

Monitoring the efficacy and retention of collagen I-Matrigel for treating myocardial infarction

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Introduction Myocardial infarction (MI) is one of the primary causes of heart failure, which is associated with severely reduced quality of life and poor clinical outcome. In recent years, stem cell therapy has emerged as a promising strategy to improve cardiac function post-MI¹. Unfortunately, the retention of stem cells after injection in the infarcted myocardium is often insufficient, leading to inadequate cell engraftment and reduced therapy efficacy². Cell engraftment may be improved by using a supporting hydrogel, which could improve delivery, retention, and survival of the injected stem cells. In addition, the hydrogel may prevent further remodelling and dilatation of the weakened heart by providing mechanical support to the myocardium post-MI³. **Goal:** Evaluate the efficacy and retention of an injectable collagen I-Matrigel for treating MI using multiple magnetic resonance imaging (MRI) techniques and histological validation.

Methods Measurements were performed on a 9.4T pre-clinical scanner (Bruker BioSpin, Ettlingen, Germany). Male Swiss mice underwent ischemia reperfusion immediately followed by intramyocardial injection of collagen I-Matrigel (n=6) or saline (n=5) in the border zone of the infarct. *In vivo* cardiac MR was performed two and eight days after injection.

Cine MRI was applied using a 2D ECG and respiratory triggered FLASH sequence to investigate improvements in cardiac left ventricle functional parameters after a treatment with collagen I-Matrigel post-MI as compared to saline treatment. Acquisition parameters Cine MRI: TR/TE=7/1.8 ms; $\alpha=15^\circ$; AcqMatrix=192x96 (zero filled to 192x192); FOV=3x3 cm²; slice thickness=1 mm; number of slices=6-8 short-axis slices from base to apex; cardiac frames=12-15.

T_2 -mapping using a T_2 -prep sequence⁴ was applied to establish the potential of quantitative T_2 -mapping as a tool for the visualization of the injected collagen I-Matrigel *in vivo*. Acquisition parameters T_2 -mapping: TR_{acc}/TE_{acc}=2.8/1.4 ms; TE_{eff}=0.8, 8.3, 15.0, 29.2, or 43.4 ms; TR_{seg}=2 s; $\alpha=30^\circ$; AcqMatrix=126x126; FOV=3x3 cm²; slice thickness=1 mm; number of slices=3; number of k-lines per segment=3; number of averages=3; total imaging time=4m30s per TE_{eff}. The acquisition took place during end-diastole. T_2 -maps were made by calculating the T_2 -values on a pixel-by-pixel basis using the signals at different TE_{eff} and the mono-exponential decay curve ($M_0 \cdot e^{-TE/T_2}$).

For histological evaluation, the collagen I-Matrigel was fluorescently labelled with 5-(4,6-dichlorotriazinyl)-aminofluorescein (5-DTAF) prior intramyocardial injection. After the last cardiac MR, mice were sacrificed and hearts were excised. Short-axis cryosections were examined for the presence of the fluorescently labelled collagen I-Matrigel with fluorescence microscopy. All sections were counterstained with Hematoxylin and Eosin (H&E) for global histological observation. For all statistics, p<0.05 was considered to be significant.

Results and Discussion The intramyocardial injections of collagen I-Matrigel in mice proved to be feasible and were successfully performed. Treatment with collagen I-Matrigel post-MI resulted in a significantly improved ejection fraction (EF) as compared to saline injection (fig.1) two days after injection (collagen I-Matrigel: 62.9±6.5% vs. saline: 54.2±4.8%; p<0.05). The EF was similar between collagen I-Matrigel and saline treatment eight days after injection post-MI (collagen I-Matrigel: 58.3±3.7% vs. saline: 57.3±5.0%; p=0.59). Fluorescence microscopy showed that the collagen I-Matrigel was retained up to eight days after injection, while the presence and retention of the hydrogel was not visible in quantitative T_2 -maps. Nevertheless, the T_2 -maps revealed a trend of increased T_2 -enhanced myocardial volume (fig.2 and fig.3) from two to eight days after collagen I-Matrigel injection (day 2: 24.7±20.5% vs. day 8: 35.8±21.9%; p=0.08). This trend was not present after saline injection (day 2: 17.4±12.0% vs. day 8: 14.2±9.7%; p=0.28). This increase in T_2 -enhanced myocardial volume between the two time points likely indicates an increased edema and/or inflammatory response after collagen I-Matrigel injection, which was corroborated by histology. The H&E-stained images showed an increased number of inflammatory cells in the infarcted myocardium surrounding the collagen I-Matrigel eight days after injection post-MI (fig. 4), which was not visible in the infarcted myocardium after saline treatment.

Conclusion This study demonstrated that collagen I-Matrigel treatment resulted in a temporary increased cardiac function post-MI, but also provoked an enhanced edema and/or inflammatory response in the cardiac tissue surrounding the collagen I-Matrigel as indicated by T_2 -mapping as well as by histology. Additional histology should indicate the nature of the biological, physical or mechanical function of the collagen I-Matrigel that provided temporary cardiac function improvement.

References 1. Singla *et al.*, Am J Physiol Heart Circ Physiol, 2007;293:1308-14, 2. Smits *et al.*, Cardiovasc Res 2009;83:527-535, 3. Traverse, J Cardiovasc Transl Res, 2012;5:67-72, 4. Coolen *et al.*, MRM, 2013 (in press)

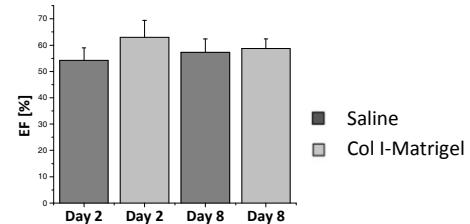


Figure 1: Ejection fraction (EF) two and eight days after treatment post-MI.

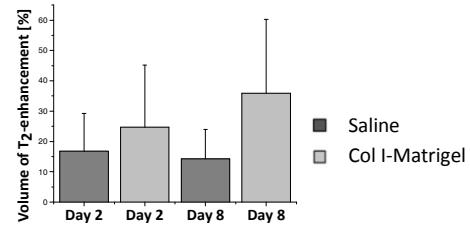


Figure 2: T_2 -enhanced myocardial volume two and eight days after treatment post-MI.

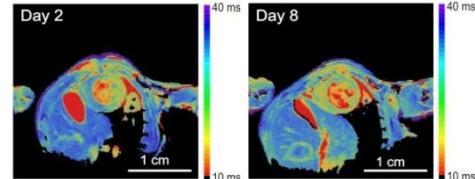


Figure 3: Short-axis T_2 -maps two and eight days after collagen I-Matrigel injection post-MI.

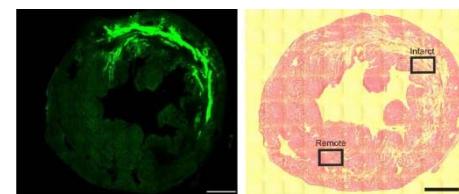


Figure 4: Left: Fluorescence images showed clear presence of 5-DTAF labelled collagen I-Matrigel within the left ventricle wall. Right: Corresponding H&E-stained sections showed an enhanced inflammatory response within the infarcted region containing collagen I-Matrigel (scale bar: 1000 μ m).