Published in final edited form as:

Biomaterials. 2012 July ; 33(21): 5406–5413. doi:10.1016/j.biomaterials.2012.04.036.

Endothelial Targeting of Polymeric Nanoparticles Stably Labeled with the PET Imaging Radiosotope Iodine-124

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Abstract

Targeting of therapeutics or imaging agents to the endothelium has the potential to improve specificity and effectiveness of treatment for many diseases. One strategy to achieve this goal is the use of nanoparticles (NPs) targeted to the endothelium by ligands of protein determinants present on this tissue, including cell adhesion molecules, peptidases, and cell receptors. However, detachment of the radiolabel probes from NPs poses a significant problem. In this study, we devised polymeric NPs directly labeled with radioiodine isotopes including the positron emission tomography (PET) isotope ¹²⁴I, and characterized their targeting to specific endothelial determinants. This approach provided sizable, targetable probes for specific detection of endothelial surface determinants non-invasively in live animals. Direct conjugation of radiolabel to NPs allowed for stable longitudinal tracking of tissue distribution without label detachment even in an aggressive proteolytic environment. Further, this approach permits tracking of NP pharmacokinetics in real-time and non-invasive imaging of the lung in mice using micro-PET imaging. The use of this strategy will considerably improve investigation of NP interactions with target cells and PET imaging in small animals, which ultimately can aid in the optimization of targeted drug delivery.

Keywords

nanoparticle; endothelial targeting; PET imaging; targeted drug delivery

1. Introduction

Molecules on the surface of endothelial cells lining the lumen of blood vessels are involved in a plethora of physiological and pathological processes and can be used as targets for drug

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delivery and medical imaging [1–4]. The luminal location of some of these surface determinants may serve as molecular signatures of physiological or pathological changes in vascular phenotype [5, 6]. Nuclear imaging modalities, such as gamma-scintigraphy [7], single photon emission computed tomography (SPECT) [8, 9], and positron emission tomography (PET) [10] have been used to non-invasively visualize vascular binding of isotope-labeled imaging probes. Vascular imaging probes have traditionally consisted of radiolabeled ligands of endothelial surface determinants, including cell adhesion molecules [11–13] and ecto-enzymes [9, 14]. Targeted nanoparticles (NPs) (e.g., polymeric carriers with diameter ranging from 20 to 500 nm) may further improve this approach to vascular imaging [6, 15]. First, multivalent binding of ligand-coupled NPs improves targeting [16–18]. Second, with a notable exception of fully PEG-coated polymersomes [19] and filomicelles [20], NPs are generally cleared from circulation faster than proteins. This helps to increase the target/blood ratio, a parameter critically important for imaging highly perfused organs. Third, NPs can carry substantially higher isotope loads without affecting the avidity to the target, providing enhanced image sensitivity.

A variety of NP probes carrying radioactive isotopes have been devised and studied *in vivo* [21–32]. However, detachment of the radionuclide bound (covalently or chelated) to the NP represents a significant problem. This can occur, for example, due to disassembly of kinetically unstable radiometal-chelate systems [33], or degradation of radiohalogen bonds that are metabolically labile such as radiolabeled peptides and proteins [34–36]. Direct conjugation of tracers to stable NP components is advantageous in this context [27, 37]. However, this approach has not been tested yet with targeted NPs. Our goal was to bridge this gap of knowledge by designing targeted polymeric NPs that can be directly radiolabeled to minimize artifacts of label detachment.

In the present study, we have developed polymeric NPs prepared from a poly(4-vinylphenol) (PVPh) polymer backbone that can be directly labeled with the longer-lived positron (β^+)-emitting radionuclide ^{124}I (half life ($t_{1/2}$) = 4.2 d). PVPh is most often associated with the electronics industry, as the dielectric layer of thin-film transistors [38–41], but in recent years PVPh has also found use in biomedical applications, including drug delivery [31, 42, 43]. As compared to the standard PET nuclide ^{18}F ($t_{1/2}$ = 110 min), the convenient 4.2 d half-life of ^{124}I allows for extended radiosynthesis and surface functionalization for NP targeting, and allows for longitudinal PET imaging of pharmacokinetics and *in vivo* targeting capabilities of nanodevices in real time. We devised PVPh-NPs coated with monoclonal antibodies (mAbs) to three endothelial determinants: platelet-endothelial cell adhesion molecule-1 (PECAM-1) [44], thrombomodulin (TM) [45], and PV1 [46]. In this report, we have characterized the salient features of these targeted PVPh-NPs (shape, size, isotope labeling and stability, mAb coating) and studied their biodistribution and targeting to the pulmonary vasculature in mice. Further, we affirmed that PECAM targeted [^{124}I]PVPh-NPs provide non-invasive real-time visualization of the pulmonary vasculature in mice using small animal PET imaging.

2. Materials and methods

2.1 Materials and Instrumentation

Deionized (DI) water (18 M Ω -cm resistivity) was dispensed by a Millipore water purification system (Millipore, Billerica, MA). Control rat IgG was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). The rat anti-mouse CD31/PECAM-1 mAb (MEC13.3 clone) was purchased from BD Pharmingen (San Diego, CA). The rat anti-mouse TM mAb (TM₂₇₃ clone) was a kind gift from Dr. Stephen J. Kennel (University of Tennessee). The PV1 mAb has been described previously [46, 47]. Poly(4-vinylphenol)

(25,000 average M_w) was purchased from Sigma-Aldrich (St Louis, MO). All other chemicals and reagents were purchased from Sigma-Aldrich and used as received.

2.2 PVPh-NP Preparation and characterization

PVPh polymer was dissolved in acetone at desired concentrations. One part PVPh/acetone solution was added (5 ml/min) to five parts DI water with vigorous stirring at the highest vortex setting (Vortex Genie 2, Scientific Industries Inc, Bohemia, NY). The mixture was continuously vortexed for 1 h following addition of polymer. Acetone was removed by evaporation under ambient conditions.

