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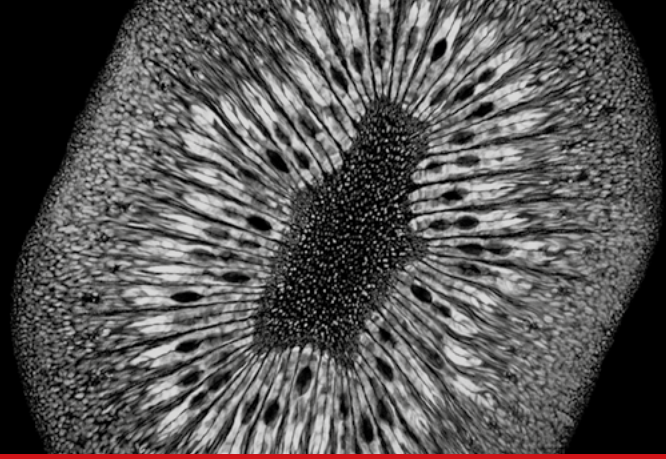
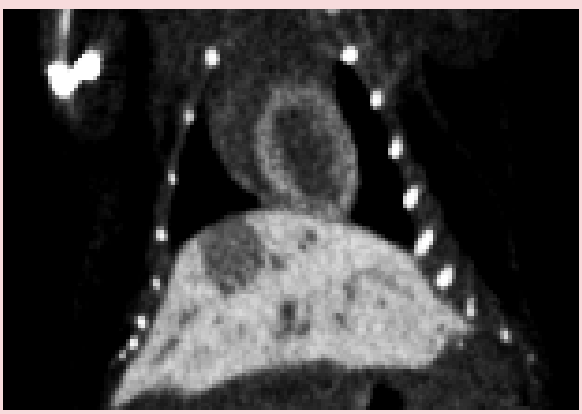


In vivo µCT of the myocardium



In vivo quantification of myocardial infarction using ExiTron™ MyoC 8000

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Introduction

Cardiovascular disease remains the leading cause of deaths worldwide, accounting for more than 17 million deaths per year[1]. With the exceedingly high number of people affected by this disease, a strong focus is being placed on the advancement of diagnostic imaging techniques that allow for improved detection of cardiovascular pathologies as well as monitoring of potential strategies for disease prevention and treatment. In recent years, *in vivo* imaging in small animal models of cardiovascular disease plays a central role in cardiovascular research and the models have certainly proven their value and clinical translatability. In this study we use a mouse model of myocardial infarction and develop a µCT method involving the use of the innovative contrast agent ExiTron™ MyoC 8000 to non-invasively detect and effectively quantify myocardial infarction *in vivo*.

Materials and methods

Experiments were performed on healthy C57BL/6J mice (n = 10) and C57BL/6J mice with myocardial infarction (n = 5) inflicted by surgical ligation of the left anterior descending (LAD) artery[2]. Following surgery, the animals were allowed to recover for a period of 2 weeks before start of the imaging experiments. µCT was performed on all animals after intravenous injection of ExiTron™ MyoC 8000 (Viscover™, *nanoPET Pharma* GmbH, Berlin, Germany), a nanoparticulate imaging agent having a high iodine content of 210 mg/mL. To investigate contrast agent kinetics, healthy anaesthetized mice were imaged before and after injection of ExiTron MyoC 8000 at a dose of 1050 mg iodine/kg body weight (i.e., 125 µL per 25 g mouse) followed by a saline flush (25 µL). Imaging was performed repetitively over 250 min with an additional scan after 24 h using a flat detector-based µCT system (Siemens Healthineers, Forchheim, Germany). Each scan was performed over 80 s with a rotation time of 19 s per revolution and image reconstructions were performed using a low-dose phase-correlated reconstruction method[3,4]. ECG data was obtained via electrodes attached to the animal's paw and the respiratory signal was derived using a pneumatic pillow (Small Animal Instruments, Stony Brook, USA). To allow for repetitive image acquisitions, the scan protocol was optimized to minimize radiation dose: each measurement comprised 50 mGy, resulting in an overall radiation dose of 600 mGy. The X-ray tube was operated using a tube voltage of 80 kV and a tube current of 50 mA. Signal enhancement was measured in different regions of interest (ROIs), including the left ventricle (corresponding to vasculature), myocardium, liver, spleen, kidney, brain and muscle.

