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Note

Synthesis and evaluation of a water-soluble polymer to reduce Ac-225 daughter migration

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Abstract: The actinium decay chain has been promoted as an *in vivo* alpha generator for therapy, but migration of daughters from the primary conjugate has lead to increased toxicity away from the target organ. To reduce daughter migration, polyethylenimine (PEI) was used with a primary chelator and secondary chelators. The primary chelator, DOTA, was used to coordinate ^{225}Actinium and secondary chelators-acetate and DTPA, were added to the polymer for coordination of daughters formed by decay. The ^{225}Actinium polymer derivatives containing secondary chelators were found to retain radioactive daughters better than the ^{225}Actinium bond to the primary alone. The retention of ^{213}Bismuth and ^{209}Thallium had the following order from highest retained to lowest DOTA-PEI-DTPA ≈ DOTA-PEI-CH_2OO- > DOTA-PEI. The data suggests this polymer approach could be used to reduce daughter migration and has potential for development of actinium labeled radiopharmaceuticals. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: actinium; alpha therapy; polyethylenimine; bismuth; PEI; daughter retention

Introduction

Alpha emitters offer high cytotoxicity with an irradiation range of only a few cells and therefore show promise for the treatment of diseases. The ^{225}Ac decay chain has four alpha emissions and two beta particle emissions and can deliver 28 MeV for each atom of ^{225}Ac that decays to a stable ^{209}Bi atom (Figure 1). Most conventional chelators, such as 1,4,7,10,13,16-hexaazacyclohexadecane-*N*,*N*′,*N*′′,*N*′′′,*N*′′′′,*N*′′′′′-hexaacetic acid (HEHA) or 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*′′,*N*′′′-tetraacetic acid (DOTA), are not designed to retain both ^{225}Ac and the daughter products. Other investigators have demonstrated ^{225}Ac localization in the liver and bone, or one of the newly formed daughter products having toxicity in other organs.[1] For example ^{213}Bi localizes in the kidney[2] and the ^{221}Fr daughter mimics K^{+1} and is trafficked through the body.[3,4] In order for the actinium decay series to be used for therapy, all daughters must be retained at the target to reduce toxicity to non-targeted cells. Brechbiel *et al.* have outlined the following major obstacles with delivering ^{225}Ac to tumors.[5] (1) Recoil energy of ^{221}Fr after the alpha particle emission (0.1 MeV) is much larger than the chemical binding energy of an ^{225}Ac conjugate. (2) The first daughter product (^{221}Fr), a 1+ ion would require significantly different chelation chemistry than actinium. The high recoil energy and different chelation chemistry of actinium and francium could lead to francium and daughters migrating in the *in vivo* studies and explain the toxicity away from the tumors.

Hypothesis/model

The problems associated with the toxicity *in vivo* at non-target sites suggest the daughters are not retained by the chelator. A limited number of studies have examined the ability of a chelator to retain a radioactive daughter that was formed as a result of alpha or beta decay. Biodistribution studies have examined the ratios of daughters to ^{225}Ac and ^{213}Bi to determine if the daughters formed by decay were retained by the DOTA or HEHA conjugates by the tumor matrix.[3,6] In both studies the daughters formed after decay were not retained by the chelates and redistribution of the daughters occurred. In a study examining the ability of DOTA to retain ^{212}Bi after beta decay of ^{212}Pb, 35% of

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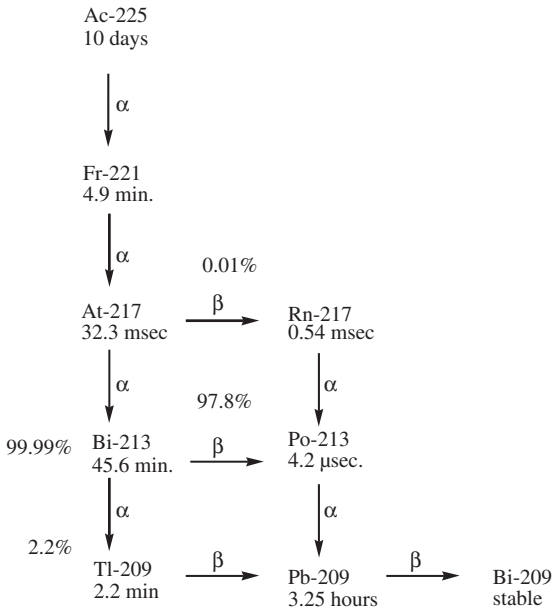