NP diameter was determined *via* dynamic light scattering (DLS, 90Plus Particle Sizer, Brookhaven Instruments, Holtsville, NY). Zeta potential measurements were performed with an adaptor in the same DLS apparatus, in order to determine effective PVPh-NP surface charge. Standard conditions were used with approximately 10 μ L of particle suspension dissolved in 1 mL of 5 mM KCl. All particle preparations were reproduced a minimum of three times and independent DLS and zeta potential measurements were made of each individual preparation.

For TEM, samples were prepared as previously described [48]. Samples were immobilized on TEM mesh grids (Formvar Film 200 Mesh, Electron Microscopy Sciences, Hatfield, PA). Staining was not necessary for visualization due to the high electron density of the phenol ring in the polymer backbone. Grids were imaged on a TEM (JEOL JEM-100CX, West Chester, PA) with an accelerating voltage set to 80 keV. Image analysis was performed with Image J software (NIH).

2.3. PVPh-NP concentration

Particle concentration was determined via a modified colorimetric assay originally used to determine phenol-containing resin concentrations [49]. This nitrous acid-based test first involves isolation of the PVPh-NP by centrifugation and subsequent lyophilization. The polymer was dissolved in butyl acetate at concentrations of 25–150 μ g/mL. To each sample, 1 mL of 3.6 N sulfuric acid and 0.2 mL 10 % aqueous sodium nitrite were added, vortexed, and heated to 70 °C for one hour with periodic vortexing. Samples were then cooled to room temperature. One mL of DI water added to precipitate salts, and the top layer aliquoted and absorbance measured at 425 nm. Unknowns were compared to a calibration standard made from dilutions of known polymer concentration.

2.4. Direct PVPh-NP radioiodination

PVPh-NPs were radiolabeled directly with either [125 I]NaI (Perkin Elmer, Boston, MA), or [124 I]NaI (IBA Molecular, Dulles, VA). Briefly, 100 μ L of PVPh-NPs (4 mg/mL, in DI water) was incubated with iodination beads (Thermo Scientific Pierce, Rockford, IL) and a radioiodine solution (40 μ Ci to 2 mCi of $^{125/124}$ I in 10–100 μ L 20 mM phosphate buffered saline (PBS, Invitrogen)). The reaction was terminated by separating radiolabeled NPs from iodination beads. Due to the high labeling efficiency, [* I]PVPh-NP (where “*” denotes either 124 I or 125 I) were used for mAb-coating without further purification. [* I]PVPh-NP labeling efficiency and free radioiodide content was determined with a standard trichloroacetic acid (TCA) assay traditionally used for characterizing radiochemical purity of labeled protein preparations.

2.5. TCA assay

Briefly, 1 μ L of [* I]PVPh-NP is added to 1 mL of 3 wt % bovine serum albumin (BSA, Sigma-Aldrich)/PBS with 200 μ L of TCA solution (6.1 N, Sigma-Aldrich). After a 15 min incubation, the suspension is centrifuged at 2500 $\times$ g for 15 min. The supernatant (containing

free radioiodine) and pellet (containing [¹²⁵I]PVPh-NP), are separated then measured individually with a Wallac 1470 Wizard gamma (γ)-counter (Perkin Elmer, Wallac Oy, Turku, Finland).

2.6. mAb coating of [¹²⁵I]PVPh-NP

[¹²⁵I]PVPh-NP coating with mAbs was performed using established adsorption techniques [50] based on interactions between the hydrophobic domains on the surface of mAbs and the relatively hydrophobic PVPh-NP surface. Ab (in aqueous buffered solution containing 0.09 % sodium azide) was added to PVPh-NP suspension in DI water, vigorously vortexed for 1 min, and then placed on a rotating shaker for 1 h at room temperature. Ab coating efficiency was determined by measuring [¹²⁵I]IgG adsorbed onto NP relative to IgG mass added. Briefly, the NP/Ab mixture was centrifuged at 12,000×g for 3 min and the supernatant (unbound [¹²⁵I]IgG) was separated from the NP pellet (with [¹²⁵I]IgG bound). Coating efficiency was then calculated as [¹²⁵I]IgG bound/[¹²⁵I]IgG added. For *in vivo* targeted NPs, mAbs were added at a theoretical maximum concentration based upon calculations of total surface area available on the PVPh-NP for protein adsorption. Briefly, using $A = 4\pi r^2$, NP surface area was approximated to ~27 μm² (where NP diameter is 92 nm, from TEM analysis). Treating mAb as a block, its footprint equates to ~120 nm². Ultimately, this corresponds to ~225 mAbs/NP. Before addition of mAbs to the particle suspension, 2 μL of tracer [¹²⁵I]PVPh-NP were mixed in with the cold PVPh-NP. After gentle shaking for one hour, particle-Ab suspensions were diluted 10× and spun down at 12,000×g for 3 min. The supernatant containing free mAb was removed, and mAb-coated PVPh-NP were resuspended in a 1 wt % BSA/PBS solution before injection.

2.7. [¹²⁵I]-NP Stability Studies

PVPh-NP protein stability was performed by preparing IgG-PVPh-NPs, incubating in 1 wt % BSA/PBS at 37 °C, and size of NPs were analyzed via DLS at specified time points (0, 2, 6, and 24 h).

Triplicate samples of radiodine-labeled NP (20 μL) were incubated in 100 μL 0.2 wt % Pronase (Sigma-Aldrich, St Louis, MO) or fresh mouse serum at 37 °C on a rotator shaker (with PBS control). To quantify the radioiodine bound to the NP, samples were surveyed over two weeks *via* digital autoradiography (DAR, FLA-7000, FujiFilm Phosphorimager, Tokyo, Japan) of SiO₂ TLC plates run in 1:1 MeOH/10 % ammonium acetate. Under these TLC conditions, the NP remains at the origin whereas free radioiodine and other radioiodine species travel near the solvent front.

