

## Vascular micro-ct imaging of mouse hind-limbs

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Limb ischemia, induced by microsurgical occlusion of the femoral vessels, is a useful model to study ischemic vascular disease in gene-manipulated mice. Revascularization after occlusion can be measured by histomorphometric analysis of blood vessels, and blood flow analysis via laser doppler imaging and fluorescent microspheres, but these methods do not reveal the three-dimensional (3D) architecture of the vasculature before and after occlusion. We have applied micro-CT analysis to overcome this limitation.

Limbs were perfusion-fixed at baseline conditions or 2 hours after vascular occlusion, and filled with contrast agent via a catheter placed in the abdominal aorta. Different dilutions of barium-gelatin mixtures were tested. A warm solution containing 30% BaSO<sub>4</sub> and 5% gelatin resulted in optimal filling of the limb vasculature, with high radio-opacity of blood vessels. Iopamide 76.9%, a non-ionic radiographic contrast medium used in clinical applications, failed to contrast blood vessels in the mouse. Limbs were dissected in toto, placed on ice to promote gelling of the contrast solution, and post-fixed with buffered paraformaldehyde solution. The crural vasculature at the level of the calf muscles was imaged at 7 $\mu$ m resolution using the SkyScan-1172 high-resolution micro-CT system, with a voltage of 50 kVp and a current of 200  $\mu$ A.

3D-reconstruction of resultant scans was carried out with the SkyScan CT-analyzer software, and allowed detailed 3D analysis of the vascular tree in the limb. The arterial trunks were homogeneously filled in all specimens (n=4). Contrast-filling of veins was not homogenous, and retrograde filling of superficial veins occurred with variable degree. Scans of ligated hind-limbs revealed almost complete abolition of blood supply. 3D morphometric analysis was performed using SkyScan CT-volume software with the calf area as pre-defined volume of interest (VOI), and allowed assessment of vessel volume (object volume in the VOI), vessel number, and vessel thickness.

We conclude that vascular micro-CT analysis is a promising method to complement analysis of revascularization in the murine limb ischemia model.