Time-course study of a gold nanoparticle contrast agent for cardiac-gated micro-CT imaging in mice

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Abstract

Although micro-computed tomography (micro-CT) images have high contrast for bone or air, between soft tissues the contrast is typically low. To overcome this inherent issue, attenuating exogenous contrast agents are used to provide contrast enhancement in the vasculature and abdominal organs. The aim of this study is to measure the contrast enhancement time course for a gold nanoparticle blood-pool contrast agent and use it to perform cardiac-gated 4D micro-CT scans of the heart. Six healthy female C57BL/6 mice were anesthetized and imaged after receiving an injected dose of MVivo gold nanoparticle blood-pool contrast agent. Following the injection, we performed micro-CT scans at 0, 0.25, 0.5, 0.75, 1, 2, 4, 8, 24, 48 and 72 h. The mean CT number was measured for 7 different organs. No contrast enhancement was noticed in the bladder, kidneys or muscle during the time-course study. However, it clearly appears that the contrast enhancement is high in both right ventricle and vena cava. To perform cardiac-gated imaging, either the gold nanoparticle agent ($n = 3$) or an iodine-based ($n = 3$) contrast agent was introduced and images representing 9 phases of the cardiac cycle were obtained in 6 additional mice. A few typical cardiac parameters were measured or calculated, with similar accuracy between the gold and iodinated agents, but better visualization of structures with the gold agent. The MVivo Au contrast agent can be used for investigations of cardiac or vascular disease with a single bolus injection, with an optimal cardiac imaging window identified during the first hour after injection, demonstrating similar image quality to iodinated contrast agents and excellent measurement accuracy. Furthermore, the long-lasting contrast enhancement of up to 8 h can be very useful for scanning protocols that require longer acquisition times.

1. Introduction

Micro-computed tomography (micro-CT) contrast agents can be separated into 3 groups (Lusic and Grinstaff 2013) depending on their active ingredients: iodine-based, gold nanoparticles and other metallic contrast agents (Rabin et al 2006). Iodine-based agents are historically the most common contrast agent used in micro-CT. They allow a high level of X-ray attenuation due to the high atomic number of iodine ($Z = 53$) which leads to an effective contrast enhancement. Gold nanoparticles as a contrast agent is a very young and interesting tool for contrast enhancement since gold has a high atomic number ($Z = 79$) and a high density. These two parameters are crucial to achieve a high level of X-ray attenuation. It has been reported (Pietsch 2017) that gold provides almost 3 times greater contrast per unit weight than iodine, which can reduce the administration dose making the agent more readily tolerated for the animal scanned. Furthermore, the shape, the size and the environment of the active molecule used in contrast media have an important impact on the image quality for several reasons (Mulder et al 2006, Li et al 2014). These factors determine the time course in blood and other organs so researchers can know at what time they should proceed with scanning to get the best data for their study (Rabin et al 2006). Larger molecules ($0.2–3\ \mu m$...
diameter) will last longer in the blood vessels and will usually be used in a blood pool contrast agent (Ashton et al 2015), although there are many other parameters to take into account. Moreover, the shape, size and environment of the media may have a huge impact on the administration dose, including the minimum dose that should be administered to achieve suitable contrast enhancement and also the maximum dose that can be injected into the rodent without altering its health (Willekens et al 2013). For mice, the injected volume should not exceed 15%–20% of the total blood volume, so the injected dose used is around 5–10 ml kg−1 body weight for mice.

In addition, contrast agents can also be distinguished by the way they are excreted (Lusic and Grinstaff 2013). Excretion depends on both the nature and the size of the active molecule used. We can split micro-CT contrast agents into 2 groups: agents excreted through the bile (liver) and agents excreted through the urinary system (kidney, bladder). Contrast agents that are excreted through the urinary system can sometimes lead to kidney failure such as contrast-induced nephropathy (Namasivayam et al 2006), which can cause serious issues even for agents that are clinically approved. However, these problems are quite rare for patients in good health. For contrast agents excreted through the bile, the biggest concern is the long retention time of the agent in the liver (Chouker et al 2008). Although this feature can be helpful in some cases to allow multiple imaging with only one injection, it is also one of the reasons why liver-excreted contrast agents are not yet used clinically.