2.8. Biodistribution studies

All animal studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the US National Institutes of Health and approved by the University of Pennsylvania IACUC. Naive C57BL/6 female mice (18–22 g; *n* = 3–5 per group) were anesthetized and injected intravenously (IV) *via* jugular vein with approximately 2 μCi [¹²⁵I]PVPh-NPs coated with anti-PECAM, TM, or PV1 mAb or control IgG. Formulations were sterilized by passing through a 0.2 μm filter prior to injection. Mice were injected with approximately 10 mg/kg mAb-PVPh-NP formulated with a tracer amount of mAb-[¹²⁵I]PVPh-NPs in 200 μL 1 wt % BSA/PBS. Anti-PECAM/[¹²⁵I]PVPh-NP blocking studies were conducted by pre-injecting mice IV with free anti-PECAM mAb MEC13.3 (30 μg/mouse in 0.3 % wt % BSA/saline). Following 30 min free Ab injection, anti-PECAM/[¹²⁵I]PVPh-NP were administered IV. At 30 min post-injection (p.i.) of NP, blood was collected from the retro-orbital sinus and organs (heart, kidneys, liver, spleen, lungs, brain, and thyroid) were collected and weighed. Tissue radioactivity was measured in a γ-counter, and PVPh-NP targeting parameters defined by percent of injected dose per

gram of tissue (% ID/g), localization ratio (LR; organ/blood ratio of % ID/g). Statistical significance was calculated using Student's *t*-test, where *p* values < 0.05 were considered significant.

2.9. PET and CT Image Acquisition and Analysis

Imaging studies were performed on a Philips Mosaic Animal PET (A-PET) scanner [51] and a MicroCATII scanner (CTI-Imtek Co.). Normal C57Bl/6 male mice (22–29 g) were anesthetized with 1–2 % vaporized isoflurane and injected *via* tail-vein with approximately 150–250 μ Ci of mAb- 124 I]PVPh-NP (approximately 10 mg/kg in 200 μ L 1 wt % BSA/PBS). To minimize resolution artifacts associated with thoracic motions, mice were affixed with a respiratory gating device. PET Image acquisition commenced 5–10 min post-injection, with dynamic scans carried out over one hour (5 min per frame; image voxel size 0.5 mm³). MicroCT images were acquired following PET scanning. Region-of-interest (ROI) analysis was performed using AMIDE (<http://amide.sourceforge.net>) on reconstructed images, guided by detailed mouse anatomy from microCT images of each imaged animal. ROI's were drawn over the lungs of each mouse.

3. Results and Discussion

3.1 Synthesis and Characterization of PVPh-NPs

Spherical polymeric NPs were synthesized from a 25 kDa poly(4-vinylphenol) (PVPh) polymer as shown in Figure 1. Particle formation was accomplished by a solvent diffusion formulation, resulting in nano-scale spherical particles suspended in water. PVPh-NP size was controlled by modifying the initial PVPh polymer/solvent concentration. As the PVPh polymer concentration increased from 3 to 100 mg/mL, the average particle hydrodynamic diameter (D_{avg}), as measured by dynamic light scattering (DLS), increased from 60 nm to approximately 200 nm (Table 1 and Fig. 2A). Further, PVPh-NPs carry a net negative charge, with a zeta potential of approximately –30 mV, due to the abundance of phenols present on the polymer backbone (Table 1 and Supporting Information Fig. S1).

For targeting studies, PVPh-NPs (D_{avg} 145 nm) were coated with mAb directed to endothelial determinants PECAM, TM, or PV1 or control IgG. Up to ~200 mAb molecules per PVPh-NP were adsorbed with > 90 % efficiency (Fig. S2). Size of protein coated NPs was stable for at least 24 hours in 1% BSA at 37°C (Fig. S3). Coating PVPh-NPs with mAb increased PVPh-NP size from 145 nm to 180 nm by DLS (Fig. 2B). Transmission electron microscopy (TEM) confirmed increased particle size and revealed that mAb coating enhances surface roughness of particles relative to uncoated PVPh-NPs (Fig. 2C,D).

3.2. PVPh-NP radioiodination

Radioiodine is typically introduced onto peptides and proteins by oxidative iodination of the phenol moiety of tyrosine residues [52]. Similarly, PVPh-NPs, comprised of phenol moieties on its polymer backbone, are easily radioiodinated by electrophilic aromatic substitution, resulting in a radioiodine *ortho* to the hydroxy group of phenol. The high concentration of phenol groups per NP results in high radioiodination efficiencies and specific activities (μ Ci/NP). Depending on the intended application, the γ -emitter 125 I ($t_{1/2}$ = 60 d) can be employed for facile measurement of isotope levels *in vitro* or in biological specimens (e.g., blood and organs obtained postmortem) using a γ -counter, whereas the β^+ -emitter 124 I can be used for PET imaging. Employing a standard protocol using commercially available isotopes [125 I]NaI or [124 I]NaI, corresponding formulations of [* I]PVPh-NPs (* denotes either 124 I or 125 I) were prepared within 30 minutes, with ~90 % yield and < 10 % residual free isotope (Fig. S4). Following mAb-coating and purification by centrifugation, mAb- * [I]PVPh-NP preparations were typically > 98 % radiochemically pure. The specific activity

of [^{125}I]PVPh-NPs typically used for *in vivo* studies is $0.1 \mu\text{Ci}/\mu\text{g}$ of polymer ($3.9 \times 10^{-11} \mu\text{Ci}/\text{NP}$) and is far from the maximum achievable specific activity. Similarly, [^{124}I]PVPh-NPs can be obtained in high yields with specific activity upwards of $10 \mu\text{Ci}/\mu\text{g}$ of polymer, sufficient for PET imaging. This approach enables a kit-ready method for preparing directly radioiodinated NPs for subsequent mAb-coating and *in vivo* targeting studies within a period of hours. Based on the general utility of the conjugation chemistry used, this platform can also be extended to other radioiodine isotopes, namely ^{123}I for SPECT and ^{131}I for β^- -therapy.

