Exploration and application of nanomedicine in atherosclerotic disease

Lobatto, M.E.

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Chapter 10

Multimodal positron emission tomography imaging to quantify uptake of $^{89}$Zr-labeled nanoparticles in the atherosclerotic vessel wall

Submitted


*= contributed equally to this work
Abstract

Nanotherapy has recently emerged as an experimental treatment option for atherosclerotic disease. To fulfill its promise, robust noninvasive imaging approaches for subject selection and treatment evaluation are warranted. In the current study we evaluated a modular procedure to label nanoliposomes with the radioisotope 89-zirconium ($^{89}$Zr). Their biodistribution and vessel wall targeting in a rabbit atherosclerosis model was evaluated up to 15 days after intravenous injection by positron emission tomography (PET)/computed tomography (CT) and PET/magnetic resonance imaging (PET/MRI). Vascular permeability was assessed in vivo using 3 dimensional dynamic contrast-enhanced MRI (3D DCE-MRI) and ex vivo using near infrared fluorescence (NIRF) imaging. The $^{89}$Zr-radiolabeled nanoliposomes displayed a biodistribution pattern typical of long circulating nanoparticles. Importantly, they markedly accumulated at atherosclerotic plaque sites in the abdominal aorta, as evident on PET/MRI and confirmed by autoradiography, which was most pronounced at sites with high vascular permeability. The method presented facilitates the development of nanotherapy for atherosclerotic disease as it provides a tool to screen for nanoparticle targeting in individual subjects’ plaques.
Introduction

Atherosclerosis, the leading cause of cardiovascular disease complications such as myocardial infarction and stroke, is a chronic inflammatory disease affecting the major arteries.1 Its progression is driven by the infiltration of lipids and immune cells into the vessel wall.2 Nanomedicine has recently emerged as a potential therapeutic option for atherosclerosis,3,4 with the first clinical trials recently conducted by our group.3 Unlike cancer, atherosclerosis is a slowly progressing —initially mild— disease process, which complicates the implementation of nanotherapeutics, as the therapeutic window might be unclear. Nanomedicine’s integration in atherosclerotic disease management can greatly benefit from companion imaging, allowing the identification of amenable subjects and providing feedback on atherosclerotic burden.4 For example, imaging may assist the development of novel nanotherapies by noninvasively examining biodistribution, the extent of disease, and finally, therapeutic outcomes.5

In previous studies we have shown that intravenously injected long-circulating nanoparticles accumulate in atherosclerotic lesions in areas of enhanced permeability in animal models, as well as in atherosclerotic lesions in patients with cardiovascular disease.5,7,8 Primarily, these nanotherapeutics were co-localized with macrophages, main contributors to atherosclerotic plaque inflammation and concurrent destabilization. Hence, the inclusion of therapeutics into nanoparticles can facilitate local enrichment of anti-inflammatory compounds, potentially increasing efficacy and decreasing adverse effects.9 Radiolabeling of nanoparticles is a valuable approach to determine pharmacokinetics and biodistution by ex vivo scintigraphic methods.10 The technique’s sensitivity requires minimal modification to nanoparticles and therefore does not—or marginally at most—compromise their function.11 More recently, PET or single-photon emission computed tomography (SPECT) imaging of radiolabeled nanoparticles has allowed the quantitative assessment of biodistribution in vivo, albeit a limitation of nuclear imaging is its low spatial resolution and dearth of anatomical information.12,13 This is especially the case in the context of atherosclerosis, where small plaques can occur in multiple regions of the arterial tree, necessitating anatomical reference. The latest advancement in clinical imaging techniques, PET/MRI in a single hybrid system, has the capability to greatly diversify acquired information. The addition of MRI allows the thorough characterization of atherosclerotic burden and the vessel wall in high resolution, a feature that is more difficult to achieve with CT due to its reduced ability to always distinguish plaque components.14 New MRI protocols that, in addition to anatomical information, can concomitantly acquire functional information, such as the quantification of arterial wall microvascular permeability through dynamic contrast enhanced (DCE) imaging, can be of great value.15 Moreover, the advent of simultaneous PET/MRI may allow a significant radiation dose reduction —by increasing the length of PET acquisition to match that of the MR scan— while preserving quantification and image quality.16

In this study, nanoliposomes were labeled with $^{89}$Zr, a positron emitter with a relatively long half-life (78.41 hours) and a favorable positron range. Clinical PET/CT and PET/MRI systems were used to improve our understanding of the nanoliposomes’ in vivo behavior, and the relation of their accumulation and plaque permeability, in a rabbit model of atherosclerosis.