A number of preclinical contrast agents have been developed for use in micro-CT imaging of rodents. To determine their utility for visualizing the vasculature or other organs, time course studies have been performed. Fenestra VC, marketed as an iodinated blood-pool agent, was found to have excellent contrast for vasculature in the first 4 h post-injection, then a second window of liver enhancement at 24 h (Ford et al 2006). Exia 160 has been characterized for vascular contrast, with a 30-minute window immediately post contrast providing suitable enhancement for cardiac imaging (Detombe et al 2012). Another group compared the contrast enhancement in the liver and spleen for Fenestra LC (a liver agent), Fenestra VC and Exia 160 (Willekens et al 2009). With the recent advancements and development of nanoparticle-based contrast agents, the clearance and time kinetics of these molecules may differ from iodinated agents. An alkaline-earth nanoparticle agent for liver imaging (ExiTron nano 6000) and for vascular imaging (ExiTron nano 12000) has been characterized, with contrast enhancement in the liver lasting for weeks post-injection (Boll et al 2011). Comparisons between the ExiTron nano 600 and 12000 with an iodinated agent Exia 160 have also been done in healthy mice and in a hepatocellular carcinoma model (Rothe et al 2015). Recently, the ExiTron nano 12000 has been tested for liver toxicity at delayed time points, with contrast enhancement continuing to 1 month post-injection with no adverse effects reported (Liu et al 2019).

The main purpose for this study is to characterize the contrast enhancement of a gold nanoparticle blood-pool agent over time using a wild-type strain of mice (C57BL/6) with a high-resolution in vivo micro-CT scanner. Additionally, we will perform cardiac-gated imaging with this contrast agent to produce a series of images throughout the cardiac cycle and compare the cardiac parameters (left ventricle volume at diastole and systole, stroke volume and ejection fraction) with a well-characterized iodinated contrast agent. We will use the results of this study to identify the imaging window for vascular and cardiac imaging tasks.

2. Materials and methods

All animal experiments were approved by the institutional ethics committee at The University of British Columbia (#A19-0097). Mice were housed 2–4 per cage in a conventional, pathogen-free animal facility with a 12 h light-dark cycle. Food and water were freely available.

2.1. Time course study

We used six healthy female C57BL/6 mice (Charles River, Wilmington, MA, USA), aged 9–10 weeks, ranging in weight from 19 to 22 g. During the study, each mouse was anesthetized using approximately 5% isoflurane in O2 in an induction chamber. Each mouse was transferred to a nose-cone at 2%–3% isoflurane in O2 and warmed to visualize the veins in the tail. To introduce a nanoparticle blood-pool contrast agent (MVivo Au, MediLumine, Montreal, QC, Canada) with an injected dose of 0.005 ml g−1 body weight, we performed a tail-vein injection. The contrast agent contains 15 nm-sized particles of gold in a concentration of 200 mg/ml. Mice were kept under anesthesia throughout the imaging session (1.5%–2% isoflurane in O2) through a nose cone. Between image acquisitions, the mice were recovered except for the first hour in which the animals were kept anesthetized even between scans. The mice were monitored and warmed before, during and after the anesthesia. They were also hydrated at 1 h and 8 h post contrast injection with a subcutaneous injection of lactated Ringer’s solution (Associated Veterinary Purchasing, Langley, BC, Canada). Total anesthesia time per mouse is estimated at 3 h (depending on time required for positioning mice on the bed, and for the tail vein injection). In our experimental setup, all injections were performed by the same veterinary technician.

All micro-CT image data were acquired using a continuous rotation protocol on a micro-CT machine (eXplore CT120, TriFoil Imaging, Chatsworth CA, USA) at 70 kVp, and 50 mA, without respiratory or
cardiac gating. The scan time was approximately 4 min to get a full body 3D image extending from the clavicles to the base of the tail. Imaging time points included immediately post-injection \((t = 0)\), and \(t = 15, 30\) and \(45\) min, and \(1, 2, 4, 8, 24, 48\) and \(72\) h after injection. The entrance dose per scan was \(0.22\) Gy (for 11 scans and \(2.42\) Gy per animal in total). Before each scan, bright and dark-field projection images were acquired in order to correct the data for detector non-uniformities. The reconstruction of the images has been performed using a cone-beam reconstruction algorithm (Feldkamp et al. 1984). The 3D image has been reconstructed with a nominal isotropic voxel spacing of 0.1 mm.