3.3. In vivo endothelial targeting of mAb-[^{125}I]PVPh-NPs

The pulmonary vasculature represents approximately 30 % of endothelial surface area in the body [53]. Further, for each systole lungs receive the entire cardiac output of venous blood *via* the pulmonary artery from the right ventricle (i.e., 50 % of total cardiac blood output), plus a fraction of arterial blood output *via* bronchial arterial circulation (all other organs share the arterial blood, i.e., < 50 % of the total cardiac output). For these reasons, compounds with endothelial affinity accumulate in the lungs after intravenous (IV) administration [54]. Therefore, to characterize endothelial targeting *in vivo*, we determined blood level and pulmonary accumulation of mAb-coated [^{125}I]PVPh-NPs and free labeled mAb ([^{125}I]-mAb) after IV injection. We tested mAbs to three endothelial surface determinants: PECAM, TM, and PV1, which localize predominantly at intercellular borders, the apical surface, and caveoli of lung endothelial cells, respectively [55].

Targeting mAb or control IgG were adsorbed onto the PVPh-NPs surface as described above at the maximal surface density of approximately 200 mAb molecules per NP. Figure 3 shows the pulmonary accumulation and blood level of PVPh-NPs coated with anti-TM, anti-PECAM, or anti-PV1 versus IgG-coated counterparts and parental [^{125}I]-mAbs. TM targeted NPs showed pulmonary accumulation similar to free TM mAb (84.6 ± 25.8 vs. 106.8 ± 7.8 % ID/g, respectively) (Fig. 3A). Anti-PECAM coated NPs showed higher pulmonary uptake than parental anti-PECAM mAb (88.5 ± 13.2 vs. 40.9 ± 9.6 % ID/g, respectively) (Fig. 3B). This effect is likely due to multivalent binding of mAb-coated NPs to the epitopes accessible on the cell surface at appropriate density, as previously described by our lab and others [16, 56, 57]. Further, lung uptake of anti-PECAM/NP was blocked when mice were injected with cold anti-PECAM mAb prior to NP administration, confirming specificity of NP targeting (Fig. S5). Of note, blood level of both anti-PECAM/NPs and anti-TM/NPs were markedly lower than that of free mAb counterparts. Thus, when corrected for residual blood level, the NP lung/blood ratio is ~20-fold greater for both PECAM and TM targeted NPs versus that of corresponding free mAb (Fig. 3A, B insets). Such a fast blood clearance is advantageous for imaging of highly perfused organs, including the lungs.

Conversely, pulmonary accumulation of anti-PV1/NPs did not exceed nonspecific uptake of control IgG/NPs (15.8 ± 2.8 vs. 10.6 ± 5.8 % ID/g, respectively), despite high pulmonary accumulation of free anti-PV1 (191.9 ± 8.7 % ID/g) (Fig. 3C). Among other potential reasons, this outcome can be attributed to the localization of PV1 in the stomatal diaphragms, structures restricted to the introits of caveolae invaginations of the endothelial plasmalemma, which have an aperture diameter < 100 nm [14, 58]. It is logical to postulate that targets in these invaginations are accessible to circulating antibodies (average size 10 nm), but not to spheres with diameters > 100 nm. Similar results have been observed with other antibodies binding to caveolar antigens: free labeled affinity peptides accumulated in the lungs extremely effectively, whereas the phage particles carrying the same ligand did not [14].

As anticipated, all tested NP formulations, both targeted and non-targeted, accumulated in liver and spleen (Table 2). As organs of the reticuloendothelial system (RES), the liver and

spleen have open sinusoids lined with discontinuous endothelium allowing for entrapment of circulating particulates [59]. Uptake in other organs was modest and similar for targeted and non-targeted NPs. The results obtained with mAb-targeted PVPh-NPs at 30 min post-IV administration have matched up with those obtained using the previous iteration of mAb-coated polystyrene (PS)-NPs of similar size indirectly traced *via* radiolabeled IgG as previously described (Table S1) [60]. This result indicates that avidity, not the chemical backbone of the comparable spherical polymeric carriers of this size define the targeting.

However, directly labeled PVPh-NPs and indirectly labeled PS-NPs showed different changes in the target over time. At 30 min and 24 h after IV injection of anti-PECAM/PVPh-NPs, the isotope level in organs did not significantly change, whereas within the same time interval anti-PECAM/PS-NP level in the lungs and RES organs dropped dramatically (Fig. 4A). The latter result agrees well with the previous study showing time dependent reduction of the pulmonary signal of anti-ICAM/PS-NP carrying ^{64}Cu -labeled tracer IgG on the surface [23]. This outcome is likely due to degradation of the isotope-labeled IgG coating of PS-NPs, reflected in the marked elevation in the thyroid uptake of radioiodine (8-fold increase in % ID relative to PVPh-NPs). In contrast, PVPh-NPs displayed < 1 % thyroid uptake even at 24 h post-injection.

In accord with this notion, no detachment of radioiodine from PVPh-NPs was detected after a 2-week incubation with a protease mixture, while PS-NPs were completely stripped of radiolabel within a few hours of protease incubation due to degradation of radiolabeled IgG (Fig. 4B). When PS-NPs were incubated with mouse serum or PBS alone, radiolabel loss was less prominent as compared to protease incubation, suggesting that mAb are not as readily dehalogenated or desorbed from the NP (Fig. S6). While the protease mixture may not represent the spectrum of enzymes found in a living animal, these results combined with the *in vivo* radiostability of PVPh-NPs demonstrate that stable conjugation of the radionuclide directly to the polymer matrix of NPs helps to avoid artifacts associated with facile radiolabel detachment observed when the label is attached to labile proteins molecules. This represents an important feature and advantage of [^{124}I]PVPh-NPs that permits longitudinal studies of redistribution and tissue trafficking of the NPs.