Figure 1 Decay scheme of actinium-225.

the formed ^{212}Bi was dissociated into Bi(III) ions.[7] No study has demonstrated the ability of a chelator to retain ^{225}Ac and all daughters.

This paper focuses on the chemistry of a new system utilizing a polyethyleneimine (PEI) polymer with a primary chelator to coordinate ^{225}Ac and secondary chelators to coordinate daughters and reduce their migration. The PEI polymer is a hyper-branched polymer made up of monomeric aziridine units with a ratio of 25% primary, 50% secondary and 25% tertiary amines. The highly branched polymer derivatives of PEI have been shown to be resistant to radiolytic degradation. Extremely high activities of ^{238}Pu (~17 mCi/mg polymer) used with the polymer have indicated the polymer is stable to fragmentation for about 8 h, after which there is a gradual increase in the migration of the activity from the polymer.[8] This loading level is substantially higher than the anticipated activity used for therapeutic dosage. A 50 K MW PEI when in aqueous solutions is ~95% water and has a solution diameter of ~90 nm.[9] A single ^{221}Fr recoil nuclei produces >4000 collisions of 5 eV (~a C–C bond) or greater,[10] most of the energy is depleted by collision with water molecules in the PEI and relatively few polymeric bonds are affected. Calculations show that the range for the recoiling ^{221}Fr in water is 60–105 nm. Ionic interactions between the very short-lived daughters such as ^{221}Fr, ^{217}At, ^{213}Po and ^{209}Tl and the anionic secondary chelators should provide physical retention within the polymer matrix. The short half lives may not be long enough for the daughter to diffuse away from the polymer after recoil. For longer-lived daughters such as ^{213}Bi and ^{209}Pb, re-complexation with the secondary chelators containing the carboxylic acid chelating groups would form complexes with stability constants[11] ($\log K_d$) similar to EDTA (Bi 27.8 and Pb 18) or DTPA (Bi 35.6 and Pb 18.6). The re-complexation of the short lived Tl as the 3+ ion would have similar stability constants to EDTA (35.3) and DTPA (46).

Results and discussion

This study was performed to determine if the polymer model outlined above could be used to reduce daughter migration which has plagued the use of the Actinium decay series for therapy. In this study a post-labeling strategy was used to label DOTA-PEI with ^{225}Ac then a secondary chelator was added and the polymer was centrifuged over 1 week to determine the amount of daughter retention (Figure 2). The basic conditions needed to deprotonate the amines of the PEI for addition of secondary chelators could cause loss of the ^{225}Ac metal in the form of metal hydroxide. The procedure for forming PEI derivatives with carboxylic acid, via bromoacetic acid, was conducted at a pH=8.3 and the percent activity retained during addition of the secondary chelators was 82% (Ac), 71% (Fr), 78% (Bi), and 76% (Tl), ($n = 2$). For the reactions with the DTPA anhydride the reaction was performed at pH=7.0 and the percent activity retained during addition of the secondary chelators was 72% (Ac), 59% (Fr), 12% (Bi), and 11% (Tl) ($n = 2$). The much lower retention of Bi and Tl during the formation of the polymer with DTPA

