SPECT imaging of fibrin using fibrin-binding peptides

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SPECT imaging of fibrin using fibrin-binding peptides

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Noninvasive detection of fibrin in vivo using diagnostic imaging modalities may improve clinical decision-making on possible therapeutic options in atherosclerosis, cancer and thrombus-related pathologies such as pulmonary embolism and deep venous thrombosis. The aim of this study was to assess the potential of a novel 111In-labeled fibrin-binding peptide (FibPep) to visualize thrombi in mice noninvasively using single-photon emission computed tomography (SPECT). FibPep and a negative control peptide (NCFibPep) were synthesized and their fibrin-binding properties were assessed in vitro. FibPep showed enhanced binding compared with NCFibPep to both fibrin and blood clots. FibPep bound to fibrin with a dissociation constant \((K_d)\) of 0.8 \(\mu\)M, whereas NCFibPep displayed at least a 100-fold lower affinity towards fibrin. A FeCl3-injury carotid artery thrombosis mouse model was used to evaluate the peptides in vivo. FibPep and NCFibPep displayed rapid blood clearance and were eliminated via the renal pathway. In vivo SPECT imaging using FibPep allowed clear visualization of thrombi. Ex vivo biodistribution showed significantly increased uptake of FibPep in the thrombus-containing carotid in comparison to the noninjured carotid \((5.7 \pm 0.7 \text{ and } 0.6 \pm 0.4\% \text{ injected dose per gram (ID g}^{-1})\), respectively; \(p < 0.01; n = 4\), whereas nonspecific NCFibPep did not \((0.4 \pm 0.2 \text{ and } 0.3 \pm 0.0\% \text{ID g}^{-1})\), respectively; \(n = 4\). In conclusion, FibPep displayed high affinity towards fibrin in vivo and rapid blood clearance in vivo, and allowed sensitive detection of thrombi using SPECT imaging. Therefore, this particular imaging approach may provide a new tool to diagnose and monitor diseases such as atherosclerosis and cancer.

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Supporting information may be found in the online version of this paper.

Keywords: fibrin; peptides; molecular imaging; thrombus; SPECT

1. INTRODUCTION

Thrombosis is the underlying pathology in a number of cardiovascular diseases, such as heart attack, deep venous thrombosis, pulmonary embolism and ischemic stroke (1). These cardiovascular diseases represent a major cause of mortality in society and therefore development of thrombus-specific detection, characterization and treatment methodologies is of paramount importance in clinical practice. Currently, detection of (intravascular) thrombi is mostly pursued using indirect imaging modalities such as computed tomography angiography, ultrasound and ventilation/perfusion scintigraphy (2,3). Molecular imaging with nuclear techniques presents an alternative way to visualize thrombi in a more specific fashion, at a higher sensitivity and with lower limitations with respect to thrombus location in the body.

A suitable target for molecular imaging of thrombi is fibrin, which is the major constituent of a thrombus and has the advantage that it is virtually absent in nonpathological situations, thus providing a beneficial target-to-background ratio. In addition to molecular imaging of intravascular thrombi, fibrin-targeted probes may allow assessment of atherosclerotic plaque vulnerability, as microthrombi on the surface of plaques are direct indicators of microfissures in the fibrous cap (4) and as fibrin is present in plaques displaying intraplaque hemorrhage, a process associated with plaque destabilization (5). Molecular imaging of cancer is another field in which fibrin-targeted probes might be of value, as fibrin deposition is associated with a variety of malignant tumors, including breast, lung, brain and prostate (6). Fibrin has a pivotal role in tumor stroma formation and deposition of fibrin has been shown to facilitate tumor angiogenesis and metastasis (6–8). The presence of fibrin in tumor stroma can also be exploited to facilitate cancer treatment, as fibrin-targeted monoclonal antibodies were recently shown to be successful vehicles for directing cytotoxic immunoconjugates to solid tumors (9). Hence, fibrin-targeted probes seem promising for diagnostic and therapeutic applications in a variety of thrombus-related cardiovascular pathologies and cancer.