3.4. PET imaging of mAb-[^{124}I]PVPh-NP

The ability of PVPh-NPs to be directly labeled with a radioiodine isotope for *in vivo* nuclear imaging is especially compelling. To test this feature of targeted PVPh-NPs we employed PET imaging, which is used clinically to detect disease states noninvasively, quantitatively, and with high sensitivity. PET detection of probe distribution *in vivo* is contingent on the presence of a β^+ -emitting radionuclide within the probe structure. In recent years, ^{124}I has emerged as a valuable radioiodine isotope for PET applications [61]. Furthermore, with a relatively long $t_{1/2}$ of 4.2 d, ^{124}I has great potential in non-invasively evaluating the longitudinal *in vivo* pharmacokinetics and targeting capabilities of NPs. In contrast, ^{18}F -labeled probes, although well established in pre-clinical and clinical PET applications, their utility is limited by their $t_{1/2}$ of 110 min. This short half-life though useful in many clinical applications, is not advantageous for the pharmacokinetic investigation of probes, such as targeted-NPs, that have long blood and tissue residence times.

To examine ^{124}I -PET imaging of the pulmonary vasculature using PVPh-NPs, we used PECAM-targeted [^{124}I]PVPh-NPs. Anti-PECAM or IgG coated [^{124}I]PVPh-NP were injected IV in mice, and imaged using dynamic PET and computed tomography (CT). PET and CT images were co-registered to facilitate anatomical definition for region-of-interest (ROI) analysis of radiotracer distribution in the thorax. Pulmonary uptake was detected within 5 minutes post-injection of anti-PECAM/[^{124}I]PVPh-NPs and throughout the 1 h dynamic scan. Control IgG/[^{124}I]PVPh-NPs provided markedly lower signal from the thorax

(Fig. 5A and Fig. S7). ROI analysis correlated with these observations and confirmed pulmonary uptake of PECAM targeted PVPh-NPs was 8-fold greater than that of IgG-coated PVPh-NPs (Fig. 5B). ROI analysis also revealed that anti-PECAM/PVPh-NP thoracic signal slowly decreased by ~15 % in the time interval from 5 to 60 min post-injection (Fig. 5C). These findings support the utility of antibody-targeted [124 I]PVPh-NPs for real-time PET imaging of the longitudinal pharmacokinetic profiles of targeted nanoparticles.

4. Conclusion

In this work, we demonstrate that polymeric nanoparticles synthesized using the PVPh-based strategy, provides sizable, targetable probes for specific detection of endothelial surface determinants non-invasively in live animals. Furthermore, the chemistry of PVPh can be exploited for direct labeling of NPs with radioisotope and this allows for stable tracking of pharmacokinetics and their real-time visualization using PET imaging in animal studies. Although not proposed for human studies, it is highly likely that targeted NPs based on the principles described in this work will find application in experimental biomedicine, in particular, for investigating NP interactions with target cells and PET imaging in small animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grant (HL-087036-01A2) to V.R.M.

REFERENCES AND NOTES

1. Staquicini FI, Cardo-Vila M, Kolonin MG, Trepel M, Edwards JK, Nunes DN, et al. Vascular ligand-receptor mapping by direct combinatorial selection in cancer patients. *Proc Natl Acad Sci U S A*. 2011; 108:18637–18642. [PubMed: 22049339]
2. Charoenphol P, Mocherla S, Bouis D, Namdee K, Pinsky DJ, Eniola-Adefeso O. Targeting therapeutics to the vascular wall in atherosclerosis--carrier size matters. *Atherosclerosis*. 2011; 217:364–370. [PubMed: 21601207]
3. Nune SK, Gunda P, Thallapally PK, Lin YY, Forrest ML, Berkland CJ. Nanoparticles for biomedical imaging. *Expert Opin Drug Deliv*. 2009; 6:1175–1194. [PubMed: 19743894]
4. Ding BS, Hong N, Christofidou-Solomidou M, Gottstein C, Albelda SM, Cines DB, et al. Anchoring fusion thrombomodulin to the endothelial lumen protects against injury-induced lung thrombosis and inflammation. *Am J Respir Crit Care Med*. 2009; 180:247–256. [PubMed: 19342415]
5. Ding BS, Dziubla T, Shuvaev VV, Muro S, Muzykantov VR. Advanced drug delivery systems that target the vascular endothelium. *Mol Interv*. 2006; 6:98–112. [PubMed: 16565472]
6. Chacko AM, Hood ED, Zern BJ, Muzykantov VR. Targeted nanocarriers for imaging and therapy of vascular inflammation. *Curr Opin Colloid Interface Sci*. 2011; 16:215–227. [PubMed: 21709761]
7. Danilov SM, Martynov AV, Klivanov AL, Slinkin MA, Sakharov I, Malov AG, et al. Radioimmunoimaging of lung vessels: an approach using indium-111-labeled monoclonal antibody to angiotensin-converting enzyme. *J Nucl Med*. 1989; 30:1686–1692. [PubMed: 2552048]
8. Chrastina A, Valadon P, Massey KA, Schnitzer JE. Lung vascular targeting using antibody to aminopeptidase P: CT-SPECT imaging, biodistribution and pharmacokinetic analysis. *J Vasc Res*. 2010; 47:531–543. [PubMed: 20431301]
9. Kennel SJ, Huang Y, Zeng WB, Stuckey A, Wall J. Kinetics of vascular targeted monoclonal antibody. *Curr Drug Deliv*. 2010; 7:428–435. [PubMed: 20950267]