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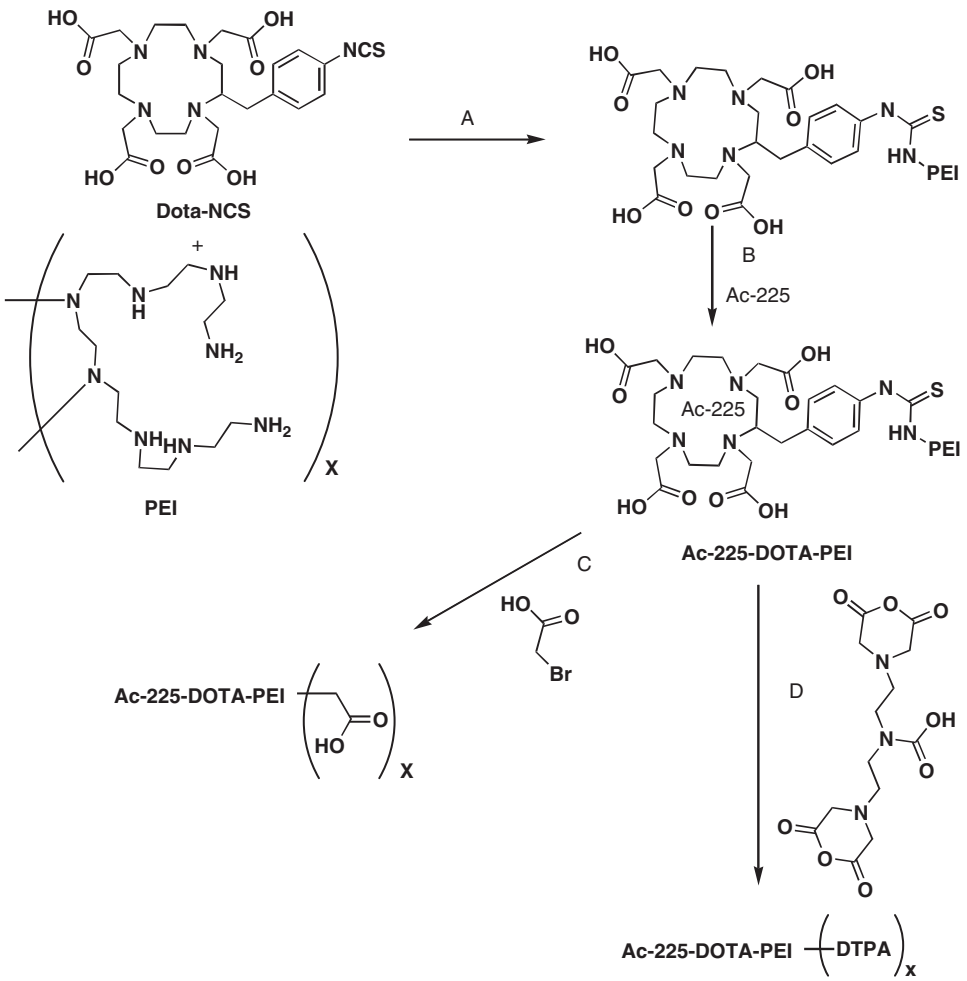

Figure 2 Preparation of Ac-225-DOTA-PEI and derivatives. Reagents and conditions: (A) Na_2CO_3, pH=8.85; (B) $NaHCO_3$, NH_4Ac or phosphate buffered saline, pH=6.0; (C) Na_2CO_3, pH=8.3, 77°C, 2 h, 40°C, 10 h; (D) $NaHCO_3$, pH=7.0, RT, 10 h.

is a result of free DTPA coordinating the metals and being retained on the size exclusion gel during purification.

Migration of actinium and daughters

To study the migration of daughters from the polymer centricon filters with a MW cut-off of 10 000 were used. This approach takes 30 min to centrifuge the samples, count times were 5 min and all samples were counted within 10 min of centrifugation. The ability of each polymer to retain each isotope is illustrated in Figures 3–6. For ^{225}Ac, ^{213}Bi and ^{209}Tl the secondary chelators had higher retention than the polymer DOTA-PEI-DTPA ≈ DOTA-PEI-CH_2OO- > DOTA-PEI (Figures 3, 5 and 6). The retention of ^{221}Fr had the following order from highest retained to lowest DOTA-PEI-DTPA>DOTA-PEI-CH_2OO- > DOTA-PEI (Figure 4). The polymers with secondary chelators had better retention of all isotopes than ones containing only primary chelators. Polymers with secondary chelators had a range of retention (average ± /1 standard deviation) with little or no overlap at or above 72 h when compared to DOTA-PEI (Table 1). This data is consistent with the high stability constant of the daughters with DTPA, and indicates the polymer needs to contain secondary chelating groups for best retention of daughters.