Volumetric images were viewed in MicroView (version ABA2.2, TriFoil Imaging, Chatsworth CA, USA) to assess the contrast enhancement achieved in each image. We placed 1 mm\(^2\) regions of interest (ROIs) in each organ (except for the vena cava, for which the ROI was 1.5 mm \(\times\) 0.3 mm). ROIs are shown for the 2 h time point in figure 1. The regions were positioned in air and left hind limb muscle as non-enhancing organs, in the right ventricle and vena cava for vascular system, and in the liver, kidney, spleen, and bladder as clearance pathways. From the ROI, mean CT number and standard deviation for all 11 images per mouse were recorded. To avoid an artificial increase of the values assigned to an organ, special attention was taken to exclude any large vessels feeding the organ from the ROI. To be more accurate and to avoid the partial volume effect for the vena cava measurement, the image was reoriented to better align the vena cava with the axes, and the ROI was positioned immediately below the diaphragm.

The mean CT numbers for each organ at each time point was compared to the 0 h time point using repeated-measures one-way ANOVA followed by Dunnett’s post-hoc test (Prism 6.0 h, GraphPad Software, La Jolla CA, USA, www.graphpad.com). Using the average values from the 6 mice for each organ at each time point, the contrast-to-noise ratio (CNR) was determined as given by:

\[
\text{CNR}[t] = \frac{\text{HU}_{\text{organ}}[t] - \text{HU}_{\text{bg}}[t]}{\sigma_{\text{bg}}[t]} \tag{1}
\]

where \(\text{HU}_{\text{organ}}[t]\) is the CT number in the organ at time \(t\) (from 0 to 72 h), \(\text{HU}_{\text{bg}}[t]\) is the CT number of the background (muscle considered as a non-enhanced tissue), and \(\sigma_{\text{bg}}[t]\) is the standard deviation in the background.

2.2. Cardiac-gating study

To compare the gold nanoparticle MVivo Au contrast agent with an iodinated contrast agent, we performed cardiac-gated imaging in 6 healthy female C57BL/6 mice. The injection during the gating scan session followed the same protocol as the time course study. Three mice were injected with MVivo Au contrast agent with a dose of 0.005 mL g\(^{-1}\) body weight and the remaining three mice were injected with a blood pool iodinated lipid-based agent (50 mg I/ml, Fenestra VC, MediLumine, Montreal, QC, Canada) with a dose of 0.01 ml g\(^{-1}\) body weight. When placing the mice on the micro-CT table, neonatal electrocardiogram (ECG) electrodes (2269 T, 3 M Health Care, St. Paul, MN, USA) were placed on three of the paws in order to measure the ECG signal. The mice were positioned prone in the micro-CT table with a pneumatic pillow under the mouse that measured the diaphragm’s motion in order to monitor the respiratory signal. Both the ECG and respiratory signals were measured and recorded using a physiological monitoring and triggering system (BioVet, m2m Imaging Corp., Cleveland, OH, USA) prior to and throughout the

![Figure 1. Locations of the ROIs in an image at the 2-hour time point. RV: right ventricle, L: liver, A: air, M: muscle, B: bladder, S: spleen, K: kidney, VC: vena cava. (a) Full FOV (b) Zoom in the abdomen, (c) Re-oriented image to align with the vena cava.](image)
scan, which enabled selection of the desired time points within the cardiac cycle. Projections were triggered by the cardiac ECG signal, and acquired at 9 time points over the cardiac cycle (every 20 ms for 180 ms) in order to get a 4D series of images representing 9 different points in the cardiac cycle. Projection images were acquired at 80 kVp, 40 mA and an exposure time of 16 ms, which leads to an x-ray dose of 2.7 Gy and a total scan time of 20–25 min (depending on the cardiac rate) for each 4D dataset. A total of 220 projections were acquired for each image using a step-and-shoot protocol and reconstructed with 0.1 mm isotropic voxel spacing.