10. Nahrendorf M, Keliher E, Panizzi P, Zhang H, Hembrador S, Figueiredo JL, et al. 18F-4V for PET-CT imaging of VCAM-1 expression in atherosclerosis. *JACC Cardiovasc Imaging*. 2009; 2:1213–1222. [PubMed: 19833312]
11. Heckl S. Future contrast agents for molecular imaging in stroke. *Curr Med Chem*. 2007; 14:1713–1728. [PubMed: 17627509]
12. Hariri G, Zhang Y, Fu A, Han Z, Brechbiel M, Tantawy MN, et al. Radiation-guided P-selectin antibody targeted to lung cancer. *Ann Biomed Eng*. 2008; 36:821–830. [PubMed: 18273706]
13. Garrood T, Blades M, Haskard DO, Mather S, Pitzalis C. A novel model for the pre-clinical imaging of inflamed human synovial vasculature. *Rheumatology (Oxford)*. 2009; 48:926–931. [PubMed: 19491304]
14. Oh P, Borgstrom P, Witkiewicz H, Li Y, Borgstrom BJ, Chrastina A, et al. Live dynamic imaging of caveolae pumping targeted antibody rapidly and specifically across endothelium in the lung. *Nat Biotechnol*. 2007; 25:327–337. [PubMed: 17334358]
15. Simone EA, Dziubla TD, Muzykantov VR. Polymeric carriers: role of geometry in drug delivery. *Expert Opin Drug Deliv*. 2008; 5:1283–1300. [PubMed: 19040392]
16. Muzykantov VR, Christofidou-Solomidou M, Balyasnikova I, Harshaw DW, Schultz L, Fisher AB, et al. Streptavidin facilitates internalization and pulmonary targeting of an anti-endothelial cell antibody (platelet-endothelial cell adhesion molecule 1): a strategy for vascular immunotargeting of drugs. *Proc Natl Acad Sci U S A*. 1999; 96:2379–2384. [PubMed: 10051650]
17. Calderon AJ, Bhowmick T, Leferovich J, Burman B, Pichette B, Muzykantov V, et al. Optimizing endothelial targeting by modulating the antibody density and particle concentration of anti-ICAM coated carriers. *J Control Release*. 2011; 150:37–44. [PubMed: 21047540]
18. Shokeen M, Pressly ED, Hagooly A, Zheleznyak A, Ramos N, Fiamengo AL, et al. Evaluation of multivalent, functional polymeric nanoparticles for imaging applications. *ACS Nano*. 2011; 5:738–747. [PubMed: 21275414]
19. Christian DA, Garbuzenko OB, Minko T, Discher DE. Polymer vesicles with a red cell-like surface charge: microvascular imaging and in vivo tracking with near-infrared fluorescence. *Macromol Rapid Commun*. 2010; 31:135–141. [PubMed: 21590885]
20. Geng Y, Dalhaimer P, Cai S, Tsai R, Tewari M, Minko T, et al. Shape effects of filaments versus spherical particles in flow and drug delivery. *Nat Nanotechnol*. 2007; 2:249–255. [PubMed: 18654271]
21. Ballot S, Noiret N, Hindre F, Denizot B, Garin E, Rajerison H, et al. 99mTc/188Re-labelled lipid nanocapsules as promising radiotracers for imaging and therapy: formulation and biodistribution. *Eur J Nucl Med Mol Imaging*. 2006; 33:602–607. [PubMed: 16450136]
22. Kennel SJ, Woodward JD, Rondinone AJ, Wall J, Huang Y, Mirzadeh S. The fate of mAb-targeted Cd(125m)Te/ZnS nanoparticles in vivo. *Nucl Med Biol*. 2008; 35:501–514. [PubMed: 18482688]
23. Rossin R, Muro S, Welch MJ, Muzykantov VR, Schuster DP. In vivo imaging of ⁶⁴Cu-labeled polymer nanoparticles targeted to the lung endothelium. *J Nucl Med*. 2008; 49:103–111. [PubMed: 18077519]
24. McDevitt MR, Chattopadhyay D, Jaggi JS, Finn RD, Zanzonico PB, Villa C, et al. PET imaging of soluble yttrium-86-labeled carbon nanotubes in mice. *PLoS One*. 2007; 2:e907. [PubMed: 17878942]
25. Gratton SE, Pohlhaus PD, Lee J, Guo J, Cho MJ, Desimone JM. Nanofabricated particles for engineered drug therapies: a preliminary biodistribution study of PRINT nanoparticles. *J Control Release*. 2007; 121:10–18. [PubMed: 17643544]
26. Cai W, Chen K, Li ZB, Gambhir SS, Chen X. Dual-function probe for PET and near-infrared fluorescence imaging of tumor vasculature. *J Nucl Med*. 2007; 48:1862–1870. [PubMed: 17942800]
27. Chrastina A, Schnitzer JE. Iodine-125 radiolabeling of silver nanoparticles for in vivo SPECT imaging. *Int J Nanomedicine*. 2010; 5:653–659. [PubMed: 20856841]
28. Choi JS, Park JC, Nah H, Woo S, Oh J, Kim KM, et al. A hybrid nanoparticle probe for dual-modality positron emission tomography and magnetic resonance imaging. *Angew Chem Int Ed Engl*. 2008; 47:6259–6262. [PubMed: 18613191]