Experimental

Ac-225 (1 mCi in 150 μL of 0.1 M HNO_3) was purchased from Isonics (Columbia, MD). The *p*-SCN-benzyl-DOTA, and *p*-NH_2-benzyl-DOTA were purchased from Macrocyclics (Dallas, TX). Econopack 10DG size exclusion column were purchased from Bio-Rad (Bio-Rad Laboratories, Hercules, CA). All other reagents were purchased from Fisher Scientific (Pittsburgh, PA), Aldrich (Milwaukee, WI), or Sigma (St. Louis, MO).

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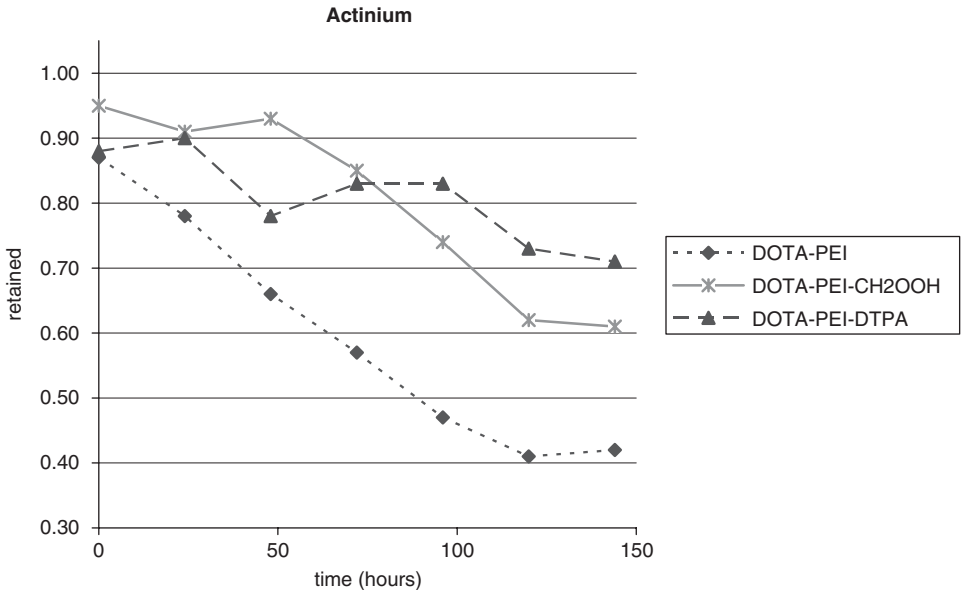

Figure 3 Retention of Ac-225. DOTA-PEI- DTPA and DOTA-PEI ($n = 3$), DOTA-PEI-CH_2OOH ($n = 2$ from time 0–120 h, $n = 1$ at 144 h).

