Radioactive Labeling of Defined HPMA-Based Polymeric Structures Using [^{18}F]FETos for In Vivo Imaging by Positron Emission Tomography

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During the last decades polymer-based nanomedicine has turned out to be a promising tool in modern pharmaceutics. The following article describes the synthesis of well-defined random and block copolymers by RAFT polymerization with potential medical application. The polymers have been labeled with the positron-emitting nuclide fluorine-18. The polymeric structures are based on the biocompatible *N*-(2-hydroxypropyl)-methacrylamide (HPMA). To achieve these structures, functional reactive ester polymers with a molecular weight within the range of 25000−110000 g/mol were aminolyzed by 2-hydroxypropylamine and tyramine (3%) to form ^{18}F-labelable HPMA-polymer precursors. The labeling procedure of the phenolic tyramine moieties via the secondary labeling synthon 2-[^{18}F]fluoroethyl-1-tosylate ([^{18}F]FETos) provided radiochemical fluoroalkylation yields of ∼80% for block copolymers and >50% for random polymer architectures within a synthesis time of 10 min and a reaction temperature of 120 °C. Total synthesis time including synthon synthesis, ^{18}F-labeling, and final purification via size exclusion chromatography took less than 90 min and yielded stable ^{18}F-labeled HPMA structures in isotonic buffer solution. Any decomposition could be detected within 2 h. To determine the in vivo fate of ^{18}F-labeled HPMA polymers, preliminary small animal positron emission tomography (PET) experiments were performed in healthy rats, demonstrating the renal clearance of low molecular weight polymers. Furthermore, low metabolism rates could be detected in urine as well as in the blood. Thus, we expect this new strategy for radioactive labeling of polymers as a promising approach for in vivo PET studies.

1. Introduction

Polymer-based therapeutics are of increasing interest in the development of nanomedical tools for medical diagnosis and treatment.[1−3] For example, micelles[4−11] and polymer drug conjugates,[12−15] containing various functionalities among a single molecule, have been applied to drug delivery applications. In this respect, polymers can interact with different biological targets selectively, carrying drugs or fulfilling biological tasks.

Functionalities can be introduced in a polymeric system either by polymerizing a mixture of monomers leading to random copolymers or by synthesizing reactive polymer structures that can be transferred into functional structures by a polymer analogous reaction afterward. The reactive ester approach offers two major advantages: On one hand only homopolymers need to be synthesized which can be precisely characterized; on the other hand copolymerization parameters can be disregarded. Copolymers based on N-(2-hydroxypropyl)-methacrylamide (HPMA) and active ester methacrylates have been applied to various medical in vivo applications.[1,4,16,17]

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However, to optimize medical application detailed knowledge about the biodistribution of polymers in the living organism is necessary. It provides insights in pharmacokinetics of the medical substance or metabolism pathways within the target tissue or other organs. The nonspecific interaction between proteins and polymer surfaces determines the in vivo fate of drug carriers.[18−20] Therefore, particle-sizes, compositions, physical properties, and surface chemistry influences the behavior of nanomaterials in vivo.[21]

To understand and finally fine-tune these parameters for in vivo therapies or diagnostics, appropriate imaging strategies are needed. In this respect, noninvasive, quantitative, and repetitive whole body molecular imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) using adequate radiolabeled derivatives would provide a significant advance in the understanding of the mentioned interactions. Compared with other imaging methods, PET and SPECT bear the advantages of high sensitivity (the level of detection approaches 10^{-12} M of tracer) and isotropism (i.e., ability to detect organ accumulation accurately, regardless of tissue depth, whereas fluorescence emission is limited by a low penetration depth), which provide reliability for in vivo quantitative imaging analysis. For macromolecules, most frequently used radioactive nuclides for in vivo imaging are chelated metals, such as ^{111}In or ^{99m}Tc for SPECT and ^{64}Cu for PET.[22−24] Nevertheless, PET offers the more precise and detailed imaging technique due to higher spatial and temporal resolution as well as quantification.[25]

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Recent research has demonstrated the use of chelators (e.g., DOTA derivatives) for the attachment of metallic PET radionuclides.[24] This strategy may have a major drawback. Typically, the chelating agent itself is rather large, bulky and charged and as a result may strongly influence the particle structure and consequently its biological behavior. This work introduces a new approach for ^{18}F-labeling polymers with a rather small synthon 2-[^{18}F]fluoroethyl-1-tosylate ([^{18}F]FETos) that should not influence the structural properties of the self-assembled nanoobject itself.

The imaging time frame of polymer-based therapeutics differs regarding their biological targeting. Due to this fact, long-term and short-term imaging is needed. An example for long-time imaging is passive polymer accumulation in tumor tissue. In contrast, there is a need for short-term in blood pool imaging. All these mentioned applications are of major interest in clinical research, for example, in tumor diagnostics[25] and therapy,[3] in certain heart dysfunctions,[26,27] or tissue perfusion.[28] In this context, ^{18}F-labeled HPMA polymers should allow precise imaging of short-term pharmacokinetics of nanostructures.