Results and Discussion

Long circulating nanoliposomes, previously applied in experimental atherosclerosis and human studies,5,7,17 were prepared by lipid-film hydration and subsequently labeled with $^{89}$Zr in a modular fashion.18 The polyethylene glycol (PEG) coating ensures long circulating properties, due to initial avoidance of capture by the mononuclear phagocyte system (MPS), mainly the liver, spleen and bone marrow, thereby avoiding rapid in vivo clearance.19 A schematic of the nanoparticle is depicted in Figure 1A. The radiochemical yield was $72 \pm 16$ % ($n = 6$) with a radiochemical purity of $> 99$ %. Radiolabeled nanoliposomes had a mean effective diameter of $106.0 \pm 5.2$ nm and a polydispersity index of $0.14 \pm 0.01$ ($n=6$). Nanoliposome composition and size exclusion chromatograms are shown in supplementary Figure 1.

Next, we performed MRI characterization of the vessel wall of New Zealand white rabbits that received double balloon injuries of the abdominal aorta and a high-cholesterol diet, a well
The animal model is of interest as it can be scanned on clinical systems and has heterogeneous atherosclerotic plaque formation along the abdominal aorta. First, T2-weighted MR images were acquired to characterize vessel wall morphology, in which rabbits subjected to the above protocol had a markedly thicker vessel wall than healthy control rabbits fed a regular chow-diet, where the vessel wall was barely discernible (0.145 vs 0.083 mm²; p<0.001: Figure 1B). Moreover, 3D DCE-MRI was performed, a technique that allows quantification of microvascular permeability of the arterial wall over an extensive vascular region by acquiring data prior to and continuously after the injection of Gd-DTPA, a clinical MRI contrast agent.²¹ From this, data kinetics can be derived, which showed that the vessel wall of atherosclerotic rabbits was highly permeable compared to the vessel wall of healthy control animals, in line with what has been observed previously (3.48 vs 1.92 a.u.; p<0.001: Figure 1B).²¹ Representative histological slides in Figure 1C reveal a normal vessel wall without macrophages and neovascularure, in contrast with the prominently thickened vessel wall in atherosclerotic rabbits with intraplaque neovessels (CD31 staining) as well as macrophage accumulation, as is evident from staining with the antibody RAM-11.

After studying the rabbit model by MRI, the animals received a single intravenous injection of $^{89}$Zr-nanoliposomes, after which they were subjected to serial in vivo PET/CT and PET/MRI sessions on clinical scanners. The study design displayed in Figure 1D indicates the time points healthy control and atherosclerotic animals were subjected to imaging.

**Figure 1.** Formulation/study design and rabbit model characteristics. (A) Labeling of $^{89}$-zirconium ($^{89}$Zr) nanoliposomes in a modular fashion. The outer layer with poly-ethylene glycol (PEG) ensures long-circulating properties. (B) The vessel wall thickness of atherosclerotic rabbits was significantly larger than in control animals (0.15 vs 0.08 mm²; p<0.001), the vascular permeability measured with 3D dynamic contrast enhanced-magnetic resonance imaging (3D DCE-MRI) was significantly higher as well (3.48 vs 1.92 a.u.; p<0.001). (C) Representative histological sections stained with hematoxylin and eosin, CD-31 antibodies for endothelial cells and RAM-11 antibodies for macrophages reveal a markedly thickened vessel wall in rabbits with atherosclerosis, with intraplaque neovessels and abundant macrophages compared to the normal vessel wall in control rabbits. Magnification 10X, scale bar 2 mm. (D) Study design shows the amount of rabbits allotted to control and atherosclerotic groups as well as which animals were scanned on which particular time points and when they were sacrificed. C= control animal, A= atherosclerotic animal.
Imaging biodistribution with PET/CT and PET/MRI. The biodistribution of $^{89}$Zr nanoliposomes in healthy control rabbits was evaluated by imaging with both PET/CT and PET/MRI 5 times in total according to the study design in Figure 1D. Representative images of the abdomen of the rabbits in coronal views in a maximum intensity projection (MIP) of PET as well as on fused PET/CT images are shown in Figure 2A, where activity on day 1 mainly derives from the blood pool and highly vascularized organs, such as the kidneys and liver. As time passes, activity in the blood pool decreases until the nanoliposomes complete vascular clearance, with eventual predominant activity in the organs of the MPS, mainly the liver and spleen up to day 15.

