3D light sheet imaging of left anterior descending artery ligation-induced myocardial infarction in mice

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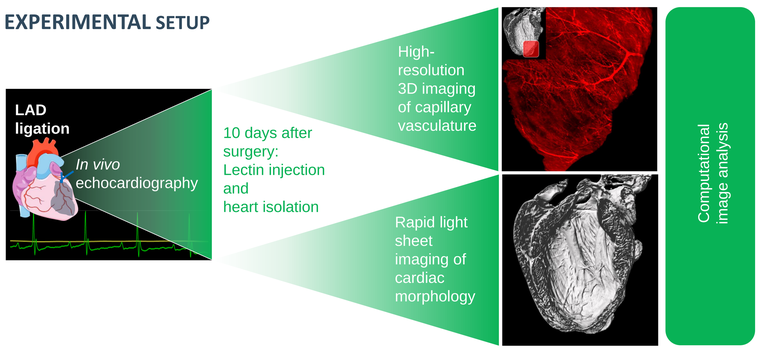
INTRODUCTION

Heart failure following myocardial infarction (MI) remains one of the leading causes of mortality worldwide. Although new treatment strategies have greatly improved the survival rate, recovery of cardiac function and prevention of progression to heart failure after MI remain challenging.

Surgical ligation of the left anterior descending artery (LAD) in mice is a frequently used animal model for MI. Whereas echocardiography and cardiac MRI are considered standard methods for the assessment of cardiac function, methods for the direct quantification of left ventricular (LV) remodelling and capillary density are still lacking.

EXPERIMENTAL SETUP



LAD ligation was performed in 8 to 9 weeks old C57BL/6 mice by transiently exposing the heart through the ribs and intercostal muscles[1]. Echocardiography using VisualSonics Vevo 3100 system was performed 9 days after surgery to evaluate the presence and location of the resultant infarction and the overall systolic function of the left ventricle. 10 days after surgery the mice were injected in the tail vein with fluorescent (DyLight-650-conjugated) tomato lectin. 10 minutes later the heart was stopped in diastole, fixed and cleared. The hearts were imaged using Lavision Ultramicroscope II. Image analysis was carried out using Bitplane Imaris and customized Python workflow. Deep learning for capillary detection was implemented using U-net platform.

Table 1 | Study groups

Study group	N
Unoperated	10
LAD ligation (echocardiographic signs of a MI in the anterior myocardial wall)	10
No infarction (no echocardiographic signs of MI after surgery)	4

RESULTS

Reduced systolic function in mice following LAD ligation

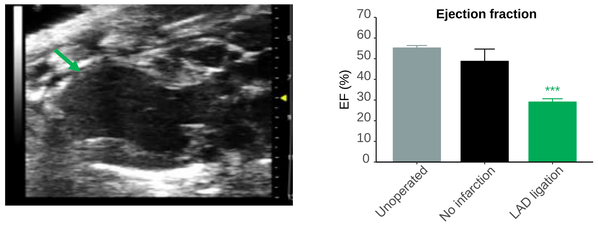


Figure 1 | Only mice exhibiting echocardiographic signs of an anterior wall MI (i.e. a thin and hypokinetic anterior wall in the parasternal long axis view) were included in the LAD ligation group (left; green arrow points at an infarcted anterior wall). Echocardiography confirmed significantly reduced ejection fraction (EF) in the LAD ligation group as compared to the No infarction group. ***: P < 0.001; Dunnett's test one-factor linear model; right.

[1] Gao et al. A novel and efficient model of coronary artery ligation and myocardial infarction in the mouse. Circ Res, 2010.

Light sheet imaging enables fast quantitative analysis of cardiac morphology

A complete protocol for heart isolation, light sheet imaging and quantitative image analysis was established. Critical parameters were optimized to achieve accurate arrest and fixation of the heart in diastole. The heart was scanned at a high resolution in 30 minutes, enabling direct quantification of left ventricular volume, shape and wall thickness.

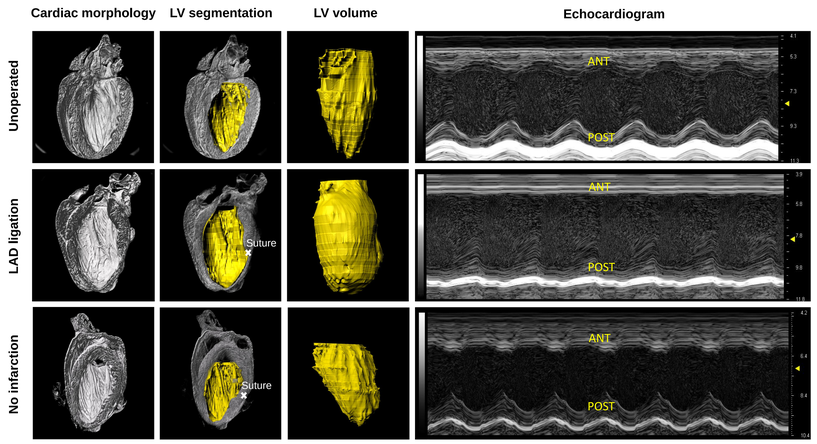


Figure 2 | Light sheet 3D imaging offers direct quantification of cardiac morphology. Images on the left demonstrate a view through the left ventricle and segmentation of the LV cavity (yellow). Corresponding echocardiographic images are shown on the right, illustrating anterior (ANT) and posterior (POST) wall thickness and motion.

