Maturation-Dependent Mechanobiology of hiPSC-derived cardiomyocytes

Dylan Mostert[1,2], Chiara Mameli[1], Rahul Bhatti[3], Paolo Contessotto[3], N.A. Kurniawan[4,5], Abhay Pandit[3] & C.V.C. Bouten[4,5]

1 Department of Cardiology, Maastricht University, the Netherlands, 2 DWI-Leibniz Institute for Interactive Materials, Germany,
3 CÚRAM, SFA Research Centre for Medical Devices, University of Galway, Ireland, 4 Department of Biomedical Engineering, Eindhoven University of Technology, the Netherlands, 5 Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology, the Netherlands

Contact: dylan.mostert@maastrichtuniversity.nl

Research motivation – the need for a cell therapy solution for heart attack patients

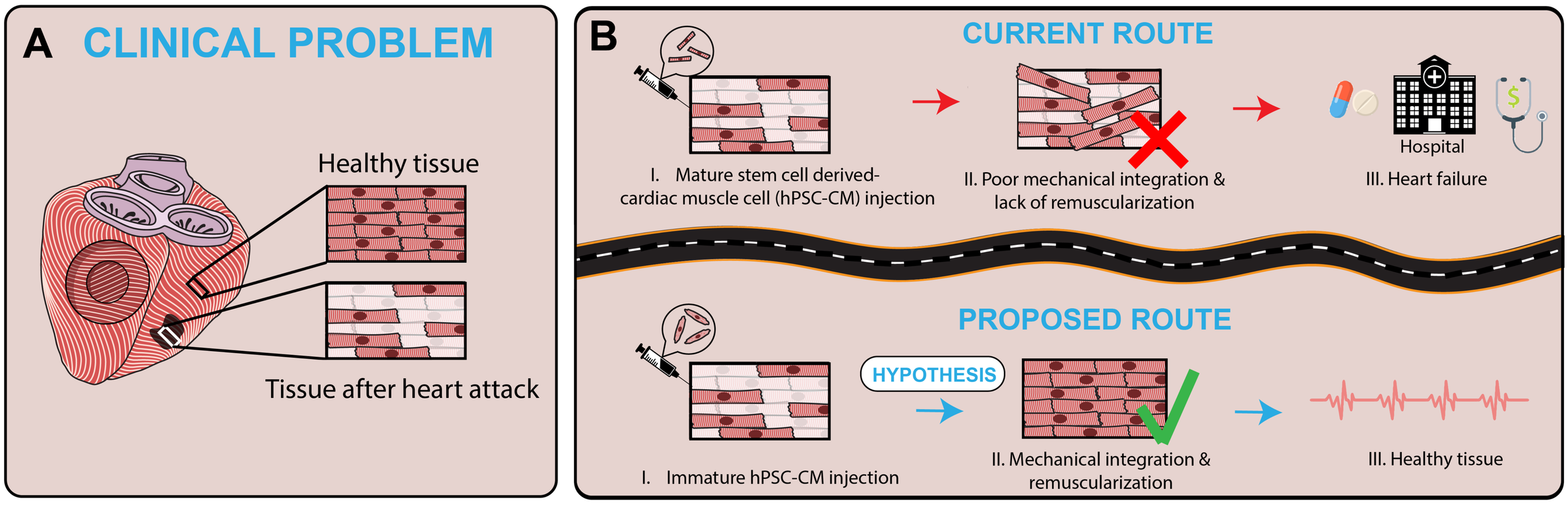

Figure 1: The concept and approach of the research project. A) Heart attacks result in a loss of cardiac muscle cells – the contractile units of the heart. **B)** The current route of treatment results in impaired mechanical integration and heart failure. The proposed route uses less mature, but mechanobiologically active hiPSC-CMs, leading to better mechanical coupling . **C)** Schematic illustration of the experimental approach. hiPSC-CMs = human induced stem cell-derived cardiomyocytes; ECM = extracellular matrix.