Analogous to PET/CT, PET/MRI showed an expected comparable distribution pattern (Figure 2B). Liver, spleen and kidneys were traced to quantify biodistribution as standardized uptake values (SUV), the standard reporting measure for PET acquired imaging (Figure 2C) using the mean SUV (SUVmean). Values were subsequently compared to atherosclerotic rabbits. Uptake in liver and spleen was markedly higher in the control compared to the atherosclerotic rabbits up until day 5 (4.6 vs. 2.8 for liver and 18.3 vs. 12.5 for spleen; values in SUV) after which the uptake was at similar levels for both liver and spleen. This difference probably occurs due to impaired liver function in atherosclerotic rabbits caused by steatohepatitis, fattening of the liver, triggered by the high-cholesterol diet.

Graphs corroborate the visible uptake in the organs shown in Figure 2A and 2B. As a confirmation of acquired data, PET/CT and PET/MRI have a high correlation in both atherosclerotic and healthy control rabbits ($\rho =0.98$ and 0.86 respectively; $p<0.0001$). Similar high correlation was found between gamma counting and PET/CT as well as PET/MRI in both atherosclerotic and healthy control animals (supplementary Figure 2).

Nanoparticle targeting of the vessel wall visualized by PET/CT and PET/MRI. After systematic interrogation of biodistribution we investigated nanoparticle vessel wall accumulation in atherosclerotic rabbits by both PET/CT and PET/MRI. Representative coronal images of both modalities at different time points are shown in Figure 3A, B, as well as a 3D reconstruction of PET activity in Figure 3C at day 1. Additional 3D volume-rendered images are shown in supplementary Figure 3 and supplementary movies S1 and S2. Interestingly, after clearance...
of activity from the blood pool at 3 days, distinct hot spots could be observed at different segments along the abdominal aorta. This patchy activity distribution was in contrast with only background activity in control rabbits. Axial PET/CT images of atherosclerotic rabbits do not allow the anatomical localization of hot spots within the vessel wall. In contrast, PET/MRI, with superb soft tissue contrast and vessel wall delineation on T2 weighted MR images, allows hot spot co-localization within the thickened vessel wall (Figure 3D). Interestingly, in focal areas without $^{89}$Zr uptake, the vessel wall was also thickened. In Figure 3E quantification of maximum SUV (SUVmax) of the entire vessel wall of the abdominal aorta in atherosclerotic rabbits reveals high uptake of $^{89}$Zr nanoliposomes in the vessel wall compared to negligible uptake in the control animals. After clearance from the blood, the uptake becomes significantly higher in the atherosclerotic compared to the control rabbits at day 3 (1.8 vs. 0.9) and remains higher throughout 15 days of observation.

**Figure 3.** Vessel wall targeting in rabbits with atherosclerosis. (A, B) Biodistribution in atherosclerotic rabbits with representative coronal images of PET/CT and PET/MRI reveal patchy uptake in the abdominal aorta, which is better appreciated at later time points after clearance of nanoliposomes from the blood pool. (C) PET maximum intensity projections (MIP) in both the PET/MRI and PET/CT group at day 1. (D) Axial images of PET/CT and PET/MRI taken through PET hot spot and non hot spots on both PET/CT and PET/MRI reveal the localization on PET/MRI compared to CT, where a thickened vessel wall corresponds to an activity hot spot. (E) Quantification of maximum standard uptake value (SUVmax) of the entire vessel wall of abdominal aortas in atherosclerotic versus control rabbits show significant higher SUVmax at 3 days and onwards. Both PET/CT and PET/MRI exhibit similar values. C= control animals, A= atherosclerotic animals.
**Pharmacokinetics and ex vivo quantification.** Pharmacokinetics were derived from blood drawn at different time points, demonstrating a long circulating half-life of nanoliposomes of 19 hours for the control rabbits, which is comparable to prior results in the same model (Figure 4A). In the atherosclerotic rabbits the circulation half-life was longer (29 hours), probably due to aforementioned liver damage.

