 and then move on to the next vial. Collect 13 drops per fraction. Once the complete volume of the siRNA-indium mixture is applied to the column, add RNase-free water. Collect 24 fractions.
6. Close the scintillation vials, and measure the counts per minute (CPM) in every vial using a gamma counter.
7. Plot the CPM versus the fraction number (Fig. 2).
8. Determine the siRNA concentration in the peak fraction using a Nanodrop spectrophotometer (*see* Note 14).

3.8 Establishment of Orthotopic Model of Ovarian Cancer

1. Culture and expand luciferase (LUC)-expressing ovarian cancer cells per established protocols.

2. Harvest cells, and inject each mouse with 1×10^6 cells resuspended in phosphate-buffered saline intraperitoneally (*see* Note 15).
3. Four weeks post-injection, place all mice on folic acid-deficient diet (*see* Note 16).
4. Monitor mice and observe tumor growth for a total of 6 weeks after cancer cell inoculation.

3.9 Monitoring Tumor Progression via Bioluminescence Imaging

1. Two weeks after cancer cell inoculation, inject mice with LUC-expressing tumors intraperitoneally with 100 μ L of a freshly prepared 15 mg/mL D-Luciferin stock solution in PBS per 10 g of their body weight.
2. After 10 min, sedate mice with 3% isoflurane (*see* Note 17).
3. Place mice inside imager cradle and take BLI images with a 3-min exposure under high sensitivity and aperture of the lens set at an f-stop of 1.1.
4. Simultaneously, take X-ray images with standard 1.2-s exposure.
5. Repeat this procedure every 2 weeks until the 6-week time point has been met (Fig. 3).

3.10 Microfluidic Preparation of Indium-Labeled siRNA Micelleplexes

1. Fill two syringes with 70% isopropyl alcohol, and pump the solution through the micromixer chip at 1 mL/min. Repeat this wash step with sterile 5% glucose solution.
2. After determining the optimal N/P ratio to be used, dilute PEI-g-PCL-b-PEG triblock copolymer and indium-labeled siRNA with sterile 5% glucose to desired concentration. In the example below, micelleplexes were prepared with 2 nmol siRNA per animal which was equivalent to approximately 3 MBq per animal (*see* Note 18).
3. Load diluted polymer and siRNA solution into separate syringes, and ensure that their plungers align (*see* Note 19).
4. Load each syringe into the syringe pump, and attach them to their corresponding female Luer adapter/FEP tubing.
5. Ensure that the syringe pump is set to the correct syringe diameter, and set the flow rate to 0.5 mL/min.
6. After steady-state flow is reached within the micromixer chip, collect assembled micelleplex solution in a separate sterile collection tube.
7. Following collection of formed micelleplexes, refill syringes with 70% isopropyl alcohol, and wash micromixer chip to avoid blockages forming during chip storage.

3.11 SPECT/CT Animal Imaging

1. Anesthetize the animals and administer the indium-labeled siRNA micelleplexes. In the example below, intravenous tail vein injection and intraperitoneal injection were chosen.
2. Place the animals, one after the other, into the SPECT mouse cradle, and start the 360° imaging program (Fig. 4) (*see* Note 20).

3.12 Pharmacokinetic Modeling

1. Immediately following administration of micelleplexes, collect a 25 µL blood sample via retro orbital collection at various time points with a microhematocrit blood tube. In the example below, blood sampling was done at 1, 3, 5, 15, 30, 60, and 120 min following both administration routes.
2. Transfer blood samples into separate scintillation vials.
3. Close the scintillation vials, and measure the counts per minute (CPM) in every vial using a gamma counter.
4. Record CPM of each blood sample for every time point.
5. To determine the percent injected dose per mL of blood, the CPMs were multiplied by 40 to account for an estimated total volume of blood in the mouse to be 2 mL.
6. Following calculations, the new CPM value was fit to a fresh ^{111}In standard curve to determine the percent injected dose of ^{111}In in the blood sample.
7. Once the values were obtained, the PK values were plotted on a logarithmic scale and fit to a two-compartment model (*see* Note 21).

3.13 Biodistribution of Micelleplexes

1. Twenty-four hours after the administration of micelleplexes, sacrifice mice in order to harvest organs.
2. Dissect the liver, kidneys, lungs, brain, spleen, bowels, and tumors.
3. Record the weight of each organ individually, and place into separate scintillation vials.
4. Close the scintillation vials and measure the counts per minute (CPM) in every vial using a gamma counter.
5. To determine the percent injected dose per gram of tissue, the CPMs were adjusted for activity to account for decay.
6. Following adjustment, the CPM of the tissue was divided by the CPM value of the total injected ^{111}In . (Note: The CPM of the injected amount was determined by fitting the value into a fresh standard curve of ^{111}In).
7. The new value was divided by the weight of each organ to determine the percent injected dose per gram of tissue (Fig. 5) (*see* Note 22).

