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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 4cm of ddH2O. A chest

wall incision, expanded with retractors, facilitated the adhesive positioning of a specially designed tis-

sue 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

centre of the stabilizer. Preliminary studies were performed in the myocardial 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 visualisation of the microvasculature. Endogenous neutrophils were labelled by

electro-arterial administration of 65g of Fluoro-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 stabiliser. Endogenous

labelled neutrophils were easily identified circulating in the cardiac microcirculation. Following car-
diac 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-

diary microscopy (IVM), 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, systematically 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

cell level.

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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 coupling (i.e. non-coupled RyRs). Previous research from our lab has shown that
these non-coupled receptors lack CaMKII and NOX2 modromian modulation. Furthermore, after

MI TTIs are lost and hence the fraction of non-coupled RyRs is increased. We investigated whether

this altered 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+; n=7) 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 (AP5 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

AP5 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), AP5 (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 densi-

ity after MI, and suggest there may be a potential for selective pharmacological targeting.