2. Materials and Methods

2.1. Materials. All chemicals were reagent grade and obtained from Aldrich. The chemicals were used without further purification unless otherwise indicated. Dioxane used in the synthesis was freshly destilled from a sodium/potassium mixture. 2,2′-Azobis(isobutyronitrile) (AIBN) was recrystallized from diethyl ether and stored at −7 °C. Lauryl methacrylate was distilled and kept at −7 °C.

2.2. Characterization. ^{1}H, ^{13}C, and ^{19}F NMR spectra were obtained at 300 or 400 MHz using a FT-spectrometer from Bruker and analyzed using the ACDLabs 6.0 software. The polymers were dried at 40 °C overnight under vacuum and afterward submitted to gel permeation chromatography (GPC). GPC was performed in tetrahydrofurane (THF) as solvent and with the following parts: pump PU 1580, auto sampler AS 1555, UV-detector UV 1575, RI-detector RI 1530 from Jasco, and miniDAWN Tristar light scattering detector from Wyatt. Columns were used from MZ-Analysentechnik: MZ-Gel SDplus 10^2 Å, MZ-Gel SDplus 10^4 Å, and MZ-Gel SDplus 10^6 Å. The elution diagrams were analyzed using the ASTRA 4.73.04 software from Wyatt Technology. Calibration was done using polystyrene standards. The flow rate was 1 mL/min at a temperature of 25 °C. Radio-TLCs (thin layer chromatography) were analyzed via an Instant Imager (Canberra Packard). HPLC was performed with a Sykam S 1100 pump and a Knauer UV-detector (K-2501), whereas SEC was performed with a waters pump (1500 Series), a Waters UV-detector (2487 λ Absorbence Detector), and a Berthold LB 509 radiodetector. μPET studies were performed with a Siemens MicroPET Focus 120 camera.

2.3. Animals. Male Wistar rats (150−300 g) housed in the animal care facility of the University of Mainz were used in this study. All experiments had previously been approved by the regional animal ethics committee and were conducted in accordance with the German Law for Animal Protection.

2.4. Synthesis of 4-Cyano-4-((thiobenzoyl)sulfanyl)pentanoic Acid. The 4-cyano-4-((thiobenzoyl) sulfanyl)pentanoic acid was used as the chain transfer agent (CTA) and synthesized according to the literature.[29]

2.5. Synthesis of Pentafluorophenyl Methacrylate (PFMA). Pentafluorophenyl methacrylate (PFMA) was prepared according to the literature.[30]

2.6. General Synthesis of the Macro-Chain Transfer Agents (CTA). The macro CTA was prepared according to the literature.[31] RAFT polymerizations of PFMA using 4-cyano-4-((thiobenzoyl) sulfanyl)pentanoic acid were performed in a Schlenk tube. The reaction vessel was loaded with 2,2′-azobisisobutyronitrile (AIBN), 4-cyano-4-((thiobenzoyl)sulfanyl)pentanoic acid (CTA) (molar ratio of AIBN/CTA = 1:8), and 15 g PFMA in 20 mL of dioxane. Following three freeze−vacuum−thaw cycles, the tube was immersed in an oil bath at 70 °C. Afterward the polymer poly(PFMA) was three times precipitated into hexane, isolated by centrifugation, and dried for 12 h at 30 °C under vacuum. In the end, a slightly red powder was obtained. Yield: 59%. ^{1}H NMR ($CDCl_3$) δ [ppm] 1.6−2.2 (br), 0.9−1.5 (br). ^{19}F NMR ($CDCl_3$) δ [ppm] −165.0 (br), −159.7 (br), −153.1 (br).

2.7. General Synthesis of Block Copolymers. The block copolymer was prepared according to the literature.[32]

2.8. Removal of Dithioester End Groups. The dithiobenzoate end group was removed according to the procedure reported by Perrier et al.[33] Typically, 200 mg polymer (M_n = 25.000 g/mol) and 40 mg AIBN (20 times higher than copolymer, mol/mol) were dissolved in 3 mL of anhydrous dioxane/DMSO (4:1). The solution was heated at 80 °C for 2 h. Finally the copolymer was precipitated three times in 100 mL of diethyl ether and collected by centrifugation. In the case of the block copolymer, the crude product was first precipitated in EtOH two times and finally one time in diethyl ether. The copolymer was dried under vacuum for a period of 24 h (yield: 92%). The absence of the dithiobenzoate end group was confirmed by UV−vis spectroscopy.