After sacrifice, organs were weighed and radioactivity accumulation was evaluated in a gamma counter confirming the in vivo measures with expectedly high uptake in tissues of the MPS (liver, spleen and bone marrow), which remained high throughout 15 days of observation (Figure 4B). Thirty minutes prior to sacrifice Evans Blue was injected, a dye that can be visualized with fluorescent imaging and extravasates at regions of vascular permeability for macromolecules. In line with the significantly higher concentration of $^{89}\text{Zr}$ in the abdominal aorta of atherosclerotic rabbits measured with the gamma-counter (0.004 vs 0.026 % injected dose per gram; $p<0.001$), near infrared fluorescence (NIRF) imaging confirmed significantly higher vascular permeability in the atherosclerotic vs. control rabbits ($2.2 \times 10^{10}$ vs. $0.7 \times 10^{10} \mu\text{W/cm}^2$; $p<0.001$) (Figure 4C). Ex vivo imaging of the aortas with NIRF imaging was compared with 3D DCE-MRI, showing a similar positive correlation between the 2 readouts of vascular permeability ($\rho=0.35$; $p=0.03$) as previously reported. Taken together, all imaging techniques –3D DCE-MRI, PET/MRI, NIRF and autoradiography– show similar regions of uptake in atherosclerotic rabbits, compared to no uptake in healthy controls (Figure 4E).

In this study we have shown that $^{89}\text{Zr}$–nanoliposomes accumulate in the vessel wall of rabbits with atherosclerosis, which we demonstrated noninvasively with multimodal imaging. To our knowledge, this is the first example of multimodal PET/MRI and PET/CT on clinical scanners that shows the accumulation of long-circulating nanoparticles in the vessel wall. Recently, a nontherapeutic biodegradable nanoparticle, a 13-nm $^{89}\text{Zr}$ labeled dextran nanoparticle was used to noninvasively assess inflammation in mice with atherosclerosis and showed that it could monitor anti-inflammatory therapy on separate PET and MRI preclinical scanners. Multimodal nanoparticles that were developed with both MRI and PET contrast agents have also been used for both PET and MRI to monitor macrophage accumulation in atherosclerotic lesions on preclinical imaging modalities.

We recently reported a method to visualize in vivo behavior of high-density lipoproteins with PET imaging. New methods to easily quantify biodistribution, characterize atherosclerotic burden and subsequent uptake of nanoparticles in the vessel wall noninvasively can be of great value for the development of nanotherapeutics. The sensitivity of PET together with the exceptional soft-tissue contrast of MRI provided vastly detailed anatomical and functional imaging. We have previously shown that liposomal nanoparticles with glucocorticoids dampened inflammation effectively in a rabbit model of atherosclerosis. Unfortunately, further development in a first-in-man small scale clinical trial showed no clinical effect in first instance, although an exact cause could not be identified. PLP’s liposomal encapsulation improved its pharmacokinetic profile in humans ($n=13$). In a small patient population with a very heterogeneous disease process it is of paramount importance to screen patients that could potentially benefit of therapy. With the tools to monitor targeting, atherosclerotic burden and biodistribution presented here, patient selection can potentially be optimized.

In conclusion, we have developed a tool to quantify nanoparticle uptake in the atherosclerotic vessel wall, image biodistribution and assess atherosclerotic burden in a single noninvasive multimodal imaging session with PET/MRI.
Figure 4. Ex vivo quantification of nanoliposome uptake. (A) Pharmacokinetics of $^{89}\text{Zr}$-nanoliposomes, the circulation half-life in control rabbits was 19 hours, versus 29 hours in atherosclerotic rabbits. (B) Ex vivo quantification of organ distribution by gamma-counter reveals high activity in the MPS as expected, predominantly the liver and spleen. (C) Quantification of Evans Blue in abdominal aortas as a marker of permeability measured by near-infrared fluorescent imaging (NIRF) revealed a significantly higher accumulation of Evans Blue in atherosclerotic rabbits versus control rabbits ($0.7 \times 10^{10}$ vs. $2.2 \times 10^{10} \mu \text{W/cm}^2$; p<0.001). A significantly higher accumulation of $^{89}\text{Zr}$ in the abdominal aortas measured by gamma-counter (0.026 vs. 0.004 % injected dose per gram; p<0.001) was found as well. (D) Area-under-the-curve (IAUC) as a unit of permeability measured by 3D DCE-MRI and correlation with ex vivo NIRF showed a significant correlation in atherosclerotic rabbits ($\rho =0.35$; p=0.03). (E) In vivo imaging with ex vivo corroboration juxtaposed reveals low permeability in control animals at 5 and 15 days measured both by 3D DCE/MRI and NIRF with focal spots of permeability in atherosclerotic rabbits. PET/MRI reveals focal hot spots similar to ex vivo autoradiography (ARG), while no focal accumulation is seen in the control rabbits. Dashed lines delineate the abdominal aortas in the images as a reference guide. BM= bone marrow.
Materials & Methods