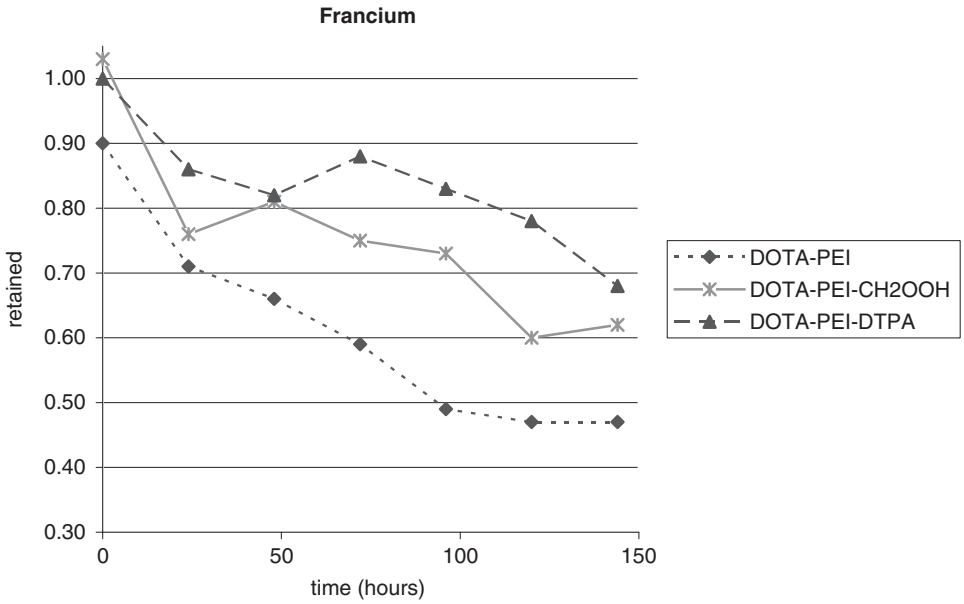

Figure 4 Retention of Fr-221. DOTA-PEI- DTPA and DOTA-PEI ($n = 3$), DOTA-PEI-CH_2OOH ($n = 2$ from time 0–120 h, $n = 1$ at 144 h).

All columns were equilibrated with 2–3 column lengths of buffer prior to sample introduction and 1 ml fractions were collected. Fractions 3–6 were checked for activity with a pancake detector prior to counting on a high purity Ge detector. All samples were counted with identical volumes. Centracon 10K centrifugal filter devices, isotemp economical dry bath incubator, Oakton Acorn pH 6 pH meter, 2 ml microcentrifuge tubes and 45 μm Nalgene sterile analytical filter unit were purchased form Fisher Scientific (Pittsburgh, PA). The centrifuge filter devices were centrifuged on setting 9 with a Fisher Centrific model 225 (Pittsburgh, PA). Dialysis was performed using Slide-A-Lyzer 10K MWCO dialysis cassettes purchased from Pierce (Rockford, IL). All aqueous solutions were made with 18 MΩ water and adjusted with either Conc. HCl or 15% NaOH to the desired pH. Then the solutions were eluted from a column of Chelex 100, the pH

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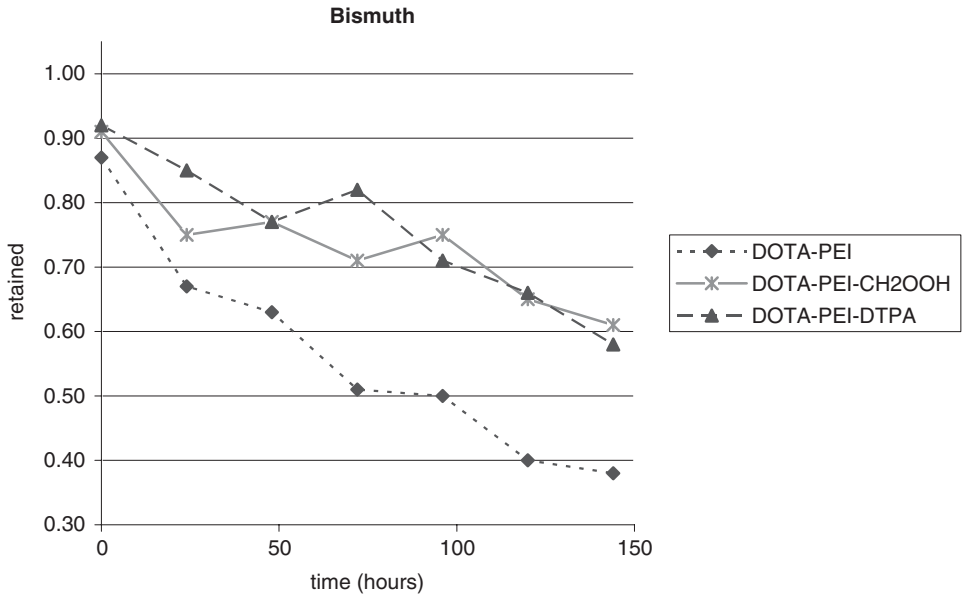