Strategies employing 99mTc-labeled fibrin-targeted antibody fragments and linear peptides have been investigated to image deep venous thrombosis and pulmonary embolism in preclinical and clinical studies in the past 15 years (10–13). Recently, the cyclic...
fibrin-binding peptide EP-2104R, containing the fibrin-binding motif Y-dGlu–C-Hyp–3CLY–GLCYIQ and four Gd–1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) moieties, was successfully used for magnetic resonance imaging (MRI) of fibrin in both preclinical and clinical studies (14–18). For detection of minute fibrin deposits, however, highly sensitive nuclear imaging using radiolabeled cyclic fibrin-binding peptides might be a more suitable option. In addition, targeted MRI-probes labeled with Gd will probably have to overcome safety issues concerning nephrogenic systemic fibrosis (19) and therefore their nuclear counterparts might be more readily translatable in the clinic. Here we describe a proof-of-concept study in which an 111In-labeled DOTA-conjugated fibrin-binding peptide (FibPep; Fig. 1A), containing the cyclic fibrin-binding motif RWQCPAESWT–Cha–CWDP (20), was developed to visualize fibrin noninvasively using single-photon emission computed tomography (SPECT). In vitro fibrin and blood clot binding of FibPep was assessed in comparison to a scrambled, linear control probe (NCFibPep; Fig. 1B). Then, we evaluated the potential of FibPep for SPECT imaging of fibrin in a preclinical study using a well-established FeCl3-induced carotid injury thrombosis model in mice (21–24). Blood kinetic and biodistribution profiles of FibPep and NCFibPep were also obtained and, finally, in vivo SPECT and computed tomography (CT) imaging was performed.

2. **RESULTS AND DISCUSSION**

2.1. **Synthesis and In Vitro Validation of the PeptideProbes**

The synthesized peptides were analyzed using liquid chromatography–mass spectrometry (LC-MS) and the found masses were in agreement with the expected ones (Fig. 1). Radiolabeling with 111In resulted in ≥95% yield and radiochemical purity for both peptides (Supporting Information, Fig. S1). In vitro binding studies showed significantly higher binding to fibrin for FibPep with respect to NCFibPep and free 111In (85.8 ± 3.8, 1.2 ± 2.5 and 2.1 ± 4.2% dose, respectively; p < 0.01; Fig. 2A). In addition, FibPep exhibited significantly higher binding to blood clots than NCFibPep and free 111In (32.2 ± 7.1, 5.1 ± 1.7 and 1.0 ± 0.7% dose, respectively; p < 0.01; Fig. 2B). To investigate the fibrin-binding properties of the probes in more detail, the affinity towards fibrin was analyzed in a microtiter-plate equilibrium binding assay (25,26). FibPep bound with good affinity to fibrin (dissociation constant, \(K_d = 0.8 ± 0.1 \mu\text{M}\), Fig. 2C) and the number of binding sites per fibrin molecule was 2.8 ± 0.1. In comparison, NCFibPep bound at least 100-fold weaker to fibrin (Fig. 2C).

**Figure 1.** Structural formulas and corresponding mass spectra of (A) fibrin-binding peptide (FibPep) and (B) negative control peptide (NCFibPep).
2.3. In Vivo SPECT Studies

In vivo SPECT was performed starting 90 min PI with a total scan time of approximately 1.6 h. Subsequently, the mice were euthanized and overnight post-mortem SPECT scans were performed. In mice injected with $^{111}$In-labeled FibPep, a high SPECT signal was observed in the thrombus-containing carotid for both in vivo and post-mortem SPECT scans, whereas this was not the case in the mice that received NCFibPep (Fig. 4). The surgical wound bed was visible in both groups of mice, most likely owing to FibPep binding to clotted blood at the wound site and to nonspecific deposition of both tracers in the surgical wound bed, possibly owing to edema formation (24) and deposition of fluid on the skin around the surgical wound. Besides these focal uptakes, only kidney and bladder were visible in the post-mortem full body scans (Fig. 5), suggesting renal elimination of both radiolabeled peptides. Quantification of the signal in volumes of interest (VOIs)

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![Figure 2](image1.png)

**Figure 2.** Binding of $^{111}$In-labeled FibPep (empty bars), NCFibPep (solid bars) and $^{111}$InCl₃ (dashed bars) to (A) fibrin and (B) blood clots. Data are the mean percentage dose ± SD ($n=4$). (C) FibPep and NCFibPep fibrin affinity curves, dashed lines plotted as described in the text.