For detection and subsequent quantification of myocardial infarction, the signal enhancement in the myocardium of infarcted mice was measured at 210 min post injection of the contrast agent. After completion of the imaging experiments, the mice were sacrificed and histological analysis was performed on paraffin-embedded sections of the heart using Masson's Trichrome[5]. Quantification of infarct size in histological stains and reconstructed 3D images was performed as previously described[6].

Results and discussion

In order to investigate the kinetics of ExiTron MyoC 8000, signal enhancements in various tissues of healthy mice were measured over a period of 24 h. The time-enhancement curve of the left ventricle (Fig. 1) illustrates that the contrast agent has a long blood half-life of ~2 h in mouse, with the maximum enhancement occurring immediately after injection. At 250 min post injection the signal enhancement in the blood has nearly reached a baseline value denoting that at this time point the contrast agent has practically cleared from the blood. Similar to the signal enhancement in the blood, the signal enhancement in the myocardium rapidly increases following injection of the contrast agent (Fig. 1). The peak enhancement is observed at 20 min post injection but the CT value at this time point is likely overestimated due to an overexposure artifact resulting from the strong contrast in the adjacent ventricles. Thereafter, the signal enhancement in the myocardium remains constant, at least until the 250 min imaging time point. As a result, ExiTron MyoC 8000 enables

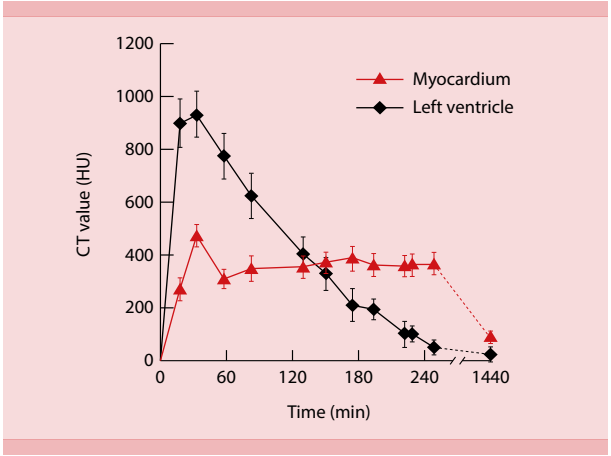

Figure 1: Time-dependent signal enhancement in the left ventricle and myocardium of healthy mice after injection of ExiTron MyoC 8000 at a dose of 1050 mg iodine/kg body weight.

not only functional cardiac imaging during the first 2 h of the blood pool phase but also imaging of the myocardium at a later phase. µCT images obtained during these two phases highlight the differences in signal enhancement within the various tissues (Fig. 2). The vessels, ventricles and aortic arch can be easily discriminated from surrounding tissues at 15 min post injection, whereas vessel contrast is diminished at 210 min post injection enabling delineation of the myocardium.

Besides providing signal enhancement in the blood and myocardium, ExiTron MyoC 8000 also results in the enhancement of the liver, spleen and kidneys, indicating