Figure 5 Retention of Bi-213. DOTA-PEI- DTPA and DOTA-PEI ($n = 3$), DOTA-PEI-CH_2OOH ($n = 2$ from time 0–120 hours, $n = 1$ at 144 h).

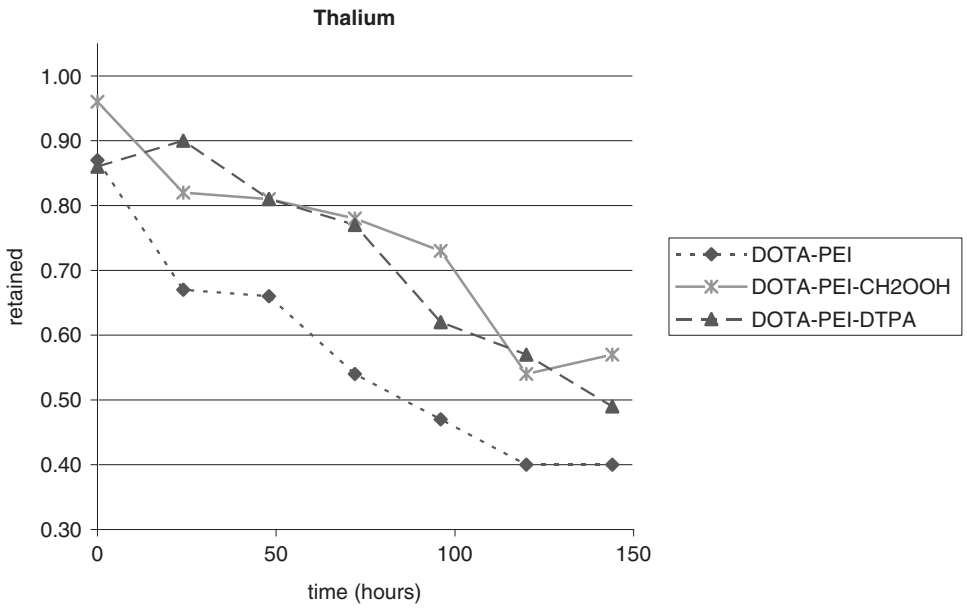

Figure 6 Retention of Tl-209. DOTA-PEI- DTPA and DOTA-PEI ($n = 3$), DOTA-PEI-CH_2OOH ($n = 2$ from time 0–120 h, $n = 1$ at 144 h).

measured, and then the solutions were filtered. Radiolabeling was performed in acid washed 2 ml microcentrifuge tubes.

DOTA-PEI

A standard solution of PEI was made by adding 50% PEI (13.4 g or 6.7 g of PEI, 0.11 mmol PEI) to a 50 ml centrifuge tube and diluting with water to a final volume of 40 ml. Then conc. HCl (10 ml) was added drop wise to the tube and the sample was sonicated until the solution became uniform. Then a working solution of PEI (1.005 g, in 30 ml, 0.0167 mmol) was made by adding the standard solution of PEI (7.5 ml) to a centrifuge tube and diluting with sodium bicarbonate (22.5 ml, 0.2 M, pH=6.5).