4 Notes

1. Wrap the metal spatula in parafilm so the DTPA does not complex to the metal ions from the spatula.
2. You should get a nice visible white pellet at the bottom of the 15 mL conical tube. A regular centrifuge that reaches a speed of $12,000 \times g$ may as well be used.
3. You should first dissolve the DTPA in DMSO before diluting in the DI-H₂O with NaOH. Make sure the DMSO is at least diluted out by a factor of 1:100.
4. Quantification of 25 kDa PEI can be achieved via a TNBS (2,4,6-trinitrobenzene sulfonic acid) assay [22].
5. The siRNA used here had a MW of 17,950.36 g/mol. Therefore, we used 0.285 μ mol of siRNA.
6. Total p-SCN-Bn-DTPA (MW = 649.9 g/mol) is 15.02 μ mol.
7. The solution turned cloudy upon the addition of the DTPA to the siRNA solution.
8. Upon addition of the ethanol, the solution should turn slightly cloudy again.
9. You should put roughly 2 mL into each column since each column can only retain 1 mg RNA and a limited volume. If you add too much, you may lose siRNA during the purification steps.
10. To make calculations easier in the future, dilute the siRNA to either 100 μ M or 50 μ M. Aliquot the samples into small portions to prevent several freeze-thaw cycles.
11. Mix the samples by gently pipetting up and down within the cuvette. Be careful not to create any bubbles.
12. Make sure you rinse out the cuvette very well. When you read the fresh 3 mL of the complex solution, verify that the values are in line with the previous measurements.
13. Since each siRNA strand has only one amine group for DTPA to complex to, if performed correctly, your ratio should be approximately a 1:1 molar equivalence of DTPA and siRNA. If the ratio of DTPA per siRNA is higher than 1:1, residual-free DTPA was not removed during the purification.
14. It may be necessary to combine two or more peak fractions based on the CPM values and RNA concentrations. If free DTPA is present in the siRNA solution when it is radiolabeled, a second small peak will appear around fraction 12, and free Indium appears around fraction 20.
15. Make sure to harvest ovarian cancer cells when they are less than 80–90% confluent. This ensures that the cells are in a period of exponential growth and will increase tumor engraftment success rate.

16. By placing mice on a folic acid-deficient diet, it reduces their serum folate to a level near that of human serum and increases the folate receptor alpha status of the ovarian cancer cells.
17. Isoflurane was used during imaging to keep the mice sedated.
18. Minimum volume of both polymer and siRNA solution must be 200 μ L.
19. When loading solutions into syringes, use a microliter pipettor that has pipette tips smaller than the diameter of the Luer tip to avoid sealing the syringe barrel. In addition, retract the syringe plunger while simultaneously ejecting the polymer or siRNA solution from the pipettor to help draw up solution.
20. The imaging procedure can be repeated at any given time. The half-life of ^{111}In is 2.6 days, and a significant amount of siRNA is typically excreted renally or even hepatically. Therefore, imaging at time points later than 48 h can become challenging.
21. Nanoparticles tend to accumulate in the liver as a deep compartment. Therefore, a two-compartment model usually fits best.
22. Make sure to collect the primary tumor first, and separate any surrounding metastatic tumors. These can then be measured separately and combined during the analysis.

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Fig. 1.

Standard curve for DTPA concentration. Scatter plot obtained from creating the standard curve of the DTPA concentrations. The amount of DTPA added to the cuvette is on the *X*-axis, and Absorbance at 652 nm is on the *Y*-axis. From here, concentration of the DTPA inside the siRNA-DTPA mixture can be obtained

Fig. 2. Elution profile of ^{111}In . Scatter plot obtained from purifying and eluting ^{111}In -labeled siRNA over a PD-10 column. The radioactivity as measured in counts per minute (CPM) are shown on the *Y*-axis as a function of the fraction eluted on the *X*-axis. A clear peak is shown in fraction 7

Fig. 3.
Assessment of tumor growth via bioluminescence. Luciferase activity is shown predominantly in the primary tumor as well as in metastases

Fig. 4. In vivo SPECT/CT images. Biodistribution analysis of nude mice 4 and 24 h post intraperitoneal injection of PEI-PCL-PEG micelleplexes loaded with ^{111}In -labeled siRNA

Fig. 5. In vivo biodistribution analysis of nude mice. Biodistribution analysis of ^{111}In after intraperitoneal and intravenous injection of PEI-PCL-PEG micelleplexes loaded with ^{111}In -labeled siRNA. Organs were harvested 24 h post injection and read under gamma scintillation counting