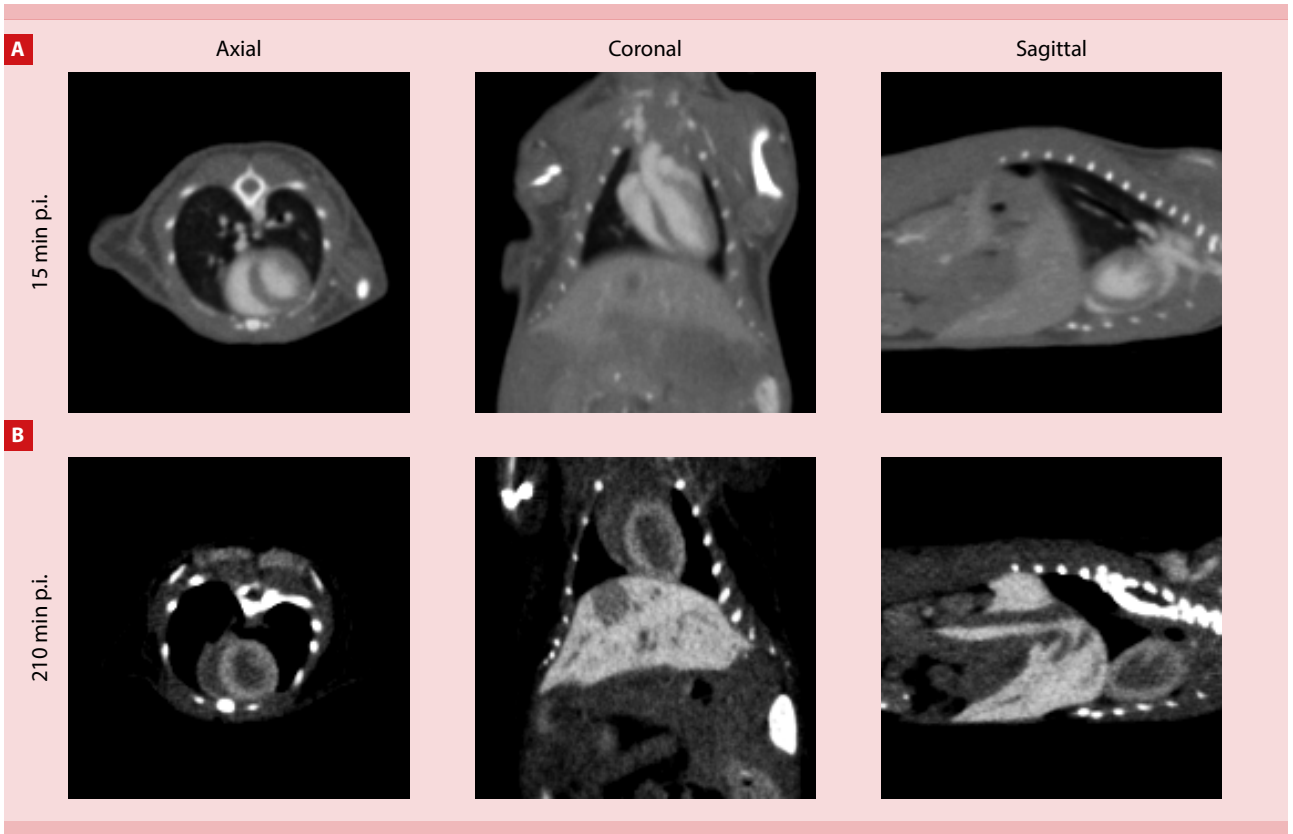

Figure 2: Axial, coronal and sagittal µCT reconstruction images obtained after injection of ExiTron MyoC 8000 in the healthy mouse at a dose of 1050 mg iodine per kg body weight.
A. In the early phase (15 min p.i.), the blood is highly contrasted allowing for functional cardiac imaging (C/W = 500/1500 HU). **B.** In the later phase (210 min p.i.), vessel contrast is diminished enabling delineation of the myocardium (C/W = 500/850 HU).