To reduce image noise in the cardiac-gated images, an edge-preserving bilateral filter was applied using Matlab (R2018b, Mathworks Inc., Natick, Massachusetts, United States). Filtering the images did not alter the greyscale values in the images or affect image resolution, but did reduce the noise induced by the short exposure times for each projection. The images were reoriented to align the long axis of the left ventricle and the aorta in the same plane. As in the studies by Detombe et al (Drangova et al 2007, Detombe et al 2008), we used the left ventricle (LV) to determine the different phases in the cardiac cycle. Using a region growing algorithm, the chamber of the left ventricle was isolated and its volume calculated. The greyscale threshold between the LV chamber and the myocardium was determined using Otsu’s thresholding algorithm (Otsu 1979). Knowing these thresholds allowed us to measure the volume of the LV in each image and determine the time points corresponding to systole and diastole. The image with the minimum LV volume was systole and the one with the maximum LV volume was diastole. Once the left-ventricular systolic volume (LVSV) and the left-ventricular diastolic volume (LVDV) were determined, the stroke volume (SV) and the ejection fraction (EF) were calculated for each mouse.

\[ SV = LVDV - LVSV \]  
\[ EF = \frac{SV}{LVDV} \]

3. Results

3.1. Time course study
The contrast-enhancement measurements were averaged for the 6 mice. Coronal and sagittal images representing the 0 h, 2 h and 72 h time points are shown in figure 2 for a single mouse. The mean and standard deviations are tabulated in table 1 for each organ and the graphs of selected organs are presented in figure 3. We can notice that the CT numbers were unchanged through time in the ROIs for air (data not shown), muscle and bladder. The contrast agent produced immediate enhancement in the right ventricle and the vena cava. In addition, the contrast agent caused an enhancement in the liver and the spleen, although it is less obvious than what we observe for the vascular system.

Concerning the vascular system, the peak enhancement occurs immediately after injection with 382 HU over background value (muscle) for the right ventricle and 343 HU over background for the vena cava. Immediately after the injection, we can visualize the vessels well, and they can be followed throughout the animal, even in the organs. Furthermore, the chambers of the heart were well defined, even without cardiac gating, as was the aortic arch. Over time, the vessels and the heart chambers were returning to their baseline values following a decreasing exponential function, and the contrast enhancement was then observed in the liver and spleen. The contrast enhancement showed a significant decrease in the vasculature at 8 h post-injection, with a half-life between 48 and 72 h.

Concerning the liver, the peak enhancement occurred 24 h after injection with a gain of 89 HU compared to the background value. After the peak, the mean value for the liver remained quite stable until the 72 h point. The spleen shows a contrast enhancement over time similar to the liver with a peak at \( T = 48 \) h and a stable value from 24 h point to 72 h. The value achieved at the peak was 193 HU above the background. The kidney showed immediate enhancement, returning to stable CT numbers at 48 h, following a similar enhancement to the vascular system. Since the bladder did not show enhancement, we believe that the observed enhancement is due to the vascular nature of the kidneys rather than the agent being cleared by the renal system.

Figure 2(a) shows that the MVivo Au agent, used within the optimal cardiac imaging window (immediately post-injection), provides sufficient contrast in the micro-CT scans to segment the vascular system. Indeed, we can observe that the major vessels are well defined and can be traced throughout the mouse. Some smaller vessels, especially in the liver, can be traced as well. Despite the absence of any kind of gating strategy, the heart also appears well defined as we can easily visualize each chamber. Figure 4 shows the volume rendered image obtained immediately after injection for the same mouse. We can observe the extraction of the skeleton, heart, and abdominal vasculature separately from the background image.

The CNRs were calculated using equation (1) relative to the background value (muscle). Table 2 shows the variation of the CNR over time. We can observe that for the first few hours, the CNR remained quite steady in the right ventricle and the vena cava before a significant drop after 8 h as the contrast agent leaves the vascular system. Concerning the liver and the spleen we note a very low CNR that increases after 8 h but never achieves the vascular system’s value.
3.2. Cardiac-gating study

Table 3 shows the average volume of the left ventricle measured in the systolic and diastolic images. It also provides the stroke volume and the ejection fraction for both MVivo Au and Fenestra VC contrast agents used in this study, calculated with equations (2) and (3). Table 3 shows similar values for the LV parameters that have been measured and calculated for both contrast agents, suggesting that both agents provide good contrast leading to accurate image-based measurements.

