PHILIPP SASSE – MVX10 APP NOTE

The Olympus MVX10 Macroscope for advanced *in vivo* imaging

Macroscopic *in vivo* analysis of transplanted cardiomyocytes

After an infarction has occurred, transplantation of cardiomyocytes can successfully facilitate the reduction of cardiac arrhythmias. The functional integration of such transplanted cells can subsequently be confirmed *in vivo* via macroscopic fluorescence imaging of a genetically encoded Ca^{2+} sensitive protein.

After a cardiac infarction, patients are at a greater risk of heart failure, as well as life-threatening cardiac arrhythmias. It has been previously demonstrated that the transplantation of cells has the potential to improve cardiac function. However, it was not known if these transplanted cells functionally engraft and couple to the native myocardium or if this could reduce the risks associated with post-infarct arrhythmia.

To investigate this issue, experiments were performed on infarcted mice in close collaboration with the Departments for Cardiac Surgery and Cardiology from the University of Bonn. This study demonstrates that the transplantation of electrically coupled embryonic cardiomyocytes, after a cardiac infarction, reduces the risk of cardiac arrhythmia occurring [1]. This effect was not observed with cells that are unable to electrically couple to each other, such as fibroblasts, bone marrow cells or normal skeletal muscle cells, as these cell types lack the coupling protein connexin. However, when skeletal muscle cells were genetically modified to express connexin 43 and used for transplantation, the same protective effect seen with cardiomyocytes was produced [1]. These results indicate that the

electrical coupling of transplanted cells with the surrounding intact myocardium is essential.

The analysis of electrical coupling in vivo

In order to directly investigate the effects of electrical coupling of transplanted cells, the genetically encoded Ca^{2+} dye GCaMP2 was used. This fusion protein is composed of a circular permutated green fluorescence protein (GFP), calmodulin and the binding peptide M13 [2]. GCaMP2 is unable to fluoresce in the absence of Ca^{2+}, but when Ca^{2+} is present it binds the calmodulin, causing a conformational change and subsequent fluorescence of the GCaMP2. Spontaneously active embryonic cardiomyocytes that stably express GCaMP2 by lentiviral transduction showed an increase in fluorescence with every contraction of the muscle. These cells were transplanted into the infarcted regions of mouse hearts and could be detected two weeks later as fully-engrafted, cross-striated and GCaMP2 positive cells (figure 1). To explore their *in vivo* activation, the mice were intubated, artificial respirated and the ribcage was removed. Careful haemostasis enabled detailed study of the beating heart over several hours. In order to successfully image the moving heart, it was stabilised using a small spoon and a glass plate.

Since the heart of an anesthetized mouse beats at 7-10 Hz, motion blurring during image capture is a problem. To overcome this, short exposure times and a particularly powerful lens are required. Microscopic imaging is unsuitable since objectives with magnifications of less than 5x do not transmit enough light for short exposure times. Therefore, the Olympus MVX10 macroscope was used with its high power 1x (NA: 0.25) and 2x (NA: 0.5) objective lenses. Unlike stereo microscopes, the MVX10 produces a well-illuminated sample using a single optical light path. For binocular preparation of the heart and surrounding tissue, special optics within the MVX10 are used to produce a sufficient stereo-like image.

GCaMP2 positive cells were identified within the infarction using a special FITC/TRITC double filter, through which the cells fluoresced green and autofluorescence was red. The GCaMP2 fluorescence of specified cells was captured at different magnifications using a selective GFP narrow-bandpass filter and a fast EMCCD camera (iXion 885KS, Andor Technologies). The camera trigger, acting as a time mark for each individual frame, and the ECG of the mouse were registered simultaneously using an AD converter (Powerlab, AD instruments). As a result, each frame was assigned to the native ECG.