![Figure 3](image2.png)

**Figure 3.** (A) blood kinetic and (B) biodistribution profiles of FibPep and NCFibPep 4 h post-injection. Data are the mean percentage injected dose per gram ± SD ($n=4$). Plotted dashed lines are the corresponding bi-exponential elimination fits.
drawn over the injured carotids showed a significantly higher uptake of FibPep with respect to NCFibPep ($p < 0.02$), suggesting specific binding to fibrin (Fig. 6). FibPep uptake in the blood clot was 9–14 times higher than that in muscle while the carotid-to-muscle ratio was lower (2–4 times) in mice injected with NCFibPep (Supporting Information, Fig. S2A). Similar differences

Figure 4. Single-photon emission computed tomography (SPECT/CT) imaging using FibPep and NCFibPep. SPECT/CT images (transversal slices, 90 min post injection) of live mice injected with (A) FibPep and (B) NCFibPep. Post-mortem maximum intensity projection of the neck area of mice injected with (C) FibPep and (D) NCFibPep. Bold arrow, thrombus; thin arrow, surgical wound area.

Figure 5. Post-mortem whole body SPECT/CT images (maximum intensity projections) of mice injected with $^{111}$In-labeled FibPep (A, B) and NCFibPep (C) showing high uptake in kidney and high activity in the urine. In (B) the bladder was emptied before the post-mortem SPECT scan to highlight the uptake in kidney. All images are on the same color scale. Arrows show urine contamination on the skin.

Figure 6. Analysis of in vivo SPECT scans. Mean count per voxel in injured carotid and muscle area plotted against the time post-injection of FibPep and NCFibPep. Data are the mean counts per voxel ± SD ($n=4$); * $p < 0.02$; # $p < 0.01$ for FibPep treated carotid vs NCFibPep treated carotid and muscle (both probes).
of the uptake ratios between the two tracers were observed using the contralateral area as normalization region (Supporting Information, Fig. S2B).

These in vivo findings were confirmed by ex vivo $\gamma$-counting of the harvested tissues (Fig. 7). FibPep accumulated significantly more in the injured carotid than in the noninjured one and in muscle (5.7 ± 0.7, 0.6 ± 0.4 and 0.2 ± 0.2%ID g$^{-1}$, respectively; $p < 0.01$). NCFibPep uptake was significantly lower than that of FibPep in the injured carotid and no major differences were observed among the carotids and muscle in mice treated with the control peptide (0.4 ± 0.2%ID g$^{-1}$ in injured carotid, 0.3 ± 0.0 in control carotid and 0.1 ± 0.1%ID g$^{-1}$ in muscle; $p < 0.01$ with respect to FibPep injured carotid uptake). Hematoxylin and eosin stained histological sections confirmed the presence of thrombus in the injured carotid (Fig. 8).