2.9. Polymer Analogous Reactions of Homopolymers. In a typical reaction, 300 mg PPFMA without ditihioester end group was dissolved in 4 mL of abs. dioxane and 1 mL of abs. dimethylsulfoxide (DMSO). A colorless solution was obtained. Afterward, 8 mg tyramine and 20 mg triethylamine were added. The mixture was kept at 25 °C for 4 h, and finally, 200 mg hydroxypropylamine and 200 mg triethylamine were added. The reaction was allowed to proceed under the above-mentioned conditions overnight. The solution was concentrated in vacuum and introduced to a column filtration using Sephadex LH-20 in dioxane and precipitated in diethyl ether, removed by centrifugation, and dried under vacuum at 30 °C for 14 h. Yield: 85%. ^{1}H NMR (DMSO-d_6) δ [ppm] 6.6−7.2 (br), 4.5−4.8 (br), 3.4−3.9 (br), 2.6−3.0 (br), 0.9−1.3 (br).

2.10. Polymer Analogous Reactions of Block Copolymers. In a typical reaction, 300 mg poly(PFMA)-*block*-poly(lauryl methacrylate) was dissolved in 4 mL of abs. dioxane and 1 mL of abs. dimethylsulfoxide (DMSO). A colorless solution was obtained. Afterward 8 mg tyramine and 20 mg triethylamine were added. The mixture was kept at 25 °C for 4 h. In the end, 200 mg hydroxypropylamine and 200 mg triethylamine were added. The reaction was allowed to run under the above-mentioned conditions overnight. The solution was concentrated in vacuum, introduced to a column filtration using Sephadex LH-20 in dioxane and precipitated in diethyl ether, removed by centrifugation, and dried under vacuum at 30 °C for 14 h. Yield: 82%. ^{1}H NMR (DMSO-*d*6): δ [ppm]) 6.6−7.2 (br), 4.5−4.8 (br), 3.4−3.9 (br), 2.6−3.0 (br), 0.9−1.5 (br), 0.8−0.9 (br t).

2.11. Synthesis of 2-[^{18}F]Fluoroethyl-1-tosylate [^{18}F]FETos. To a dried Kryptofix2.2.2./[^{18}F]fluoride complex, 4 mg ethyleneglycol-1,2-ditosylate in 1 mL of acetonitrile was added and heated under stirring in a sealed vial for 3 min. Purification of the crude product was accomplished using HPLC (Lichrosphere RP18-EC5, 250 × 10 mm, acetonitrile/water 50:50, flow rate: 5 mL/min, R_f: 8 min). After diluting the HPLC fraction containing the [^{18}F]FETos with water (HPLC fraction/water 1:4), the product was loaded on a C18-Sepac cartridge, dried under a nitrogen stream, and eluted with 1.2 mL of DMSO. The whole preparation time was about 40 min and the overall radiochemical yield was between 60 and 80%.[34]

2.12. Radioactive Labeling of Polymers Using [^{18}F]FETos. In a typical reaction, 3 mg polymer was dissolved in 1 mL of DMSO. A clear solution with a concentration of 3 mg/mL was obtained. To this solution, 1 μL of 5 N sodium hydroxide solution and [^{18}F]FETos solution were added. The clear solution was kept at temperatures from 80−150 °C for 20 min. For kinetic measurements, samples were taken from the solution every 5 min. The decay-corrected radiochemical yield (RCY) was checked by TLC (Merck 60 F_{254}) and SEC (HiTrap Desalting Column, Sephadex G-25 Superfine, column volume 5 mL; flow rate: 1 mL PBS-buffer solution) leading to comparable results.

Scheme 1. Synthetic Pathway to Functional Precursor Polymers via RAFT Polymerization