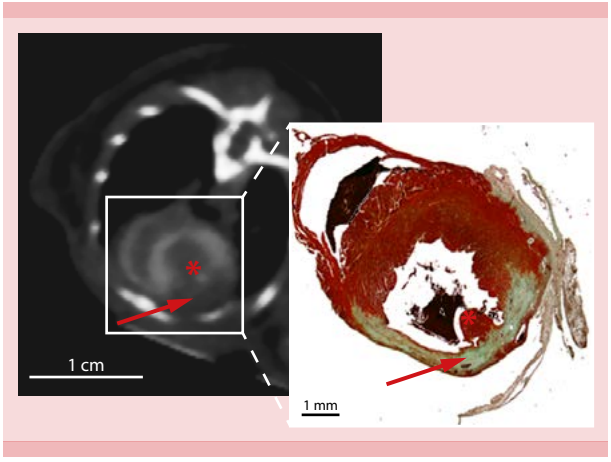

Figure 3: Qualitative comparison of µCT and histology images of mouse with myocardial infarction after injection of ExiTron MyoC 8000.
Axial µCT image at 210 min p.i. and corresponding histology image showing myocardial infarction (red arrows). In the histology image, viable tissue is depicted in red and infarct scar as well as local collagenous tissue, in green. Note the viable papillary muscle (red asterisks), which can be identified in both the µCT and histology image.

that these organs play a role in contrast agent elimination[7]. As expected, the muscle and brain tissues do not show any significant uptake of the contrast agent. At the 24 h time point (1440 min) the signal enhancement curves of all measured tissues converge with the baseline indicating that the contrast agent is practically eliminated from the body, offering the possibility of multiple injections[7].
Since imaging at 210 min post injection of ExiTron MyoC 8000 allows for effective visualization of the myocardium, this time point was selected for subsequent studies in a mouse model of myocardial infarction. Through µCT reconstruction images, infarct sizes were quantified and compared to those obtained via histopathology. A qualitative comparison of the µCT reconstruction images and microscopy images of histological sections depicts that the contrast agent is homogeneously distributed in the healthy myocardium, but is not taken up by areas of myocardial infarction (Fig. 3). The infarct sizes obtained via imaging as well as via histology vary in the range of 14 % and 60 % (Fig. 4), and are similar to those previously reported[6]. A quantitative comparison of infarct sizes obtained via the two methods shows a significantly strong correlation ($R^2 = 0.98$), demonstrating that the developed method allows for the detection and effective quantification of myocardial infarction *in vivo*.

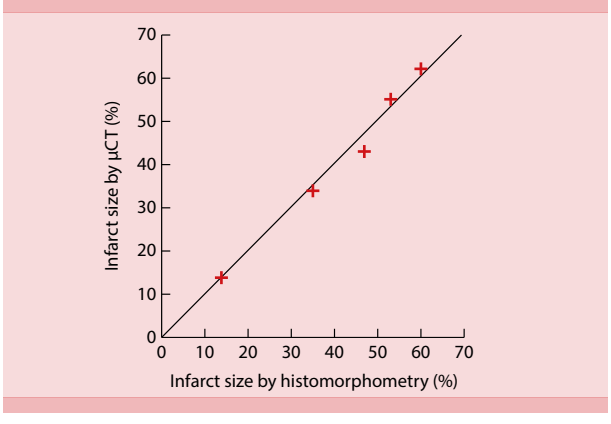

Figure 4: Comparison of infarct size obtained via µCT reconstruction images and via histomorphometry shows a significantly strong correlation ($R^2 = 0.98$).

Conclusion

In this study we develop a µCT method involving ExiTron MyoC 8000 and a low-dose phase-correlated reconstruction algorithm to monitor cardiac processes in small animals *in vivo*. The method allows for functional cardiac imaging as well as imaging of the myocardium, offering an accurate and reproducible method for *in vivo* detection and quantification of infarct size. Thus, the approach presents researchers with an innovative tool for the monitoring of cardiovascular pathologies in small animal models in order to evaluate the response to therapy.

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ExiTron™ P, 5 x 5 injections	130-095-145
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ExiTron™ nano 12000, 5 x 5 injections	130-095-700
ExiTron™ MyoC 8000, 1 x 5 injections	130-095-701
ExiTron™ MyoC 8000, 5 x 5 injections	130-095-702

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