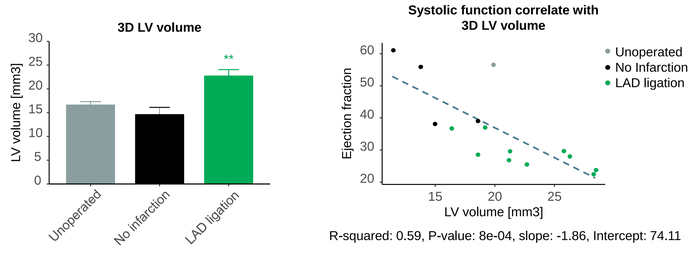


Figure 3 | Light sheet acquired 3D volume of the LV cavity was increased by approx. 35% in the LAD ligation group, as compared to the no infarction group (left; **P < 0.01; Dunnett's test one-factor linear model). 3D volume of the LV cavity was inversely correlated to ejection fraction calculated from echocardiography (right).

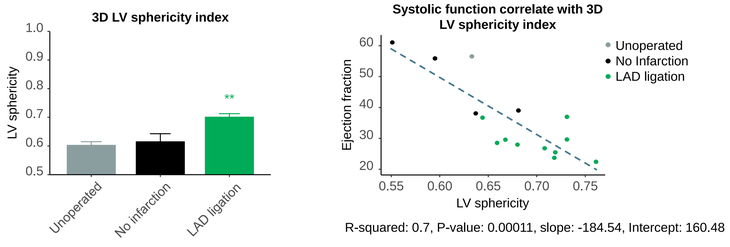


Figure 4 | Myocardial infarction induced a more spherical shape of the LV cavity (LV sphericity index 0 to 1, with 1 being perfectly spherically shaped). The LV sphericity index was increased by approx. 13% in the LAD ligation group, as compared to the no infarction group (left; **: P < 0.01; Dunnett's test one-factor linear model). Moreover, LV sphericity index was inversely correlated with ejection fraction derived from echocardiography (right).

Disclosures: All authors are employees of Gubra ApS.

3D analysis for accurate measurement of capillary density in the infarcted heart

In the setting of a MI, rapid restoration of microcirculation may limit cardiac pathology and help recover cardiac function. Intravenous injection of fluorescent lectin (to label blood vessels in the mouse heart) before light sheet imaging enabled high resolution volumetric analysis of the microvasculature. Next, a method for quantification of capillary density was established in the LV anterior wall including the apex, which is the main area at risk after LAD ligation.

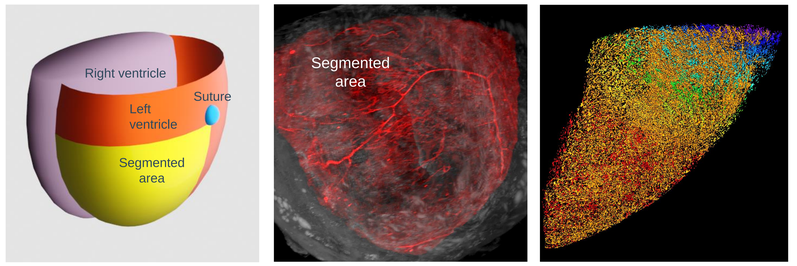


Figure 5 | Volumetric segmentation of the anterior wall of the left ventricle and automated 3D quantification of capillaries. A schematic demonstration with the segmented area, representing the anterior wall and the apex, shown in yellow (left). The segmented area spans distally from the suture, excluding the septum and the epicardium. A 3D volumetric view of the vasculature in the segmented area is shown in the middle panel and 3D segmented capillaries on the right, color coded from proximal to distal.

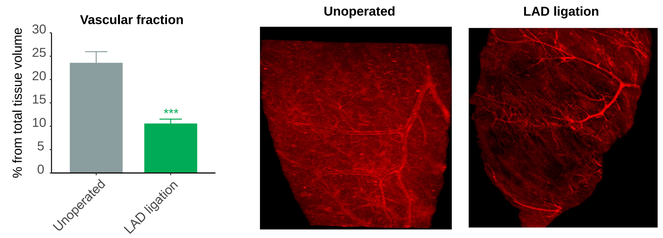


Figure 6 | Volumetric analysis demonstrated significantly reduced capillary density in the anterior wall of the left ventricle in the LAD ligation group as compared to unoperated mice (***P < 0.001; unpaired t-test). 3D volumes of representative unoperated and LAD ligated mice are shown on the right.

CONCLUSION

- **Light sheet microscopy provides a complete imaging methodology to quantify not only the myocardial remodelling but also the capillary density in a murine model of MI.**
- **Combined with *in vivo* echocardiography, quantitative data describing morphological and functional changes in the heart is provided, which enable a more accurate analysis of the effect of novel pharmaceuticals on e.g. angiogenesis and myocardial recovery in mouse models of MI.**