Computed tomography imaging of macrophage phagocytic activity in abdominal aortic aneurysm

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Figure S1

Radiodensity of CT contrast agents. CT images and radiodensity quantification of serial dilutions of selected nanoparticle contrast agents. PBS: Phosphate buffered saline, HU: Hounsfield units.
Figure S2

RAW 264.3 macrophage polarization and impact of Exitron nano 12000 exposure on polarization. (A) Gene expression analysis of RAW 264.7 cell polarization markers Nos2 and Mrc1 in untreated cells (M0), after LPS and IFNγ stimulation [M(LPS+ IFNγ)] and IL-4 stimulation [M(IL-4)] (n = 7–8). Statistical significance was assessed by Kruskal-Wallis test with Dunn’s corrections and one-way ANOVA for Nos2 and Mrc1 gene expression, respectively. (B) Evaluation of polarization markers Nos2 and Mrc1 in RAW 264.7 cell without and after 1 h, 4 h, 18 h and 48 h of Exitron nano 12000 exposure (n = 3–4 for Exitron, n = 10 for Control). Statistical significance was assessed by Dunn’s multiple comparison test.
Figure S3

In vitro evaluation of Exitron nano 12000 uptake in unpolarized (M0) and polarized [M(LPS+IFNγ) and M(IL-4)] RAW 264.7 macrophages (A), b.END3 endothelial cells (B) and MOVAS aortic smooth muscle cells (C) at 4 and 37 °C (n = 3). Statistical significance was assessed by Dunn’s multiple comparison test (left panel in A) or one-way ANOVA (all other panels). Ex: Exitron nano 12000; HU: Hounsfield units.
Figure S4

Exitron nano 12000 biodistribution in Ang II-infused Apoe−/− mice. Average tissue radiodensity quantified on in vivo CT images of Apoe−/− mice pre-, and at 5 min and 24 h post-Exitron administration. Statistical significance was assessed by one-way ANOVA with Tukey’s correction (n=13). ** \( P < 0.01 \), *** \( P < 0.001 \), **** \( P < 0.0001 \), ns: not significant. HU: Hounsfield Units.
Figure S5

Exitron nano 12000 uptake in topical elastase-induced murine AAA. (A) Brightfield images of serial sections of infra-renal abdominal aorta (proximal to distal from left to right) from a C57Bl/6J mouse at 6 weeks after topical elastase application (scale bar: 1 mm), and maximal external diameter of the infra-renal aorta at 4 to 6 weeks after topical elastase application in β-aminopropionitrile-treated C57Bl/6J mice. (B) CT images of a representative C57Bl/6J mouse at
4 weeks after topical elastase application pre- (left panels), and at 5 min (middle panels) and 24 h (right panels) post-Exitron nano 12000 administration. White arrows: AAA; red arrows: inferior vena cava. Scale bar: 1 cm. CT scale: -750 to 1250 HU. (C) Quantification of the CT signal presented as enhancement volume (top panel) and maximal radiodensity (bottom panel).

Statistical analysis was performed using Wilcoxon signed-rank test. ** \( P < 0.01 \). HU: Hounsfield Units.
**Figure S6**

Evolution of CT signal with AAA progression. Serial CT images obtained weekly before (top panels) and 24 h after Exitron nano 12000 administration (bottom panels) from an *Apoe<sup>-/-</sup>* mouse infused with Ang II for 4 weeks. White arrows: AAA. Scale bar: 1 cm. CT scale: -750 to 1250 Hounsfield Units.
Figure S7

Flow chart of animal studies.

- Biodistribution over 24 h
- Uptake in abdominal aorta (Con)
- Uptake in abdominal aorta (AAA)
- Gene expression analysis in abdominal aorta (AAA)
- Uptake in abdominal aorta (AAA) at 5-8 days
- Predictive value of uptake in AAA (survival & external diameter at 4 weeks)
- Uptake in abdominal aorta (AAA)
Thresholding of CT images acquired 24 h post-Exitron administration. CT images of a representative Ang II-infused Apoe⁻/⁻ mouse acquired at 5 min and 24 h after Exitron nano 12000 administration (left panels). The pixels with radiodensities ≥ 150 Hounsfield Unit (HU), 200 HU and 250 HU thresholds are colored in green on delayed CT images (right panels). White arrow: AAA. CT scale: -750 to 1250 HU. Scale bar: 1 cm. HU: Hounsfield units.