3. DISCUSSION

The present study demonstrates the feasibility of highly sensitive noninvasive SPECT imaging of fibrin with the $^{111}$In-labeled fibrin-binding peptide FibPep. Previous studies investigating EP-2104R, a paramagnetic labeled fibrin-binding peptide, have shown that this compound holds potential to visualize fibrin deposition using MRI in preclinical models of pulmonary embolism, atherosclerosis, coronary and carotid artery thrombosis, as well as in patients (14–18,28). In a phase II clinical trial, EP-2014R allowed the detection of thrombi which were not readily visible in pre-contrast MRI screening and enhanced signal intensity of thrombi that were already detected on pre-contrast scans. However, 33% of the thrombi located in the venous system remained undetected. This relatively high number of false negatives can probably be attributed to lack of sensitivity of molecular MRI. In a subsequent preclinical study, part of the Gd in the DOTA-chelator of EP-2104R was replaced with the positron-emitter $^{64}$Cu for multimodal PET/MR imaging and the obtained hybrid tracer was evaluated in rats injected with preformed thrombi (29). In this model, $T_1$-weighed molecular MRI was able to distinguish the occluded carotid artery 10 min after contrast agent injection, while the focal uptake of $^{64}$Cu was clearly visible in PET images acquired 30–90 min PI. Such an approach might allow for more sensitive detection of fibrin deposition and also could eliminate the need for a baseline MR examination, which would eradicate issues with respect to co-registration of pre- and post-contrast images, complex workflow and patient compliance.

With these promising preclinical and clinical results in mind, we set out to evaluate the cyclic fibrin-binding amino acid sequence RWQCPAESWT–Cha–CWDP, previously identified via phage-display using fibrinogen-binder depleted libraries and subsequently optimized for fibrin-binding (20,30), for highly sensitive fibrin detection using nuclear imaging. For this purpose, the fibrin-binding sequence was linked to a DOTA chelator, yielding FibPep (Fig. 1), which could be readily labeled with $^{111}$In. FibPep exhibited a good affinity towards fibrin ($K_d = 0.8 \mu M$) in an in vitro microtiter-plate equilibrium binding assay as opposed to the scrambled, linear peptide NCFibPep ($K_d > 100 \mu M$) that was
used as negative control. The affinity of FibPep was slightly improved with respect to that of EP-2104R ($K_d = 1.8 \mu M$) (25); however, the low micromolar affinity was not expected to be a major problem in vivo owing to the high abundance of fibrin on clots.

The pharmacokinetics, biodistribution and in vivo fibrin targeting capabilities of the candidate tracer were evaluated in a mouse carotid injury model. Notably, FibPep cleared rapidly from the circulation, which is key to detecting intravascular targets, as high blood levels of radioactivity might obscure thrombus-specific signal. Therefore, in this proof-of-concept study a 90 min delay after tracer injection was chosen for SPECT imaging to ensure sufficiently low FibPep blood levels. FibPep also exhibited a favorable biodistribution with low uptake (<1.4% ID g$^{-1}$) in most considered nontarget tissues. Only kidney retained a significant amount of radioactivity 4 h PI (7.1 ± 0.9%ID g$^{-1}$), probably owing to the negative charge of FibPep. However, this value is lower than that of other radiopeptides used in the clinic, such as $^{111}$In-DOTATOC (31), and therefore it is not expected to raise dosimetry concerns. The rapid blood clearance and low retention in most organs in conjunction with the finding that FibPep is able to extravasate supports the hypothesis that FibPep will probably be able to visualize its fibrin-target in most parts of the body within a useful time-frame.

Subsequently, in vivo SPECT imaging using the fibrin specific and nonspecific peptides was performed to gauge the potential of FibPep for in vivo detection of fibrin deposition. $^{111}$In-labeled FibPep allowed clear visualization of the small (~1 mm$^3$) thrombi generated in mice while the injured carotids of the mice injected with NCFibPep could not be distinguished from the surrounding tissues. Quantitative analysis of SPECT data was performed by drawing volumes of interest on the SPECT images, as the carotids were not visible on the CT scans. In the live mice the clot-to-muscle and clot-to-background (contra-lateral area) ratio did not change significantly during the assessed time frame (90–162 min PI, Supporting Information, Fig. S2), suggesting that <90 min elapse between injection and imaging might be feasible with short-lived radionuclides. However, the mean counts per voxel in the injured carotid area exhibited a declining trend during the assessed time frame for FibPep (Fig. 6), with an approximately 2 h half-life at the clot site. This half-life is ca 7 times longer than that of FibPep in circulation, suggesting specific FibPep retention in the clot.