Figure 5 shows the average variation of the left ventricle volume within the 9 images obtained throughout the cardiac cycle superimposed upon a sample of one cardiac trace from near the midpoint of the scanning session. By using the cardiac signal to trigger image acquisition and collecting projections from 9 time points within the cardiac cycle, we are able to determine the volume of the left ventricle at each point of the cardiac cycle.

Figures 6(a) and (b) shows typical diastolic and systolic images acquired for a single mouse imaged with the MVivo Au agent. Similar images of a mouse injected with Fenestra VC are shown for comparison in figures 6(c) and (d). We can clearly distinguish the difference in volume between the systolic and diastolic images. The images were reoriented to get the long axis of the left ventricle and the aorta in the same plan. In figure 6, we can observe the variation in LV volume through the cardiac cycle with both contrast agents. The edges are well visualized with both contrast agents. In the systole image however, the gold nanoparticle image looks to be more accurate as we can better visualize the contours of the chambers, which is consistent with the reduced standard deviations given in table 3 for the MVivo Au agent.

| Time (h) | Muscle (HU) | Bladder (HU) | Right Ventricle (HU) | Vena Cava (HU) | Kidney (HU) | Liver (HU) | Spleen (HU) |
|---------|-------------|--------------|----------------------|----------------|-------------|------------|------------|
| 0       | 112 ± 13    | 174 ± 35     | 494 ± 91             | 455 ± 65       | 172 ± 52    | 193 ± 15   | 260 ± 34   |
| 0.25    | 112 ± 14    | 169 ± 27     | 471 ± 73             | 436 ± 69       | 175 ± 15    | 185 ± 27   | 242 ± 37   |
| 0.5     | 105 ± 15    | 186 ± 31     | 473 ± 75             | 437 ± 68       | 172 ± 16    | 189 ± 21   | 233 ± 28   |
| 0.75    | 114 ± 17    | 189 ± 20     | 468 ± 64             | 437 ± 55       | 170 ± 18    | 189 ± 18   | 236 ± 21   |
| 1       | 115 ± 13    | 192 ± 29     | 456 ± 59             | 429 ± 60       | 183 ± 15    | 191 ± 21   | 238 ± 25   |
| 2       | 120 ± 7     | 180 ± 33     | 443 ± 57             | 411 ± 57       | 176 ± 14    | 204 ± 18   | 235 ± 16   |
| 4       | 120 ± 11    | 189 ± 23     | 444 ± 49             | 408 ± 44       | 181 ± 14    | 211 ± 14   | 240 ± 32   |
| 8       | 127 ± 9     | 196 ± 34     | 422 ± 37             | 386 ± 52       | 167 ± 18    | 212 ± 18   | 286 ± 35   |
| 24      | 125 ± 8     | 207 ± 31     | 310 ± 58             | 298 ± 52       | 158 ± 24    | 214 ± 15   | 304 ± 39   |
| 48      | 126 ± 20    | 199 ± 34     | 250 ± 45             | 245 ± 48       | 143 ± 20    | 213 ± 25   | 319 ± 40   |
| 72      | 120 ± 15    | 203 ± 40     | 212 ± 47             | 197 ± 61       | 136 ± 17    | 211 ± 26   | 315 ± 37   |
4. Discussion