Characterization of hiPSC-CM mechanobiology at various stages of maturity

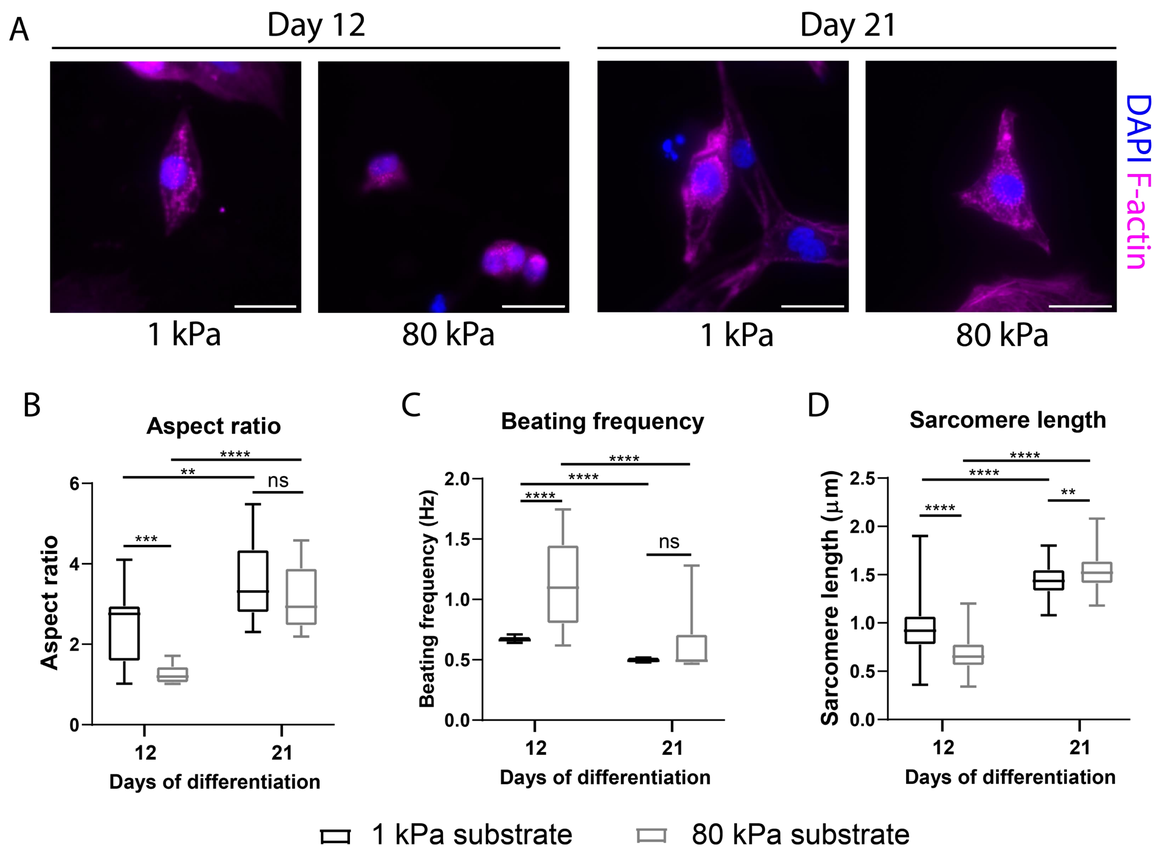

Figure 2: Early-stage hiPSC-CM are more sensitive to substrate stiffness than their more mature counterparts. A) hiPSC-CM morphology at day 12 and day 21 of differentiation on 1 kPa and 80 kPa polyacrylamide gels. Scale bar = 20 µm. **B-D)** The effect of substrate stiffness and differentiation stage on hiPSC-CM aspect ratio, beating frequency, and sarcomere length. Student t-test (n = 50 cells from three independent experiments). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

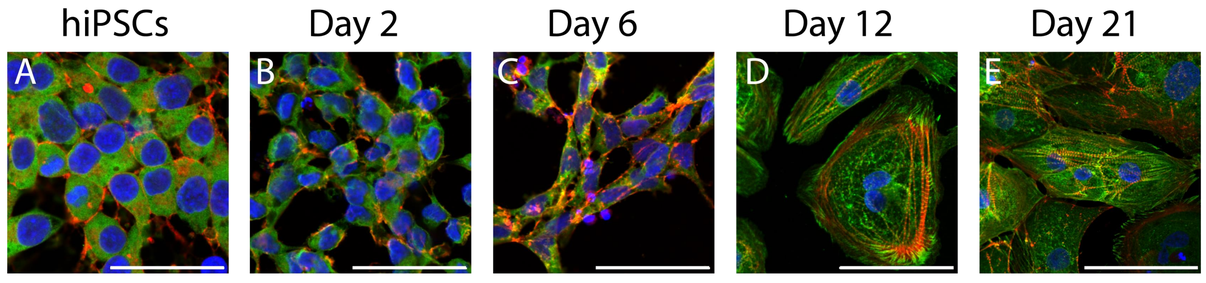

Figure 3: hiPSC-CM maturation alters the expression of cellular adhesion complexes and contractile components. A-E) Representative immunofluorescence at various stages during the CM maturation of hiPSCs. Scale bar = 50 µm.

Conclusive remarks

Our findings underscore the significant role of hiPSC-CM maturity in shaping hiPSC-CM mechanobiology. Late-stage hiPSC-CMs, while therapeutically promising, exhibited diminished mechanosensitivity, which suggests that early-stage hiPSC-CMs hold promise in cell therapy and tissue engineering applications, where mechanical cues are frequently employed to promote cell adhesion, integration, and functionalization. Future studies will investigate i) the expression of cell-cell interactions, ii) the mechanoresponse of hiPSC-CMs to other cues inherent in the myocardium, and iii) assess the integration and functionalization when seeded in *in vitro* models of varying complexity.

ACKNOWLEDGEMENTS

This research was financially supported by the Gravitation Program "Materials Driven Regeneration," funded by the Netherlands Organization for Scientific Research (No. 024.003.013). Dr. N. A. Kurniawan acknowledges financial support from the European Research Council (No. 851960). This publication has emanated from research supported by a research grant from Science Foundation Ireland (SFI), co-funded under the European Regional Development Fund through Grant number 13/RC/2073 and 13/RC/2073_2. The authors acknowledge the facilities and the scientific and technical assistance of the Centre for Microscopy & Imaging at the University of Galway (www.imaging.nuigalway.ie). Moreover, the authors thank Romee van Schijndel for her contribution to the research. D.M. acknowledges