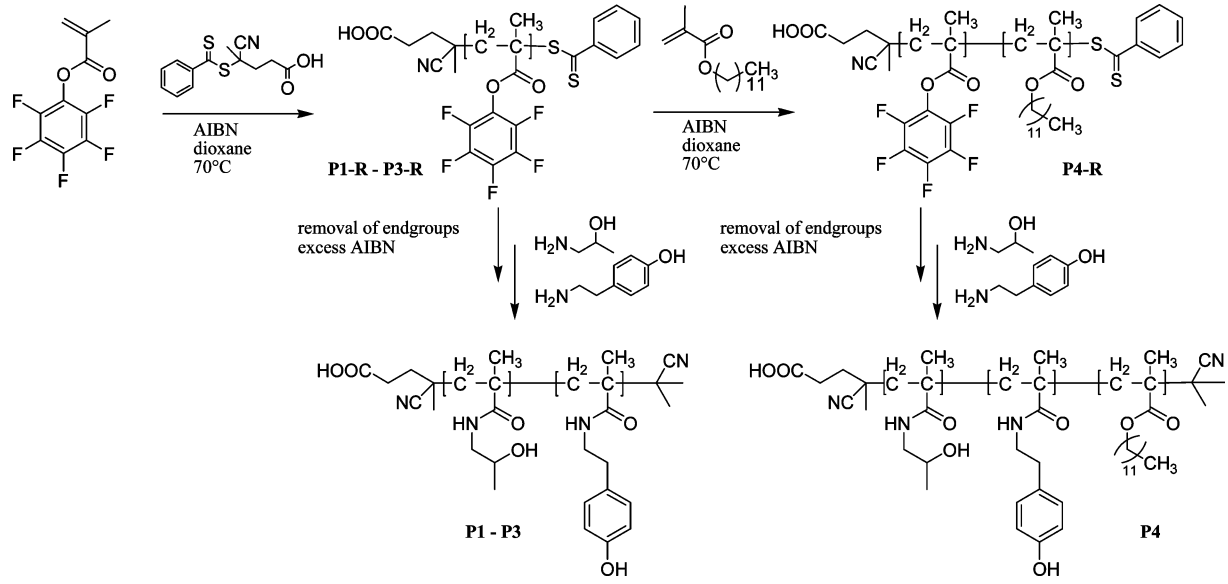


Table 1. Synthesized Reactive and Functional Polymers as Precursors for Radioactive Labeling

polymer	block ratio	ratio of tyramin units at the polymer %	M_n (number average of molecular weight)	M_w (weight average of molecular weight)	PDI
P1-R			21090[b]	25090[b]	1.19[b]
P2-R			50260[b]	60840[b]	1.21[b]
P3-R			103900[b]	134100[b]	1.29[b]
P4-R	87:13[a]		22680[b]	27920[b]	1.25[b]
P1		3[a]	10980[c]	13050[c]	1.19
P2		3[a]	26140[c]	31640[c]	1.21
P3		3[a]	54030[c]	69730[c]	1.29
P4	87:13[a]	3[a]	12570[c]	15880[c]	1.25

[a] As determined by ^{1}H NMR spectroscopy after aminolysis with hydroxypropylamine yielding **P1** to **P4**. [b] As determined by GPC in THF as solvent for the activated ester polymers **P1-R** to **P4-R**. The value for **P1** and **P2** is recalculated from the molecular structure. [c] Calculated from the block ratio obtained by ^{1}H NMR spectroscopy and GPC data of **P1-R** to **P4-R**.

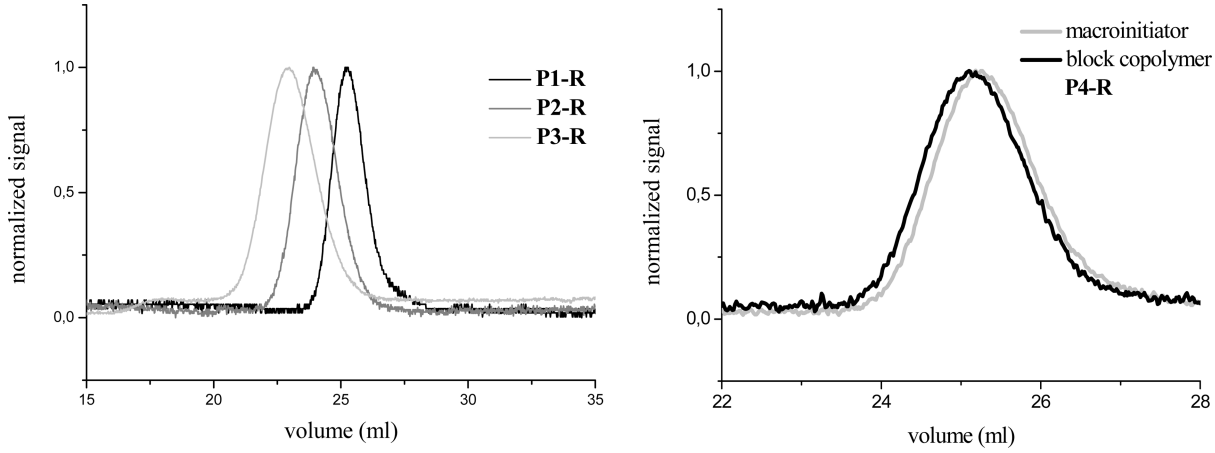


Figure 1. GPC elugram of reactive homopolymers **P1-R**, **P2-R**, and **P3-R** (a) and GPC elugram of the macro initiator and final block copolymer **P4-R** (b).

2.13. Stability. In a typical test, labeled polymers were reinjected into a SEC column (HiTrap Desalting Column, Sephadex G-25 Superfine, column volume 5 mL; flow rate: 1 mL PBS-buffer solution) and checked for impurities.