Preparation of 89Zr-labeled nanoliposomes. Ready-to-label liposomes were prepared using a sonication method as previously described.14 Briefly, a lipid film composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), cholesterol, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[aminomethyl(polyethylene glycol)-2000] (DSPE-PEG2000) and the phospholipid-chelator 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-deferoxamine B (DSPE-DFO) was produced by evaporation of a chloroform solution. The film was hydrated with phosphate buffered saline (PBS) and the resulting suspension sonicated for 25 min. For radiolabeling, a solution of deferoxamine B (DFO)-bearing liposomes in PBS was reacted with 89Zr-oxalate at 40 °C for 2 h.14 The labeled liposomes were purified by spin filtration using 100 kDa molecular weight cut-off tubes (Millipore, Billerica, MA). The concentrate was washed with sterile PBS and finally diluted with sterile PBS to the desired volume.

Animal model preparation. Eight New Zealand male white rabbits were fed a high-cholesterol diet and received two balloon angioplasties of the abdominal aortas to induce atherosclerotic lesions according to a well-established method.20 Eight New Zealand male white rabbits fed a normal chow diet were used as healthy control animals. All intravenous injections were administered through 22 gauge catheters positioned in the marginal ear vein. Imaging was performed under anesthesia with a combination of Ketamine (35 mg/kg) and Xylazine (5 mg/kg), and Isoflurane was used as maintenance anesthesia. All animal experiments were approved by the Institutional Animal Care and Use Committee at the Icahn School of Medicine at Mount Sinai.

Imaging acquisition. After intravenous co-injection of 27.1 ± 3.6 MBq (mean ± SD) 89Zr-nanoliposomes and plain, non-radioactive nanoliposomes in 5 ml PBS solution (at a combined dose of 50 μmol total lipid/kg body weight), rabbits were positioned in either the Siemens mCT Biograph PET/CT or Siemens mMR 3T PET/MRI in a body matrix coil. Animals were scanned according to study design in Figure 1D. PET was acquired in 3D mode, 10 minutes for PET/MRI and 7 minutes for PET/CT.

The parameters for PET/CT were set at: 140 kVp voltage; 43 mA tube current; 1000 ms exposure time; 1 mm slice thickness. Image reconstruction was performed with the algorithms point-spread function and time-of-flight (TOF). For PET/MRI, the scan was started after scout scans and were acquired along a radial VIBE MR sequence with the following parameters: 20 ms TR: 1.89 ms TE: 10 degree flip angle: 1.1 mm3 slice thickness. PET image attenuation correction was performed with a built-in MR based attenuation correction (MR-AC) map and image reconstruction using OP-OSEM algorithm.

A time-of-flight angiography was acquired to seek anatomical landmarks (renal arteries and iliac bifurcation). T2-weighted 3D anatomical MRI was acquired using the Sampling Perfection with Application optimized Contrasts using different flip angle Evolution (SPACE) sequence, in the sagittal plane, starting from the left renal artery to the iliac bifurcation, with isotropic voxels of 0.6 mm3. Other relevant imaging parameters were: repetition time (TR), 1600 ms; echo time (TE), 118 ms; number of slices, 56; number of signal averages, 2; echo train length (ETL), 83.

Delayed enhancement and 3D DCE-MRI were acquired using a segmented fast low angle shot (FLASH) sequence, in the sagittal plane, also starting from the left renal artery to the iliac bifurcation, with isotropic voxels of 0.6 mm3. Relevant imaging parameters were: TR, 1111 ms; TE, 5.7 ms; number of slices, 20; flip angle, 25 degrees. For delayed enhancement, 8 signal averages were acquired, while for DCE-MRI only 1 signal average per time frame was acquired.