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Table 1 Retention of labeled polymers at times greater than 70 h

		Time (h)							
Polymer conjugate		72 h	SD	96 h	SD	120 h	SD	144 h	SD
DOTA-PEI	Ac-225	0.57	0.12	0.47	0.1	0.41	0.08	0.42	0.07
DOTA-PEI-CH2OOH	Ac-225	0.85	0.21	**0.74**	0.08	**0.62**	0.08		
DOTA-PEI-DTPA	Ac-225	0.83	0.14	**0.83**	0.16	**0.73**	0.16	**0.71**	0.22
DOTA-PEI	Fr-221	0.59	0.16	0.49	0.05	0.47	0.06	0.47	0.1
DOTA-PEI-CH2OOH	Fr-221	0.75	0.21	**0.73**	0.14	**0.60**	0.04		
DOTA-PEI-DTPA	Fr-221	**0.88**	0.09	**0.83**	0.04	**0.78**	0.07	**0.68**	0.07
DOTA-PEI	Bi-213	0.51	0.16	0.5	0.09	0.40	0.09	0.4	0.07
DOTA-PEI-CH2OOH	Bi-213	0.71	0.14	0.75	0.18	**0.65**	0.12		
DOTA-PEI-DTPA	Bi-213	**0.82**	0.04	**0.71**	0.01	**0.66**	0.07	0.58	0.12
DOTA-PEI	Tl-209	0.54	0.12	0.47	0.08	0.4	0.08	0.38	0.06
DOTA-PEI-CH2OOH	Tl-219	0.78	0.25	**0.73**	0.25	0.54	0.25		
DOTA-PEI-DTPA	Tl-219	**0.77**	0.04	**0.62**	0.04	0.57	0.13	0.49	0.18

Numbers that are bold indicate the range of data (average $\pm$ /SD) for the polymers with secondary chelators do not overlap with the polymer containing the primary chelator. DOTA-PEI- DTPA and DOTA-PEI ($n = 3$), DOTA-PEI-CH_2OOH ($n = 2$).

Sodium bicarbonate (1 L, 0.2 M, pH=6.5) and Chelex 100 (6.35 g) were added to a 1.5 l container, and dialysis was performed on a portion of the working solution of PEI (12 ml, 0.00668 mmol). The dialysis was carried out at RT for 21 h with stirring, and the buffer was changed and dialysis was repeated for 50 h. Then a PEI sample (6 ml, 0.00334 mmol) was transferred to a 50 ml centrifuge tube and sodium carbonate (5 ml, 0.2 M, pH=9.5) was added and the final pH was 8.85. Next 2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid (0.0074 g, 0.011 mmol) was placed in a 2 ml vial, dissolved in water (1 ml), and added to the PEI solution. The vial was rinsed with sodium carbonate (1 ml, 0.2 M, pH=8.3) and added to the reaction solution. Then the sample was mixed for 24 h at RT. The sample was transferred to a dialysis chamber and dialysis was performed with sodium carbonate (2X1L, 0.2 M, pH=8.3), chelex 100 (6.35 g) and the sample was stirred at RT for 24 h. The number of chelates per PEI was determined by converting the UV absorbance at 280 nm (1.6796) and 290 nm (1.4836), to concentrations of the chelator using a standard curve for a propyl derivative of DOTA-NCS.[12] The concentrations of chelator was divided by the concentration of PEI to determine the average number of chelators per PEI (1.54 chelates/PEI, $n = 2$). Yield=51%.

^{225}Ac-DOTA-PEI

Various solvents and reactions volumes were used to label the DOTA-PEI conjugate with actinium. Radiolabelings with sodium bicarbonate (0.2 M, pH=6.0) were 42–53%. The DOTA-PEI (1 ml) in sodium bicarbonate was buffer exchanged five times with ammonium acetate (0.2 M, pH=6.0) using a centricon filter device. The DOTA-PEI (0.030 ml, 7×10^{-6} mmol), ammonium acetate (0.03 ml) and the actinium (0.010 ml) were added to a vial and the sample was placed in a dry heating block at 77°C for 2 h. The volume was checked every 0.5 h and 0.090 ml of buffer was added if the solution was less than 0.05 ml. After 2 h 0.090 ml of buffer was added and the vial was heated until no solvent was visible. The labeled DOTA-PEI samples were dissolved in sodium bicarbonate (total volume =1 ml, 0.2 M, pH=6.0) and the initial activity was determined. Then the samples were added to a size exclusion column and eluted with sodium bicarbonate (0.2 M, pH=6.0) and the activity in fractions 3–6 was determined. Fractions with activity were pooled and concentrated to 1 ml by centrifugation and the radiolabeling yield of the DOTA-PEI sample was 79%.

