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Establishing a model for intravital imaging of the beating murine heart
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Introduction: While stem cell (SC)-based therapy following heart injury holds promise, clinical suc-
cess is compromised by poor SC recruitment to the heart following infusion. One solution is to iden-
tify strategies that may increase SC capture in cardiac microvessels. We have routinely used intravital microscopy (IVM), a method of live in vivo imaging of the microcirculation, to monitor cell trafficking. To do so in the heart would help facilitate research aimed at improving SC delivery. However, IVM of the beating mouse heart remains challenging due to difficulties with organ access and impractical de-
grees of tissue motion. The study presents a method that allows for cardiac IVM using a 3D-printed stabilizing device.

Methods: Anaesthetized mice were artificially ventilated using a small rodent ventilator which deliv-
ered medical oxygen. PEEP was applied by immersing the ventilator outlet in 4°C of ddH2O. A chest-
wall incision, expanded with retractors, facilitated the adhesive positioning of a specially designed tis-
ue stabilizer onto the heart surface. This limited respiratory and cardiac contractile motion in a small area without compromising overall heart function. Imaging was performed through a small hole in the center of the stabilizer. Preliminary studies were performed in the myocardioma ischemia-reperfusion injury model. This injury was established by occlusion of the left anterior descending artery (LAD). The vessel was occluded for 45 minutes using a small piece of plastic tubing within a loop which was placed under the LAD. Identification of vascular dynamics (e.g. cell recruitment, monitoring of vascular no-reflow) took place at various points during reperfusion. Administration of FITC-BSA al-
lowed for visualization of the microvasculature. Endogenous neutrophils were labelled by intra-arterial administration of 648 Fluor-460 labelled anti-Gr-1 antibody.

Results: Infusion of FITC-BSA permitted visualization of the coronary microcirculation. Vascular in-
tegrity was not disturbed and flow was visible as a result of application of the stabilizer. Endogenous (labelled) neutrophils were easily identified circulating in the cardiac microcirculation following cardiac IR. We identified significantly enhanced neutrophil recruitment as early as 60 minutes post-
reperfusion, when compared to sham-operated control animals. Furthermore, using FITC-BSA visu-
alization of the coronary microcirculation, we identified areas of vascular no-reflow following cardiac IR. Finally, systemically administered SCs (fluorescently labelled HSCs) could be identified trafficking through the cardiac microvasculature following cardiac IR injury.

Conclusion: We present a model for intravital imaging of the mouse beating heart in vivo. This meth-
od may be useful in monitoring cell dynamics and microvascular disturbances in the heart at the single

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Altered RyR microdomains lead to more Ca2+ waves in non-coupled RyRs after myocardial infarction
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Purpose: In ventricular myocytes of large mammals, a significant number of ryanodine receptors (RyRs) are not in couplers (i.e. non-coupled RyRs). Previous research from our lab has shown that these non-coupled receptors lack CaMKII and NOX2 microdomain modulation. Furthermore, after MI TT1s are lost and hence the fraction of non-coupled RyRs is increased. We investigated whether this affected microdomain signaling and diastolic Ca2+ waves which are potentially arrhythmogenic.

Methods: Using an established pig model of chronic ischemia and myocardial infarction (MI, N=16), we studied myocytes from the area adjacent to the MI and compared these to myocytes from sham pigs (N=13) using whole-cell voltage clamp with Fluo-4 as a (Ca2+), indicator together with confocal line scan imaging. Myocytes were stimulated at a high frequency (2 Hz) in the presence of isoproterenol (10 nM ISO). Ca2+ waves were recorded during a 15 s rest period following stimulation and assigned to different subcellular regions categorized as coupled or non-coupled RyRs using a specific algorithm.

Results: Conditioning myocytes at high frequency in the presence of ISO induced more Ca2+ + waves in MI myocytes (0.21 ± 0.1 #waves/100μm²; n=62) compared to SHAM myocytes (0.15 ± 0.1 #waves/100μm²; n=59). No differences were found in wave foci, but a significant reduction in foci recruitment occurred after MI (57% in MI vs. 70% in SHAM). Moreover, in healthy myocytes more Ca2+ + waves occurred in coupled compared to non-coupled in a 2:1 ratio. In MI myocytes, however, the subcellular site of wave occurrence was reversed to non-coupled sites resulting in a 12 ratio for coupled-non-coupled RyRs. Specific blockers directly (S107) or indirectly (AIP inhibiting CaMKII, mitoTEMPO scavenging mitochondrial ROS) targeting RyRs were investigated. In healthy myocytes, none of the blockers significantly affected wave frequency, neither at the global nor in the coupled versus non-coupled RyRs. However, in myocytes from MI animals, S107, mitoTEMPO and AIP all reduced the global increase in wave frequency. Subcellular analysis of different RyR subsets showed that in MI S107 (0.06 ± 0.1 #waves/100μm²; n=27), AIP (0.06 ± 0.1 #waves/100μm²; n=25) and mitoTEMPO (0.08 ± 0.1 #waves/100μm²; n=22) reduced the wave frequency in non-
coupled RyRs without significantly affecting coupled RyRs.

Conclusion: After MI, Ca2+ + waves arise predominantly in non-coupled RyRs. The non-coupled RyRs after MI are modulated via local microdomains of CaMKII and mitochondrial ROS. These data highlight the arrhythmogenic nature of the non-coupled RyR clusters, which are at a higher dens-
ity after MI, and suggest there may be a potential for selective pharmacological targeting.