4.1. Time course study
All of the C57BL/6 mice in our study tolerated the nanoparticle agent well, with a survival rate of 100% (for contrast agent injection, the multiple doses of inhaled anesthesia for the various time points and the accumulated radiation exposure) indicating that this agent can surely be considered, in term of low toxicity, for prospective studies with contrast enhanced micro-CT. The mean values in our sample of 6 mice shows a peak contrast enhancement over 340 HU in the vascular system (343 HU for vena cava and 382 HU for the right ventricle). The peak enhancement in the spleen and the liver are 193 HU and 89 HU over baseline background value. Concerning the blood, these enhancement values are slightly higher than the ones observed by Ford et al (Ford et al 2006) using Fenestra VC, an iodinated agent (326 HU for the right ventricle and 344 HU for vena cava). However, regarding the values for the spleen and the liver, we observe a lower contrast enhancement compared to Fenestra VC values (Ford et al 2006) (302 HU in the liver, and 446 HU in the spleen). The values obtained in the vasculature with the nanoparticle contrast agent are also higher compared with liposomal iohexol (Kao et al 2003) (109 HU in blood and 200 HU in liver), dysprosium-DTPA dextran (Vera and Mattrey 2002).
agent for the liver (Ford et al 2006), as the CNR never reaches or exceeds CNR = 5, the Rose criterion for visualization by the human eye (Rose 1948). In the previous study (Ford et al 2006), the CNR in the liver ranged from 11 immediately post-injection to 40 at the 24 h time point, which equaled that of the vasculature at injection, suggesting that all of the contrast agent remained in the body at 24 h post-injection. The MVivo Au agent may be excreted more completely, as the amount that has accumulated in the liver at each time point never reaches the same CNR as the vasculature. This suggests that some of the MVivo agent is being excreted throughout the time points studied, and as the total contrast enhancement across the body as-a-whole decreases over time. The excretion rate of this agent is beyond the scope of an imaging study, but should be investigated.

For imaging studies investigating disease models where visualization of the vasculature is needed (blockages, aneurysms, etc), scans should be preferably performed immediately after the injection or within the first hour; however, the long residence period of the contrast agent (between 48 and 72 h in the vascular system) allows a wide scanning window, enabling multiple image acquisitions with a single injection of contrast agent. This long vascular residence period may also be an advantage for prospectively-gated cardiac acquisitions, with scan times ranging from 7–20 min (Badea et al 2005, Nahrendorf et al 2007, Sera et al 2008, Cao et al 2010), requiring a stable contrast enhancement for optimal image quality. The images shown in figure 2 shows the same quality as the previous studies acquired with fast scans of only a few seconds (Ford et al 2006).

4.2. Cardiac-gating study

In the cardiac-gating imaging study, the gold nanoparticle and iodine-based agents were both well tolerated by the C57BL/6 mice, with a survival rate of 100% (including the contrast agent injection, radiation exposure and fairly long duration of inhaled anesthesia). The injected volume of the MVivo Au agent was approximately ½ of the Fenestra VC dose.

Measurements of the left ventricle systolic and diastolic volumes, along with the ejection fraction and stroke volumes, that we obtained with MVivo Au and our cardiac-gating protocol are similar to those obtained by Detombe et al (Drangova et al 2007, Detombe et al 2008), which suggests the measurement accuracy is similar for our imaging protocol. No significant difference between the nanoparticle and the iodine-based contrast agents can be noticed in the measured values. The image quality provided by the MVivo Au nanoparticle agent is slightly better than the Fenestra VC iodinated contrast agent especially in the systole phase. The results of these 2 studies suggest the MVivo Au nanoparticle agent seems to be a very suitable contrast enhancer for cardiovascular disease.

### Table 2. Contrast-to-noise ratios for the right ventricle, vena cava, kidney, liver, and spleen.

| Time (h) | Right Ventricle | Vena cava | Kidney | Liver | Spleen |
|---------|----------------|------------|--------|-------|--------|
| 0       | 11             | 10         | 2      | 2     | 4      |
| 0.25    | 10             | 9          | 2      | 2     | 4      |
| 0.5     | 10             | 9          | 2      | 2     | 3      |
| 0.75    | 10             | 9          | 2      | 2     | 4      |
| 1       | 10             | 9          | 2      | 2     | 4      |
| 2       | 10             | 9          | 2      | 3     | 3      |
| 4       | 10             | 9          | 2      | 3     | 4      |
| 8       | 8              | 7          | 1      | 2     | 5      |
| 24      | 6              | 5          | 1      | 3     | 5      |
| 48      | 4              | 3          | 0.5    | 3     | 6      |
| 72      | 3              | 2          | 0.5    | 3     | 6      |