The duration of each time frame for DCE-MRI was approximately 30s, for a total of 18 dynamic frames acquired. After acquisition of 3 images, 0.1 mmol/Kg of gadolinium (Gd) DTPA (diethylenetriaminepentacetate) (Magnevist, Bayer Schering Pharma), were injected at the rate of 0.5 ml/s, and flushed with 15 ml of saline solution at the same rate.

Ex vivo methods. To perform the pharmacokinetic analysis, blood was withdrawn from the marginal ear vein at 30 min, 24, 48, 72 and 120 h post injection of nanoliposomes. Next, samples were weighed and radioactivity concentration was measured on a Wizard® 2480 automatic gamma counter (Perkin Elmer, Waltham, MA). Following the final in vivo imaging session and 30 min prior to sacrifice animals were injected with 0.5% of 5 mL of Evans Blue (Sigma-Aldrich, St. Louis, MO) in PBS. Afterswards rabbits were sacrificed using an overdose of pentobarbital and saline perfused. Next, pieces of organs were harvested, weighted and counted using a Wizard® 2480 automatic gamma counter for assessment of 89Zr-nanoliposome biodistribution.

Near infrared fluorescence imaging. Directly post sacrifice, the abdominal aortas were positioned on thick black paper and fluorescence images were acquired with an IVIS Spectrum Preclinical Imaging System (Perkin Elmer, Waltham, MA). Excitation and emission wavelengths were set at 605 and 680 nm, respectively. The data were then measured as total radiant efficiency (μW/cm2). Ten ROIs were placed from the left renal artery to the iliac bifurcation.

Autoradiography. Tissue samples were placed in a film cassette against a phosphor imaging plate to perform digital autoradiography (BASMS-2325, Fujifilm, Valhalla, NY) for 48 hours at -20 °C. The phosphor imaging plates were set at a pixel resolution of 25 μm with a Typhoon 7000IP plate reader (GE Healthcare, Pittsburgh, PA).

Histology. A selection of healthy and atherosclerotic aortas were cut into 5 mm thick sections and placed in 4% paraformaldehyde. A day later sections were paraffin embedded and cut into sections of 5 μm thickness and placed on slides. Slides were subsequently stained with hematoxylin and eosin, RAM-11 and CD-31 antibodies according to standard immunohistochemistry techniques.

Imaging data analysis. Osiris 7.0 MD was used for image analysis, by tracing regions of interest (ROI) on liver, kidneys, spleen and abdominal aortas on fused PET and radial VIBE MRI images to quantify biodistribution. From these ROIs SUVmax was derived. Plaque burden was analyzed by drawing ROIs on the abdominal aortas on T2 weighted images, from here the vessel wall area was calculated. 3D DCE-MRI was analyzed by drawing ROIs on the delayed enhancement scan and superimposing these on the dynamic scan. IAUC maps were analyzed in MatLab using a custom made program.

For T2W scans, assessment of vessel wall thickness was calculated as outer vessel wall area minus inner vessel wall area.

Statistical analysis. Data are presented as mean ± standard error of the mean. GraphPad Prism version 5.0 was used for data analysis. Tests used were unpaired student t-tests and Mann Whitney U tests for comparison of group differences. Pearson’s r coefficients were calculated to determine correlation between in vivo and ex vivo readings. P values < 0.05 were deemed as significant.
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Supporting information

**Supplementary figure 1.** (A) Composition of nanoliposomes used in this study, in mol %. (B) HPLC size exclusion chromatograms showing elution of 89Zr-nanoliposomes (absorption at 220 nm, black trace; and radioactivity, blue trace), and 89Zr-oxalate (radioactivity, red trace).

**Supplementary figure 2.** Correlation between SUVmean values acquired from gamma counting, PET/CT and PET/MRI in control (A, B, C) and atherosclerotic rabbits (D, E, F).
Supplementary Figure 3. (A) 3D Volume rendered PET images from 1 hour to 15 days post-injection reveals predominant uptake in liver and spleen. Uptake in the heart is clearly visible until day 3 whereas accumulation in bone marrow and intestines is evident from day 1 and at least until day 5. All images are from the atherosclerotic group acquired on the PET/MRI system. (B) Percentage of initial radioactivity remaining in the abdominal aorta of atherosclerotic (A) and control (C) rabbits. A higher fraction of the nanoliposomes is retained in the aorta of the atherosclerotic compared to the control animals from 1 day post-injection until the end of follow-up. Data are acquired on the PET/MRI scanner.