29. Park JC, Yu MK, An GI, Park SI, Oh J, Kim HJ, et al. Facile preparation of a hybrid nanoprobe for triple-modality optical/PET/MR imaging. *Small*. 2010; 6:2863–2868. [PubMed: 21104828]
30. Shao X, Zhang H, Rajian JR, Chamberland DL, Sherman PS, Quesada CA, et al. ¹²⁵I-labeled gold nanorods for targeted imaging of inflammation. *ACS Nano*. 2011; 5:8967–8973. [PubMed: 22003968]
31. Lee CC, Yoshida M, Frechet JM, Dy EE, Szoka FC. In vitro and in vivo evaluation of hydrophilic dendronized linear polymers. *Bioconjug Chem*. 2005; 16:535–541. [PubMed: 15898719]
32. Sun Y, Yu M, Liang S, Zhang Y, Li C, Mou T, et al. Fluorine-18 labeled rare-earth nanoparticles for positron emission tomography (PET) imaging of sentinel lymph node. *Biomaterials*. 2011; 32:2999–3007. [PubMed: 21295345]
33. Brechbiel MW. Bifunctional chelates for metal nuclides. *Q J Nucl Med Mol Imaging*. 2008; 52:166–173. [PubMed: 18043537]
34. Akizawa H, Uehara T, Arano Y. Renal uptake and metabolism of radiopharmaceuticals derived from peptides and proteins. *Adv Drug Deliv Rev*. 2008; 60:1319–1328. [PubMed: 18508156]
35. Haubner R, Wester HJ. Radiolabeled tracers for imaging of tumor angiogenesis and evaluation of anti-angiogenic therapies. *Curr Pharm Des*. 2004; 10:1439–1455. [PubMed: 15134568]
36. Vaidyanathan G, Zalutsky MR. Protein radiohalogenation: observations on the design of N-succinimidyl ester acylation agents. *Bioconjug Chem*. 1990; 1:269–273. [PubMed: 2096920]
37. Herth MM, Barz M, Moderegger D, Allmeroth M, Jahn M, Thews O, et al. Radioactive labeling of defined HPMa-based polymeric structures using [¹⁸F]FETos for in vivo imaging by positron emission tomography. *Biomacromolecules*. 2009; 10:1697–1703. [PubMed: 19425549]
38. Lee YJ, Jang Y, Cho K, Pyo S, Hwang DH, Hong SC. Preparation of poly(4-hydroxystyrene) based functional block copolymer through living radical polymerization and its nanocomposite with BaTiO₃ for dielectric material. *J Nanosci Nanotechnol*. 2009; 9:7161–7166. [PubMed: 19908749]
39. Park JH, Kim E. Effect of electric-field-assisted thermal annealing of poly(4-vinylphenol) film on its dielectric constant. *Appl Phys Lett*. 2008; 92
40. Gao JK, Cui YJ, Yu JC, Lin WX, Wang ZY, Qian GD. Design and synthesis of bichromophores for nonlinear optical applications in polymer films. *React Funct Polym*. 2011; 71:496–501.
41. Li S, Chen W, Chu D, Roy S. Self-aligned high-resolution printed polymer transistors. *Adv Mater*. 2011; 23:4107–4110. [PubMed: 21800381]
42. Stepanek M, Hajduova J, Prochazka K, Slouf M, Nebesarova J, Mountrichas G, et al. Association of poly(4-hydroxystyrene)-block-poly(Ethylene oxide) in aqueous solutions: block copolymer nanoparticles with intermixed blocks. *Langmuir*. 2012; 28:307–313. [PubMed: 22107340]
43. Lee NS, Lin LY, Neumann WL, Freskos JN, Karwa A, Shieh JJ, et al. Influence of nanostructure morphology on host capacity and kinetics of guest release. *Small*. 2011; 7:1998–2003. [PubMed: 21678552]
44. Newman PJ. The role of PECAM-1 in vascular cell biology. *Ann N Y Acad Sci*. 1994; 714:165–174. [PubMed: 8017765]
45. Kennel SJ, Lankford T, Hughes B, Hotchkiss JA. Quantitation of a murine lung endothelial cell protein, P112, with a double monoclonal antibody assay. *Lab Invest*. 1988; 59:692–701. [PubMed: 2460698]
46. Stan RV, Tkachenko E, Niesman IR. PV1 is a key structural component for the formation of the stomatal and fenestral diaphragms. *Mol Biol Cell*. 2004; 15:3615–3630. [PubMed: 15155804]
47. Stan RV. Multiple PV1 dimers reside in the same stomatal or fenestral diaphragm. *Am J Physiol Heart Circ Physiol*. 2004; 286:H1347–H1353. [PubMed: 14630628]
48. Simone EA, Dziubla TD, Colon-Gonzalez F, Discher DE, Muzykantov VR. Effect of polymer amphiphilicity on loading of a therapeutic enzyme into protective filamentous and spherical polymer nanocarriers. *Biomacromolecules*. 2007; 8:3914–3921. [PubMed: 18038999]
49. Swann MH, Weil DJ. Colorimetric determination of phenolic resins. *Anal Chem*. 1956; 28:1463–1465.
50. Muro S, Cui X, Gajewski C, Murciano JC, Muzykantov VR, Koval M. Slow intracellular trafficking of catalase nanoparticles targeted to ICAM-1 protects endothelial cells from oxidative stress. *Am J Physiol Cell Physiol*. 2003; 285:C1339–C1347. [PubMed: 12878488]

51. Surti S, Karp JS, Perkins AE, Cardi CA, Daube-Witherspoon ME, Kuhn A, et al. Imaging performance of A-PET: a small animal PET camera. *IEEE Trans Med Imaging*. 2005; 24:844–852. [PubMed: 16011313]
52. Salacinski PR, McLean C, Sykes JE, Clement-Jones VV, Lowry PJ. Iodination of proteins, glycoproteins, and peptides using a solid-phase oxidizing agent, 1,3,4,6-tetrachloro-3 alpha,6 alpha-diphenyl glycoluril (Iodogen). *Anal Biochem*. 1981; 117:136–146. [PubMed: 7316186]
53. Davies MG, Hagen PO. The vascular endothelium. a new horizon. *Ann Surg*. 1993; 218:593–609. [PubMed: 8239773]
54. Shuvaev VV, Ilies MA, Simone E, Zaitsev S, Kim Y, Cai S, et al. Endothelial targeting of antibody-decorated polymeric filomicelles. *ACS Nano*. 2011; 5:6991–6999. [PubMed: 21838300]
55. Oh P, Li Y, Yu J, Durr E, Krasinska KM, Carver LA, et al. Subtractive proteomic mapping of the endothelial surface in lung and solid tumours for tissue-specific therapy. *Nature*. 2004; 429:629–635. [PubMed: 15190345]
56. Scherpereel A, Wiewrodt R, Christofidou-Solomidou M, Gervais R, Murciano JC, Albelda SM, et al. Cell-selective intracellular delivery of a foreign enzyme to endothelium in vivo using vascular immunotargeting. *FASEB J*. 2001; 15:416–426. [PubMed: 11156957]
57. Danilov SM, Gavrilyuk VD, Franke FE, Pauls K, Harshaw DW, McDonald TD, et al. Lung uptake of antibodies to endothelial antigens: key determinants of vascular immunotargeting. *Am J Physiol Lung Cell Mol Physiol*. 2001; 280:L1335–L1347. [PubMed: 11350815]
58. Stan RV. Endothelial stomatal and fenestral diaphragms in normal vessels and angiogenesis. *J Cell Mol Med*. 2007; 11:621–643. [PubMed: 17760829]
59. Wisse, E.; de Leeuw, AM. Structural elements determining transport and exchange process in the liver. In: Davis, SS.; Illum, L.; McVie, JG.; Tomlinson, E., editors. *Microspheres and Drug Therapy, Pharmaceutical, Immunological and Medical Aspects*. Amsterdam: Elsevier; 1984. p. 1-23.
60. Garnacho C, Shuvaev V, Thomas A, McKenna L, Sun J, Koval M, et al. RhoA activation and actin reorganization involved in endothelial CAM-mediated endocytosis of anti-PECAM carriers: critical role for tyrosine 686 in the cytoplasmic tail of PECAM-1. *Blood*. 2008; 111:3024–3033. [PubMed: 18182571]
61. Chacko AM, Divgi CR. Radiopharmaceutical chemistry with iodine-124: a non-standard radiohalogen for positron emission tomography. *Med Chem*. 2011; 7:388–405.