2.14. In Vitro Binding of Polymers to Human Serum Albumine. Solutions with a concentration of 40 mg/mL of human serum albumine (HSA; **S1**), 1 mg/mL of polymer **P2** (**S2**) as well as a mixture of 0.2 mg **P2** and 40 mg HSA in 1 mL of **S3** were prepared. A TLC in MeOH/H_2O (4:1) using RP-18$F_{(254s)}$ TLC plates was performed by spotting the prepared isotonic solutions. R_f values (**S1**, 0.8; **S2**, 0; **S3**, 0.8 and 0).

2.15. Ex Vivo Metabolism Studies ^{18}F-Labeled Particles. Male Wistar rats were anaesthetized with pentobarbital (40 mg/kg, i.p., Narcoren, Merial, Hallbergmoos, Germany) and a catheter was inserted into the left jugular vein for radiotracer application, a second catheter was inserted into the left carotic artery and a tube was placed in the trachea. The radiotracer was injected i.v. at a dose of ~10 MBq of the labeled ^{18}F-polymer. At 5, 10, 20, 30, and 60 min post-injection, blood samples were collected and analyzed. Whole blood was centrifuged at 7500 rpm for 5 min at 4 °C to separate plasma and blood cells. Plasma and blood cell fractions were obtained and radioactivity was measured with an automatic γ-counter (2470 Wizard;[2] Perkin-Elmer). The percentage of radioactivity bound to plasma

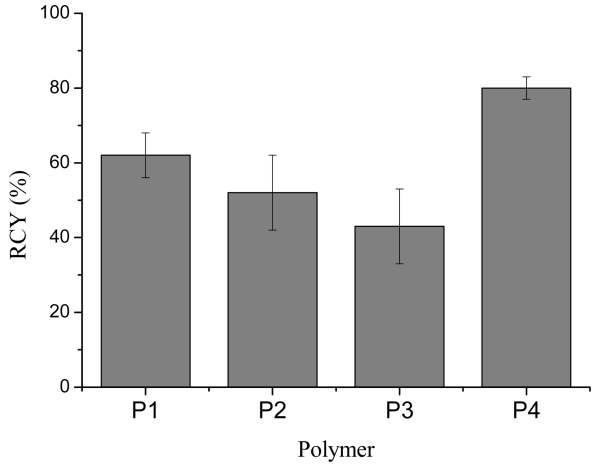


Figure 2. Corrected radiochemical labeling yields (RCY) of the random copolymers **P1**, **P2**, and **P3** and the block copolymer **P4** after 20 min at 100 °C using 3 mg of each precursor polymer.

(μPET) scanner. Animals were anaesthetized with pentobarbital (40 mg/kg, i.p., Narcoren, Merial, Hallbergmoos, Germany) and a catheter was inserted into the left jugular vein for radiotracer application and a tube was placed in the trachea. During PET measurements the animals were placed in supine position and breathed room air spontaneously through a tracheal tube. Listmode acquisition was started with the tracer injection of 15−25 MBq (specific activity: 1.5−2.5 × 10^{-3} GBq/μmol). The ^{18}F-labeled tracers were applied via i.v. injection into the jugular vein catheter. The tracer distribution was followed for up to 4 h after injection. Thereafter, a whole body scan of the rat was performed.

3. Results and Discussion

The synthesis and ^{18}F-labeling of functional HPMA-copolymers and block copolymers is based on precisely characterized active ester polymers,[30] which can be easily modified using primary amines. The synthetic route to functional block copolymers based on the clinically approved