Selecting small volumes of interest based on SPECT images is an inherently biased process. Therefore, in future studies we are planning to administer a long-circulating CT contrast agent (32) to the mice in addition to FibPep, which should allow clear visualization of the arteries by CT and better quantification of the SPECT data in the co-registered images. Also, the dosage and specific activity of the tracer were not optimized in this proof-of-concept study and, therefore, it is plausible that the pharmacokinetics, targeting efficiency and time between injection and detection can be further improved by optimizing these parameters. A limitation of the employed scrambled negative control peptide NCFibPep is the fact that the peptide has a linear structure, whereas FibPep is cyclic. Even though FibPep and NCFibPep are very similar in chemical nature, and have similar blood kinetic and biodistribution profiles, the current study setup does not allow to investigate whether part of the binding property of the peptide is actually owing to the cyclic nature of the peptide, and not because of the specific amino acid sequence. Interestingly, Uppal and co-workers showed recently that for the EP-2104R peptide, linearization of the peptide obliterates any specificity towards fibrin (33). It is not unlikely that a linearized, nonscrambled version of FibPep also has virtually no affinity towards fibrin.

The presented results show promise for FibPep as a SPECT agent for thrombus detection and provide a solid foundation for further exploration of the potential of FibPep in animal models of, for example, atherosclerosis and cancer. Employing SPECT as imaging modality for detection of fibrin deposition is especially of interest for future applications concerning the evaluation of atherosclerotic plaque vulnerability and cancer, as multi-isotope SPECT imaging allows simultaneous assessment of multiple biomarkers (34,35), which is vital for obtaining reliable and robust read-outs of complex pathological systems such as tumors and atherosclerotic lesions. Furthermore, the use of DOTA allows a rapid translation of this SPECT tracer into a PET tracer, for example, by using $^{68}$Ga- or $^{18}$F-labeling approaches (36,37). FibPep does not contain Gd$^{3+}$ ions and therefore avoids the risk of inducing nephrogenic systemic fibrosis, which is linked to extended retention of Gd in the body in combination with less stable Gd-chelates (19,38). Thanks to the high sensitivity of nuclear imaging, radiotracers can be administered at very low doses, reducing the risk of toxicity or pharmacological effects. Thus nuclear probes like FibPep may have to overcome lower hurdles for clinical translation and commercialization compared with MRI probes.

4. CONCLUSIONS

The present proof-of-concept study demonstrates the ability of $^{111}$In-labeled FibPep to noninvasively image minute thrombi in vivo using SPECT. If further successfully validated and translated, this particular imaging approach may provide a new tool to diagnose and monitor atherosclerosis, cancer and thrombus-related pathologies such as pulmonary embolism and deep venous thrombosis.

5. EXPERIMENTAL

5.1. Synthesis of the Peptide Probes

9-Fluorenylmethoxy carbonyl-protected L-amino acids and Rink amide resin were purchased from either Novabiochem(Merck) or Bachem. The fibrin-binding peptide Ac–RWQPCPAESWT–Cha–CWDPGGGK–NH$_2$ and a scrambled negative control Ac–WPTAD–Cha–RAWPSQEWPAGGGK–NH$_2$ with C-A substitutions were synthesized on 4-methylbenzhydrolamine hydrochloride salt rink amide resin by the use of standard 9-Fluorenylmethoxy carbonyl solid-phase peptide synthesis. The fibrin-binding peptide contains the fibrin-binding motif RWQCPAESWT–Cha–CWDPGGGK–NH$_2$, which was previously identified via phage-display using fibrinogen-binding-depleted libraries and subsequently optimized for fibrin-binding (20,30). The peptides were cleaved from the resin with a trifluoroacetic acid–triisopropylsiliane–H$_2$O–ethanedithiol (90:5:5:2.5 v/v) mixture for 3 h and purified by preparative reversed-phase high-pressure liquid chromatography (RP-HPLC) using an Agilent 1200 apparatus equipped with a C$_{18}$ Zorbax column (150 × 21.2 mm; 5.0 μm particle size). The peptide Ac–RWQCPAESWT–Cha–CWDPGGGK–NH$_2$ was cyclized via the formation of a disulfide bond between the cysteine residues in dimethylsulfoxide–H$_2$O (9:1 v/v) for 5 days with the pH set to 8 using N-methyl-D-glucamine (Sigma Aldrich) (20), and subsequently purified using preparative RP-HPLC. Next, the peptides were functionalized at the lysine ε-amino group with DOTA by mixing the peptides and a 7-fold excess of DOTA–NHS ester (Macrocyclus)
in dimethylformamide containing 3.6% (v/v) triethylamine at room temperature overnight. Finally, the peptides were purified using preparative RP-HPLC, yielding the DOTA-functionalized FibPep and NCFibPep (Fig. 1). The peptide structures were analyzed by LC-MS on an Agilent 1200 apparatus, equipped with a C8 Eclipse plus column (100 × 2.1 mm; 3.5 μm particle size) and an electrospray mass spectrometer (time-of-flight LC-MS model 6210; Agilent Technologies).