Results

Recordings from the infarcted region *in vivo* provided an indication of electrical activation because the transplanted cells demonstrated periodic changes in fluorescence. Further processing was required because heart movement could not be completely suppressed, despite mechanical stabilisation. Off-line analysis with the "turbo-reg" algorithm [3] led to a further reduction of movement artefacts. This algorithm shifts and rotates each frame to fit a reference image, producing fixed GCaMP2 positive cells. After normalisation (F/F_0), the GCaMP2 fluorescence was compared with the native ECG. From this, custom written algorithms (LabView, National Instruments) were used to calculate the delay for each heartbeat, the results of which are shown in Figure 2.

GCaMP2 signals were not significantly affected by motion. After every other native heart beat shown in the ECG, an increase in GCaMP2 fluorescence was observed. The transplanted cells were therefore coupled at a ratio of 2:1 to the native heart beat. A 4:1 and 6:1 coupling was also observed in other experiments and only if the native heart frequency was lower than 5 Hz, a 1:1 coupling could be seen.

After *in vivo* heart analysis, further investigation took place *ex vivo*, using the Langendorff isolated perfusion system. Figure 3 shows an example with

spontaneously active transplanted cells that were not coupled to the native myocardium. The high resolution of the 2x lens enabled identification of individual cells and their sequential activation.

Conclusion

The MVX10 macroscope, in combination with the Ca^{2+}-sensitive protein GCaMP2 enables precise investigation of the electrical coupling of transplanted cells *in vivo*.

Bibliography

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Images:

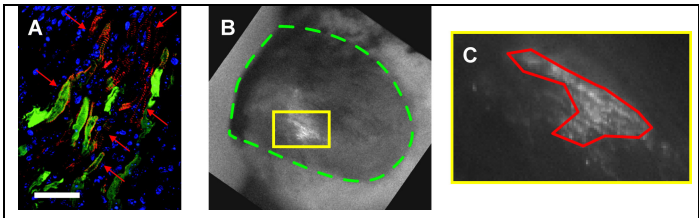

Figure 1

Transplanted GCamP2 positive (A, green) embryonic cardiomyocytes show α-actinin positive (A, red, arrows) cross-striation and can be macroscopically detected in the infarct region (highlighted in B by green line). C: Enlargement of yellow box in B showing the transplanted cells in detail. Bar 35 µm (A), 1.5 mm (B) 360 µm (C). Modified from [1].

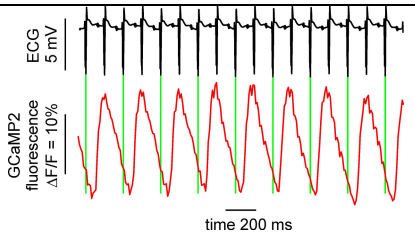

Figure 2

In vivo imaging of GCaMP2 fluorescence from transplanted embryonic cardiomyocytes (red, analysis performed from marked region in figure 1C) and the ECG (black): GCaMP2 fluorescence in the transplanted cells increases after every other native heart beat (green lines) proving 2:1 coupling. Modified from [1].

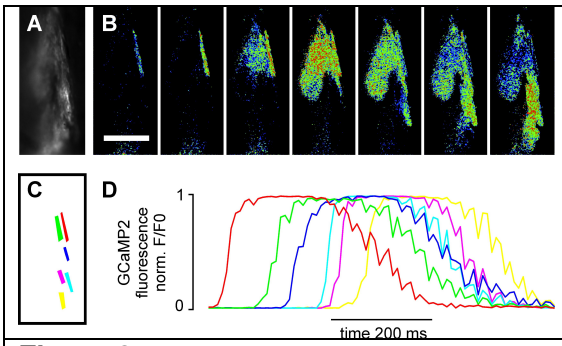

Figure 3

High resolution image of transplanted GCaMP2 positive embryonic cardiomyocytes (A) and single images showing their activation (B, 53 ms between 2 fames). Normalised GCaMP2 fluorescence of individual cells (marked in C) show the coupling among them with sequential activation (D). Bar 360 µm. Modified from [1].

Figure 4

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