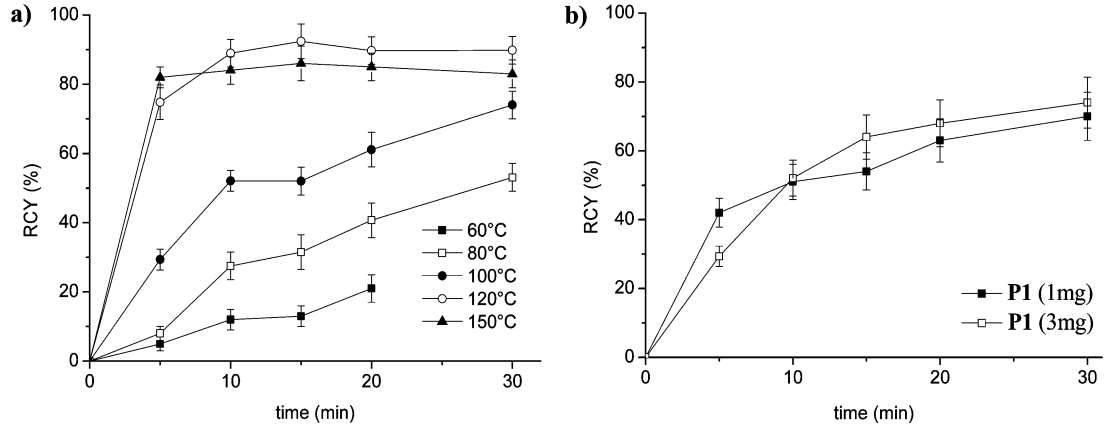
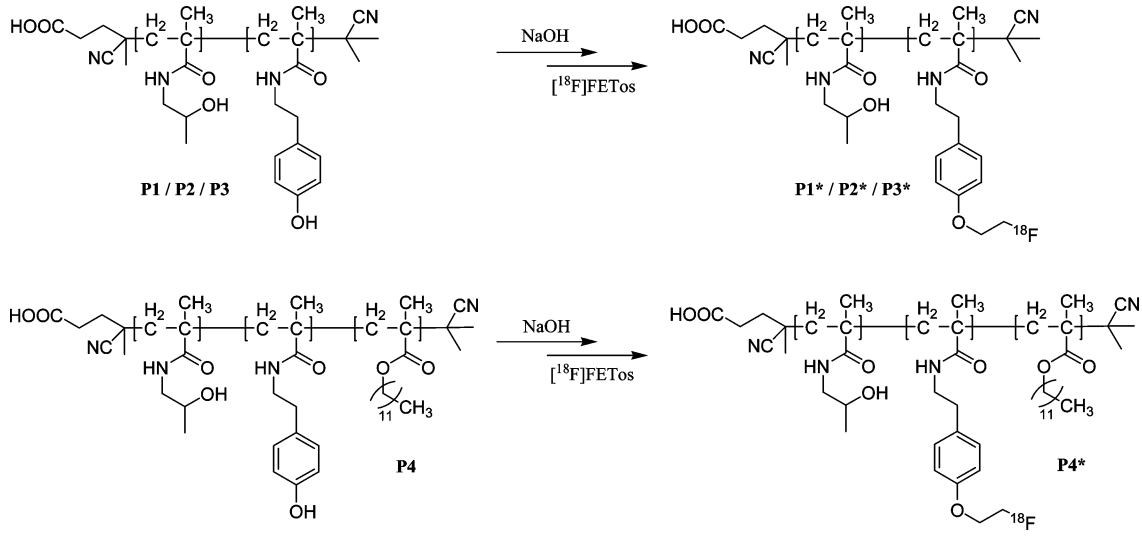


Figure 3. Corrected radiochemical labeling yields (RCY) of **P1** depending on temperature (a) and amount of precursor at 100 °C (b).

Scheme 2. Radioactive Labeling of Polymers Using [^{18}F]FETos



and blood cells was calculated thereafter. In addition, at 60 min p.i. samples of the urine were obtained from puncture of the animal's bladder. For metabolic studies the blood plasma and urine fractions were analyzed via reverse phase thin layer chromatography applying the same conditions described in section 2.14.

2.16. Initial In Vivo PET Studies of ^{18}F-Labeled HPMA-Polymers. Positron emission tomography scans were performed with a Siemens/Concorde Microsystems microPET Focus 120 small animal PET

N-(2-hydroxypropyl) methacrylamide (HPMA) was recently described by Barz et al.[32] Random and block copolymers based on HPMA with phenolic hydroxyl groups in the polymer backbone (scheme 1) were synthesized using the active ester approach. The reactive ester homopolymers and block copolymers have been synthesized by the RAFT polymerization method[29] leading to well-defined polymers

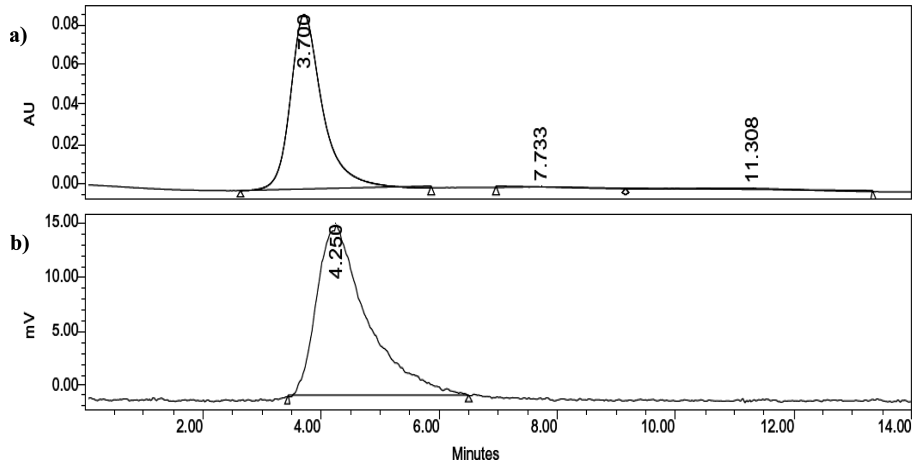


Figure 4. SEC elugram of the ^{18}F-labeled polymer **P2*** proofing stability 2 h after the initial purification in isotonic solution indicating any low molecular weight contamination due to decomposition: UV detector (a) and gamma counter (b).