### Table 3. Average functional parameters (mean ± SD) measured in free-breathing mice (n = 3).

| Parameter                  | MVivo Au    | Fenestra VC |
|----------------------------|-------------|-------------|
| LV systole volume (μl)     | 19.0 ± 0.7  | 19.5 ± 4.3  |
| LV diastole volume (μl)    | 49.5 ± 6.2  | 49.1 ± 1.9  |
| Stroke volume (μl)         | 30.4 ± 5.0  | 29.6 ± 6.0  |
| Ejection fraction (%)      | 61.2 ± 3.1  | 60.0 ± 10.2 |
Further improvements in the image quality for cardiac-gated imaging have been described for advanced image reconstruction using more sophisticated algorithms, including iterative reconstruction techniques (Badea et al 2008, Badea et al 2010), deconvolution techniques (Badea et al 2011), sparsity techniques (Karimi et al 2015) or by applying denoising algorithms (Karimi et al 2016). These improvements are not standard on commercially-available micro-CT scanners, but would enable reduction in the radiation dose, and potentially allow for a reduction in the number of projections acquired, thereby reducing the scan time and the anaesthesia time for the animal. These techniques would improve the noise characteristics of the image, making the features more easily observed by the human eye, and improving the ability to segment desired features, but the mean HU values reported here for various organs should remain unchanged by the use of different reconstruction algorithms.

4.3. Study novelty
In this study, we have used healthy mice to monitor the time course of contrast enhancement and to demonstrate both vascular and cardiac imaging with the MVivo Au contrast agent. Using healthy animals ensures that we can identify the source of any adverse reaction as being due to the contrast agent. Using a disease model, knockout animals or those that are receiving other interventions introduces alternate reasons for adverse events, including interactions between the contrast agent and other treatments or
drugs. Furthermore, some researchers have noted strain-related differences in the pharmacokinetics of contrast agents that resulted in different contrast enhancement time courses or uptake in different organs (Suckow and Stout 2008, Detombe et al 2012), so characterizing the contrast agent in a commonly used strain is widely applicable for many researchers.

Other nanoparticle-based and iodine-based contrast agents have been reported to remain in the body for weeks or months post injection (Boll et al 2011, Mannheim et al 2016). Although some authors perceive this as an advantage for longitudinal imaging without reintroduction of the contrast agent, there may be implications on animal well-being (Detombe et al 2012) or the progression or response to treatment of the disease model. Our results show less accumulation in the liver than other nanoparticle agents, as given by the peak contrast enhancement, suggesting clearance from the body during the time course of our study. Others have shown peak liver enhancements equal to the peak vascular enhancement, at delayed time points for Exitron agents (Boll et al 2011), Fenestra agents (Ford et al 2006, Willekens et al 2009) and eXia agents (Willekens et al 2009, Detombe et al 2012), which implies that the entire dose remained in the body at these delayed time points.

From our study, we can identify the first hour as the optimal time point for cardiac imaging, with consistent contrast enhancement throughout the vasculature for more than 4 h post injection, which would enable repeated imaging throughout a single day from a single dose of contrast agent. Furthermore, the workflow of the imaging study may be optimized by injecting the entire cage of mice at once. Imposing a short delay between injection and scan time would not affect the contrast enhancement in the vasculature. This is particularly important for researchers that rely on animal facility staff to administer the contrast agent, as injecting each mouse immediately prior to scanning will take the animal facility staff away from other tasks for an extended period of time.

5. Conclusion

In this study, we have characterized the contrast enhancement of a nanoparticle blood-pool contrast agent, MVivo Au, in C57BL/6 mice over time. The injection of this nanoparticle contrast agent provides a high contrast enhancement in the heart and throughout the vasculature (340 HU over background), with a residence period near to 72 h. MVivo Au allows small injection volumes in the range of 0.1 ml for a 20 g mouse, which is very interesting in serial small-animal in vivo studies. Furthermore, it has been shown in this study that the MVivo Au contrast agent is an excellent candidate to consider for cardiac-gated imaging since it exhibits high, immediate and long-lasting contrast enhancement, which allows accurate image-based measurements and comparable image quality to traditional iodine-based agents.

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