5.2. Radiolabeling

111InCl3 (PerkinElmer) was mixed with FibPep or NCFibPep in ammonium acetate buffer (0.2–0.25 M, pH 6, 35–200 μl) and shaken (350 rpm) for 60 min at 75 °C. For in vivo experiments the mixture was subsequently diluted with saline and challenged with an equivalent dose of diethylene triamine pentaacetic acid (DTPA; Sigma-Aldrich).

The 111In-labeling yield was determined using radio thin-layer chromatography (TLC) and the radiochemical purity was determined by radio-HPLC. TLC was performed on instant TLC silica gel strips (Varian Inc.) eluted with 200 mM ethylenediaminetetraacetic acid (EDTA) in saline. The strips were imaged on a phosphor imager (FLA-7000; Fujifilm) and the labeling yields were quantified with AIDA Image Analyzer software. Analytical radio-HPLC was performed on an Agilent 1100 system equipped with a C18 Eclipse XBD column (150 × 4.6 mm; 5 μm particle size) and a Gabi radioactive detector (Raytest). The radiolabeled peptides did not undergo any purification procedures prior to use.

5.3. In Vitro Fibrin and Blood Clot Binding Assays

5.3.1. Fibrin binding

Fibrin coated tubes were prepared by adding 4 μl of 100 U ml⁻¹ human thrombin (Sigma Aldrich) in ultrapure water (Millipore) to 200 μl of 2.9 mg ml⁻¹ human fibrinogen (Sigma Aldrich) in HEPES-buffered saline (HBS, pH 7.4) and incubated overnight at 37 °C. Prior to further use, the tubes were rinsed with HBS.

111In-labeled FibPep, NCFibPep or 111InCl3 (0.75 ± 0.10 MBq, 2 nmol peptide, n=4 per probe) were diluted with HBS to a total volume of 150 μl added to the tubes and incubated for 2 h at 37 °C. Subsequently, the solution was removed and the fibrin clot was washed three times with HBS. The fibrin-bound radioactivity was measured using a dose calibrator (VDC-405; Veenstra Instruments) and expressed as percentage dose.

5.3.2. Blood clot binding

Blood clots were prepared by incubating a mixture of 5 μl human tissue factor (Dade Behring), 6 μl of 1 M CaCl2 in ultrapure water and 400 μl of citrated human blood plasma (Sanquin) for 30 min at 37 °C. Subsequently, the blood clots were washed three times with HBS. Next, 111In-labeled FibPep, NCFibPep or 111InCl3 (0.76 ± 0.17 MBq, 2 nmol peptide, n=4 per probe) were diluted with HBS to a total volume of 450 μl added to the blood clots and incubated for 30 min at 37 °C. After incubation, the solution was removed and the blood clots were washed three times with HBS. The clot-bound radioactivity was measured and expressed as percentage dose.