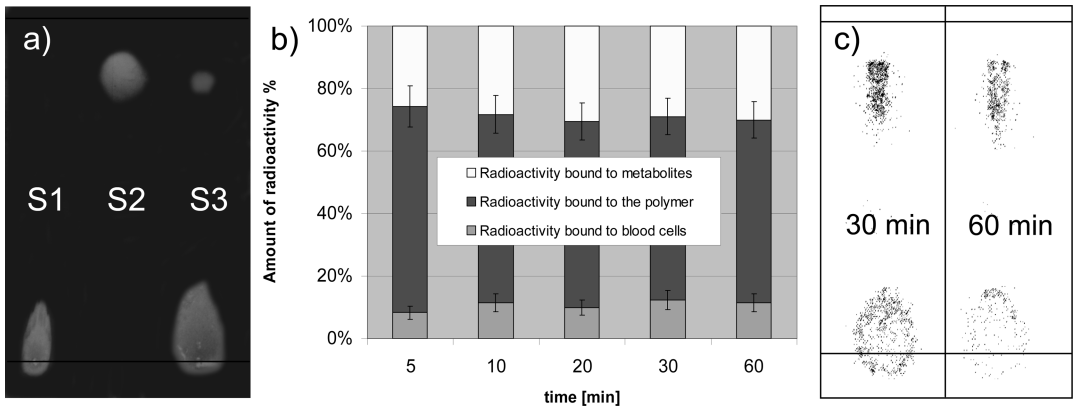


Figure 5. Metabolic analyses of ^{18}F-labeled HPMA. (a) RP-TLC in MeOH/H_2O (4:1) of isotonic solutions: 40 mg/mL of human serum albumine (HSA; **S1**), 1 mg/mL of polymer **P2** (**S2**), as well as a mixture of 0.2 mg **P2** and 40 mg HSA in 1 mL of **S3**. Staining was performed using Seebach Reagent. (b) Distribution of radioactivity among blood cells, proteins, and free polymer ^{18}F-labeled **P2*** in plasma water from 5 to 60 min p.i. determined by radio RP-TLC and automatic γ-counter. (c) Comparison of metabolism of polymer ^{18}F-labeled **P2*** in blood 30 and 60 min p.i. monitored by radio RP-TLC in MeOH/H_2O (4:1), ensuring a small amount of metabolite.

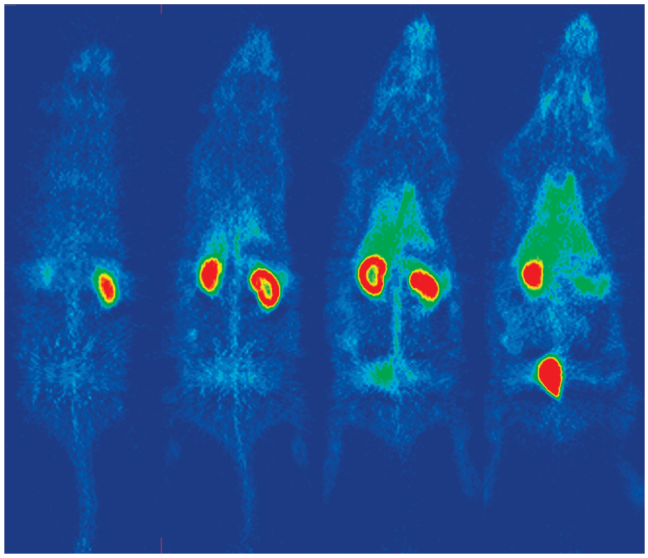


Figure 6. A representative whole body μPET summed image 140−150 min p.i. of the ^{18}F-labeled polymer **P2*** proofing the renal clearance. The most prominent accumulation is seen in the kidneys and the bladder.

with narrow molecular weight distributions. In general, the obtained polymers exhibit a polydispersity index (PDI) of 1.2−1.3 (Table 1) and have been characterized by NMR and GPC (Figure 1). The reactive polymers have been transferred to HPMA based polymeric structures as previously reported.[32]

To achieve regio-selective introduction of an ^{18}F-label, the reactive polymers have been reacted with 2-hydroxy-1-aminopropane and besides with a rather small amount (3%) of tyramine (4-(2-aminoethyl) phenol) to the hydrophilic block to minimize the influence on the polymer's structure. For ^{18}F-labeling purposes, the phenolic tyramine moieties were first deprotonated using a smaller amount of base compared to the introduced phenolic hydroxy groups and subsequently labeled using [^{18}F]FETos (Scheme 2).