Fig. 1. Schematic diagram of the synthesis of poly-(4-vinyl)-phenol (PVPh) nanoparticles (PVPh-NP) by a solvent diffusion method (I), and their subsequent radiolabeling directly with ^{125}I or ^{124}I radioiodine (*I) (II). Coating of the [*I]PVPh-NP with targeting antibodies (mAb) is achieved by adsorption (III).

Fig. 2. Control of PVPh-NP size. (A) Average size of PVPh-NPs, as measured by DLS, increases with increasing concentration of PVPh monomer added. (B) DLS of 145 nm formulation of PVPh-NP before and after Ab coating, Representative TEM of PVPh-NPs before (C) and after (D) Ab-coating.

Fig. 3.

In vivo targeting of mAb-[¹²⁵I]PVPh-NPs to the pulmonary endothelium. [¹²⁵I]PVPh-NPs were targeted to three distinct determinants: (A) TM, (B) PECAM, or (C) PV1. Radioactivity levels of mAb-[¹²⁵I]PVPh-NPs in the lung and blood (% ID/g) were compared to IgG-[¹²⁵I]PVPh-NP controls and parental free [¹²⁵I]-mAbs to determine targeting parameters. Lung localization ratio, corrected for blood uptake, is pictured in an inset for each formulation. Data represented as mean $\pm$ S.D. (n = 3–5).

Fig. 4.

In vivo and *in vitro* tracing of radiolabeled-NP stability over time. (A) Organ uptake of PECAM targeted [¹²⁵I]IgG-PS-NPs and IgG-[¹²⁵I]PVPh-NPs were analyzed at 30 min and 24 h post-IV administration (inset: thyroid levels of free radioiodide (% ID) at 24 h post-injection). (B) *In vitro* assessment of radiolabel stability of [¹²⁵I]IgG-[¹²⁵I]PVPh-NPs vs. [¹²⁵I]IgG-PS-NPs when incubated with 0.2 wt % pronase protease mix at 37 °C over 4 d.

Fig. 5.

Coronal and transverse sections of real-time *in vivo* CT and PET images acquired after administration of Ab-[¹²⁴I]PVPh-NP targeting in naïve C57/Bl6 mice, coated with IgG or anti-PECAM. (A) CT images of mice with visible lung space (left). 1 h summed PET image (5–65 min post intravenous injection) co-registered with CT images of IgG-[¹²⁴I]PVPh-NP (left) and antiPECAM-[¹²⁴I]PVPh-NP (right). Arrows points to lungs, liver and spleen. (B) Average lung uptake of IgG and PECAM groups extrapolated from regions-of-interest (ROIs) drawn on lung volumes from PET images at 1 hour p.i. *, $p < 0.05$. (C) Lung uptake extrapolated from ROIs drawn on lung volumes from PET images in real-time during the 1 hour scan time. Data represented as mean $\pm$ S.D. (n = 5).

TABLE 1Characterization of PVPh-NP^a

[PVPh] added (mg/mL)	D_{avg} (nm)	Polydispersity (PD)	Zeta potential (mV)
3.125	60	0.12	-32
6.25	80	0.10	-32
12.5	120	0.10	-25
25	145	0.16	-30
50	195	0.20	-30
100	185	0.18	-25

^a Average particle size, D_{avg} , PD, and zeta potential for PVPh-NP were determined by DLS.

TABLE 2
a *In vivo* organ uptake of mAb-[¹²⁵I]PVPh-NPs compared to free [¹²⁵I]-mAb at 30 min post-injection in naïve mice.

mAb-[¹²⁵ I]PVPh-NP					
mAb	Liver	Spleen	Kidney	Heart	Brain
IgG	69.2 ± 2.0	79.9 ± 22.0	3.0 ± 0.4	0.4 ± 0.1	0.1 ± 0.0
PECAM	79.4 ± 5.0	101.2 ± 11.3	4.8 ± 0.8	4.5 ± 0.9	0.6 ± 0.1
TM	89.4 ± 8.2	111.8 ± 12.4	1.4 ± 0.1	1.5 ± 0.4	0.3 ± 0.1
PV1	80.9 ± 4.9	87.2 ± 7.0	3.4 ± 2.5	0.4 ± 0.3	0.08 ± 0.06

[¹²⁵ I]-mAb					
IgG	6.9 ± 0.4	7.5 ± 2.1	8.4 ± 1.0	2.7 ± 0.6	1.2 ± 0.1
PECAM	34.1 ± 4.8	17.2 ± 1.8	9.6 ± 3.4	7.2 ± 0.6	1.7 ± 0.2
TM	12.9 ± 0.2	15.1 ± 2.1	16.0 ± 0.5	6.6 ± 0.7	1.2 ± 0.3
PV1	14.8 ± 0.3	21.3 ± 0.2	28.5 ± 0.9	2.0 ± 0.2	0.3 ± 0.1

a Mice were injected *via* jugular vein with formulations, and organs harvested 30 min post-injection. Isotope level in organs was measured to calculate % of injected dose per gram of tissue (% ID/g). The data are shown as mean % ID/g ± S.D. (n = 3–5)