A sample of DOTA-PEI (0.030 ml, 7×10^{-6} mmol) in ammonium acetate (0.2 M, pH=6.0) was heated at 77°C until no solvent was visible. Then phosphate buffered saline (0.06 ml 0.1 M phosphate, 0.15 M sodium chloride pH=6.8) and 0.010 ml of actinium solution was added and the procedure above was followed. The radiochemical yield was 67%.

Addition of secondary chelators

To prepare the labeled DOTA-PEI for addition of bromoacetic acid the sample was purified with a size exclusion column and the elution buffer was sodium

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carbonate (1.0 M, pH=8.3). In a vial containing labeled PEI (1 mL, 7×10^{-6} mmol) was added bromoacetic acid (0.0063 g, 0.045 mmol) and the solution was heated for 2 h at 77°C then the heat was set for 40°C and the sample was left for 10 h. The reaction was purified by size exclusion chromatography and the samples were eluted with sodium bicarbonate (0.2 M, pH=6.0). The amount of activity retained was 82% (Ac), 71% (Fr), 78% (Bi), and 76% (Tl), ($n = 2$).

For reactions with DTPA anhydride the labeled PEI was eluted from the size exclusion column with sodium bicarbonate (1 M, pH=7.0). In the vial containing the labeled PEI (1 ml, 7×10^{-6} mmol) was added DTPA anhydride (0.0051 g, 0.014 mmol) at RT. The sample was reacted for 10 h and the purification mentioned for the previous derivative was followed. The amount of activity retained was 72% (Ac), 59% (Fr), 12% (Bi), and 11% (Tl) (n=2).

Daughter migration

To test daughter migration, the radiolabeled polymer derivatives were first counted (A0) with a germanium detector. Then added to a centrifuge device, followed by addition of sodium bicarbonate (1 ml, 0.2 M, pH=6.0) and the sample was centrifuged for 30 min. The retentate (0.3–0.7 ml) was diluted to the initial volume (1 ml) and the activity (A_1) was determined. The process was repeated every 24 h and the fraction of daughters retained (f_d) was determined (f_d=A_1/A_0). The samples were done in triplicate. A decay correction was made using a standard which was 0.010 ml of ^{225}Ac solution in sodium bicarbonate (0.990 ml, 0.2 M, pH=6.0). An initial activity (A_{s0}) of the standard was determined for all isotopes then the standard was counted (A_{s1}) every 24 h. The fraction of activity of the standard (f_s) was determined over time (f_s=A_{s1}/A_{s0}). Then the decay corrected retention of daughters (R_d) was determined (R_d=f_d/f_s) for each metal at each time point. In the daughter retention graphs, the data at time=0 corresponds to 24 h after the actinium PEI derivatives were purified by size exclusion chromatography and is the amount retained after centrifugation.

Conclusion

This paper uses a post-labeling approach with a polyethyleneimine containing primary chelators and secondary chelators with carboxylic acid groups. The data indicates the polymers retain daughters and this method has the potential to reduce daughter migration *in vivo*. Using this approach the synthetic procedure to make a bioconjugate would follow these steps: (1) addition of a protected sulfhydryl to the PEI; (2) addition of the primary chelator to the PEI; (3) radiolabeling with ^{225}Ac; (4) addition of the secondary chelator containing phosphate groups or carboxylate groups to the PEI; (5) deprotection of the sulfhydryl; and (6) formation of the thiol-maleimide linkage to the targeting molecule. Future research will be conducted to determine the retention of daughters by PEI conjugates with targeting molecules.

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