The radioactive labeling kinetics for 4 different polymers (**P1**−**P4**) have been evaluated and optimized. A clear dependence of the molecular weight on the decay-corrected radiochemical yield (RCY) at constant temperature could be observed. Higher molecular weight of the polymer led to minor RCY (Figure 2). This expected effect could be explained by the decrease in the surface−volume ratio. Less phenolic moieties should therefore be able to better interact with [^{18}F]FETos. This effect is obvious at all temperatures.

Five different temperatures from 60 up to 150 °C were studied and resulted in the expected tendency of increasing RCY with rising temperature until a maximum yield at ∼120 °C reached. At all temperatures, the polymer itself is stable and does not decompose. The results are plotted in figure 3. The optimal RCY could be obtained at 120 °C in a reaction

time of 10 min. However, even at 60 °C a suitable RCY of ~20% was observed after 15−20 min. This offers the possibility of labeling at ambient temperatures which is necessary for incorporating temperature sensitive molecules into the polymer. The impact of sample mass of the polymer samples turned out to be rather small due to the fact that the amount of labeling agent [^{18}F]FETos is 10^4 to 10^7 times lower compared to the amount of polymer used. This leads to quasi-first order reaction kinetics, explaining the results.

Purification was carried out by size exclusion chromatography (SEC) workup in PBS-buffer leading to a pure, labeled polymer and took ~10 min.

The highest RCYs of 50% for random and ~80% for block copolymers have been obtained for the block copolymer **P4**. An explanation can be micellization in polar DMSO. Tyramine units are trapped in the hydrophilic part of the superstructure. Therefore, the local concentration of phenolic hydroxyl groups is higher compared to the random coil structures. This may result in high labeling yields. The specific radioactivity (A_s) of the polymers was found to be 30 MBq/$g_{polymer}$.

The stability of the polymer was tested 2 h after initial purification and no decomposition occurred (see Figure 4).

These promising results enabled initial PET experiments to determine the in vivo fate of these polymers in healthy rats. PET scans with polymer **P2*** were performed. As expected for slightly negative charged low molecular weight HPMA based polymers,[35] a strong accumulation was observed in the kidneys and in the bladder. Figure 5 shows a representative whole body μPET image of the ^{18}F-labeled polymer **P2***.

A metabolism study demonstrated the in vivo stability of the labeled HPMA-particles. Only a maximum content of ~20% radioactive metabolites could be detected in the blood. Interestingly, this amount does not change during 1 h hour of investigation hinting on a stable ^{18}F-labeled polymer after initial metabolism. In vitro, the labeled polymer was not bound to human serum albumine (Figure 6a), indicating that the synthesized polymer does not show physical interaction with proteins. However, in vivo analysis of the metabolism showed that approximately 20−30% of the radioactivity was found bound to plasma proteins (Figure 6b, "radioactivity bound to metabolites"), indicating that the polymer undergoes some kind of metabolism and the ^{18}F-label may detach from the polymer. A more detailed study has to be carried out to investigate the stability of the O-fluoro-ethyl label and the influence of structural diverse polymers on in vivo metabolism.

The accumulation of radioactivity in the kidneys and the bladder in the PET study can be explained by the renal clearance of the ^{18}F-labeled HPMA-polymers. A metabolism study of the urine demonstrated only the existence of ^{18}F-labeled macromolecules within the bladder indicating the polymer per se is not metabolized in the organsim.

Altogether, total ^{18}F-fluorination including [^{18}F]FETos synthesis, polymer labeling, and polymer purification via SEC took no longer than 90 min and resulted in an ^{18}F-labeled polymer which can now be used for PET imaging over a period from 5–10 h. This time scale appears reasonable to study the particle distribution and accumulation regarding short-term pharmacokinetics.

4. Conclusion

A new versatile ^{18}F-labeling strategy for polymeric particles has been introduced. Defined and functional HPMA-based random and block copolymers have been synthesized by RAFT polymerization and labeled in high RCY of >50% using [^{18}F]FETos in a reaction time of ~ 10 min. Overall synthesis, including [^{18}F]FETos synthesis, polymer labeling, and polymer purification via SEC, was carried out in less than 90 min. The labeled polymer showed no decomposition. First metabolism and μPET experiments showed promising results concerning the in vivo behavior of the ^{18}F-labeled polymer **P2***. The accumulation of radioactivity in kidneys and bladder is due to the renal clearance of the intact compound. Slight metabolism was observed in the blood. However, after initial metabolism, no further degradation could be detected.

In summary, a new method to label polymer precursors by ^{18}F-fluorine has been carried out enabling biological evaluation of polymeric systems in vivo via μPET in the close future. This approach will provide the possibility of precise in vivo imaging of polymeric nanoparticles over a period of 5−10 h. Furthermore, this approach may lead to a detailed understanding in which way alterations in physical properties of the nanostructures such as size, surface chemistry or core material will influence the fate of nanoparticles in living systems.

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