5.3.3. Fibrin binding affinity assay

Human fibrinogen (Sigma-Aldrich) was dissolved in HBS pH 7.4 (2.5 mg ml⁻¹ fibrinogen) and CaCl2 was added to a final concentration of 7 mM. The fibrinogen solution (50 μl) was dispensed into a 96-well polystyrene microplate (Nunc). A 50 μl aliquot of human thrombin (2 U ml⁻¹; Sigma-Aldrich) was added to the wells to induce the clotting of fibrinogen and then the plates were dried at 37 °C overnight, yielding a thin fibrin layer. Subsequently, the plates were sealed and stored at −20 °C until further use.

The assayed solutions ranged from 0.1 to 80 μM and from 0.1 to 200 μM peptide for FibPep and NCFibPep, respectively. A 100 μl aliquot of 111In-labeled FibPep or NCFibPep (5–45 kBq nmol⁻¹ peptide) in HBS pH 7.4 was added to the wells and the microplate was shaken at 500 rpm and room temperature for 2 h. After incubation, the solution was removed and the radioactivity was measured in a γ-counter (Wizard 1480; PerkinElmer) along with standards to determine the concentration of free peptide. The fibrin-bound peptide concentration was calculated by subtraction

\[
\frac{\text{[peptide bound]}}{\text{[fibrin]}} = \frac{\text{[peptide total]} - \text{[peptide free]}}{\text{[fibrin]}} + K_d
\]

according to Overoye-Chan and co-workers (25). Total fibrin concentration was estimated to be 3.7 μM by calculating the concentration of polymerized fibrinogen monomer using a molecular weight of 340 kDa for fibrinogen.

5.4. In Vivo Experiment

All animal procedures were approved by the ethical review committee of Maastricht University (The Netherlands) and were performed according to the principles of laboratory animal care (39) and the Dutch national law ‘Wet op Dierproeven’ (Stb 1985, 336). For in vivo thrombus formation a well-established FeCl3-induced carotid artery thrombosis model in mice was used (21–24). C57BL/6 mice (23 ± 3 g body weight; Charles River Laboratories) were housed under standard conditions and acclimatized for at least 1 week. Food and water were freely available. Thirty minutes prior to surgery, the mice were subcutaneously injected with buprenorphine hydrochloride (0.1 mg kg⁻¹; Schering-Plough). The mice were anesthetized with isoflurane and a segment of the right carotid artery was exposed through an incision of the skin and blunt dissection of the fascia over the vessel. Wall-adherent thrombus formation in the right carotid artery was induced by applying a small piece of cleaning cloth soaked in 10% FeCl3 on the carotid. After 5 min, the cloth was removed, the carotid was washed with saline and the skin was closed with a suture.

5.5. Blood Kinetics and Biodistribution

The mice were allowed to recover from anesthesia after the surgery. One hour post-thrombus formation, 111In-labeled FibPep or NCFibPep (15 nmol, 100 μl, 1.13 ± 0.09 MBq, n=4 per probe) was injected into the tail vein of the mice. At selected time points PI (2, 5, 10, 30, 60 and 120 min), blood samples (25 ± 4 μl) were withdrawn from the vena saphena, weighed, and diluted to 1 ml with water. The mice were euthanized 4 h PI using pentobarbital (Alfasan). A blood sample and tissues of interest were collected, weighed and subsequently 1 ml water was added. The radioactivity...
in the muscle (front limb) and contralaterally of the injured carotid drawing a VOI around the hot spot in the injured carotid area. To m\(200\) per gram.

along with standards to determine the percentage injected dose differences with t

sets were compared using either a paired or an unpaired two-tailed multiple comparisons. For differences between two groups, data

were stained with hematoxylin and eosin.

arteries were cut in transversal sections of 5

formaldehyde and subsequently embedded in paraf

The injured as well as the contralateral arteries were

fixed in 4%

Contrast Media Mol. Imaging

were stained with hematoxylin and eosin.

The injured as well as the contralateral arteries were

Histology

The injured as well as the contralateral arteries were fixed in 4%

formaldehyde and subsequently embedded in paraffin. The arteries were cut in transversal sections of 5 \(\mu\)m and the sections were stained with hematoxyn and eosin.

Supporting Information

Supporting information can be found in the online version of this article.

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