Acoustic patterning of vascularized engineered cardiac tissues

N. Khoshdel Rad[1], O. O'Dwyer Lancaster-Jones[1], M. Wisbar[1], R. Quinn[1], M. Bencun[2], D. Duarte Campos[1]

[1] Bioprinting and Tissue Engineering Group, Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), University of Heidelberg, Germany
[2] Section of Bioinformatics and Systems Cardiology, Klaus Tschira Institute for Integrative Computational Cardiology (IKIM), University Hospital Heidelberg, Germany

DC bioprintinglab

Introduction

Cardiac Anisotropy:
Native myocardium exhibits a highly organized **anisotropic architecture**, in which cardiomyocytes (CMs) are aligned to support coordinated contraction. This structural organization enables **spatially organized cell-cell interactions** and efficient force generation at the tissue level [1-3].

Multicellular Structure:
Cardiac tissue is multicellular, comprising CMs together with **supporting cells**, including fibroblasts, endothelial cells, and pericytes. These cells contribute to extracellular matrix (ECM) organization, structural stability, and local signaling, collectively shaping tissue organization and functional behavior [4]. In addition, cellular orientation can influence not only cardiomyocytes but also supporting cell populations, guiding their organization and interactions within the tissue environment.

Research Aim:
To investigate whether introducing anisotropic organization in CMs-only and multicellular cardiac systems improves tissue structure and function, toward the development of a more physiologically relevant human cardiac model.

Methods

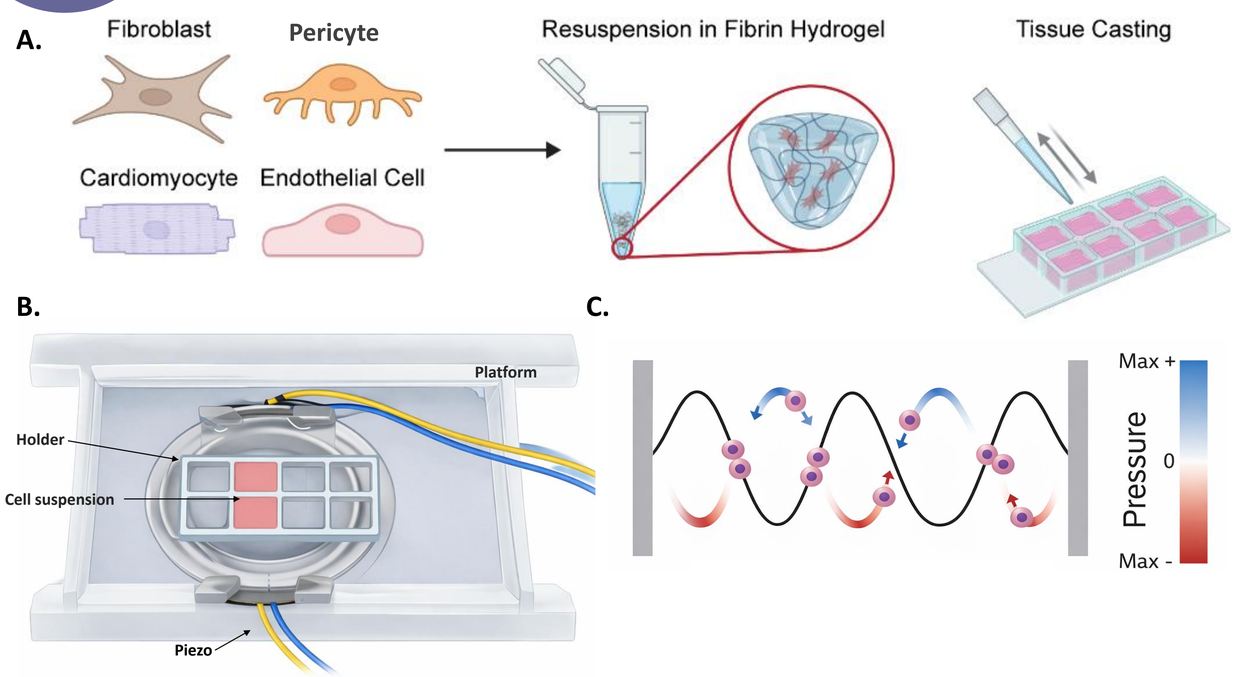


Figure 1. Schematic approach for the creation of acoustic patterning of 3D vascularized cardiac microtissues. (A) Cells were resuspended in a fibrin hydrogel (5 mg/mL fibrinogen, 2.5 U/mL thrombin) and cast into chamber slides. **(B)** Constructs were subjected to standing acoustic waves (1.66 MHz) to induce spatial alignment of cells prior to gel polymerization. **(C)** Standing wave schematic of cell manipulation where pressure oscillations shift the cells into the node positions. Following alignment, microtissues were stabilized and cultured under standard conditions for subsequent analysis.

Result

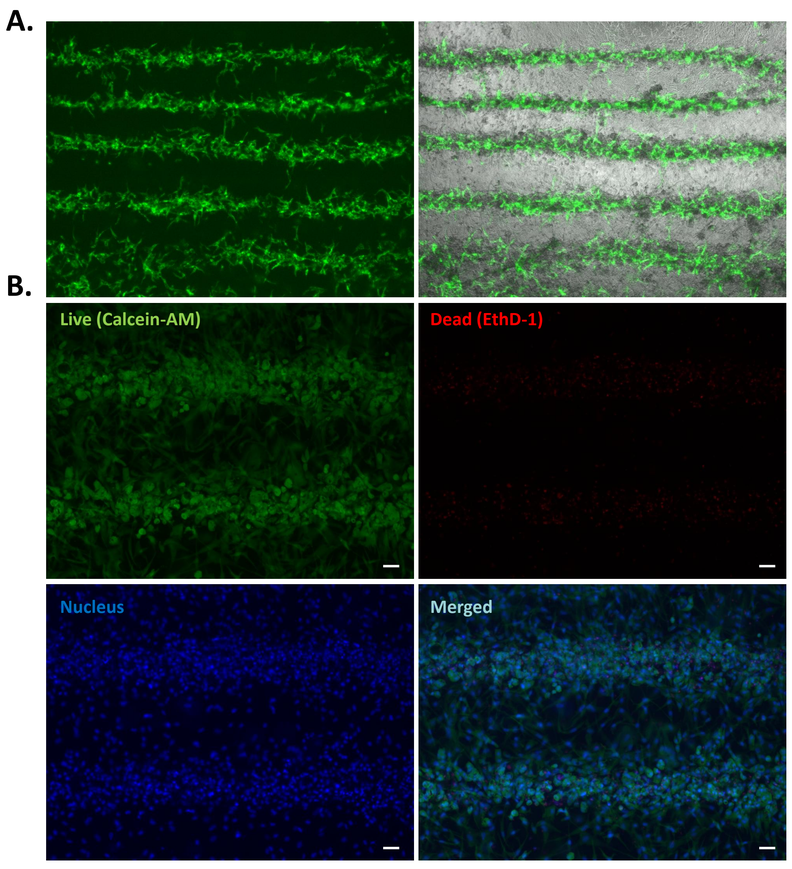


Figure 2. Alignment and viability of multicellular cardiac microtissues. (A) Representative fluorescence images showing the organization of GFP-positive endothelial cells (green) within aligned multicellular cardiac microtissues. Alignment promotes spatial organization of cells within the microtissue (Scale bar: 100 µm).
(B) Live/dead staining of aligned multicellular cardiac microtissues. Live cells are shown in green (Calcein-AM), dead cells in red (EthD-1), and nuclei in blue (DAPI). (Scale bar: 50 µm).

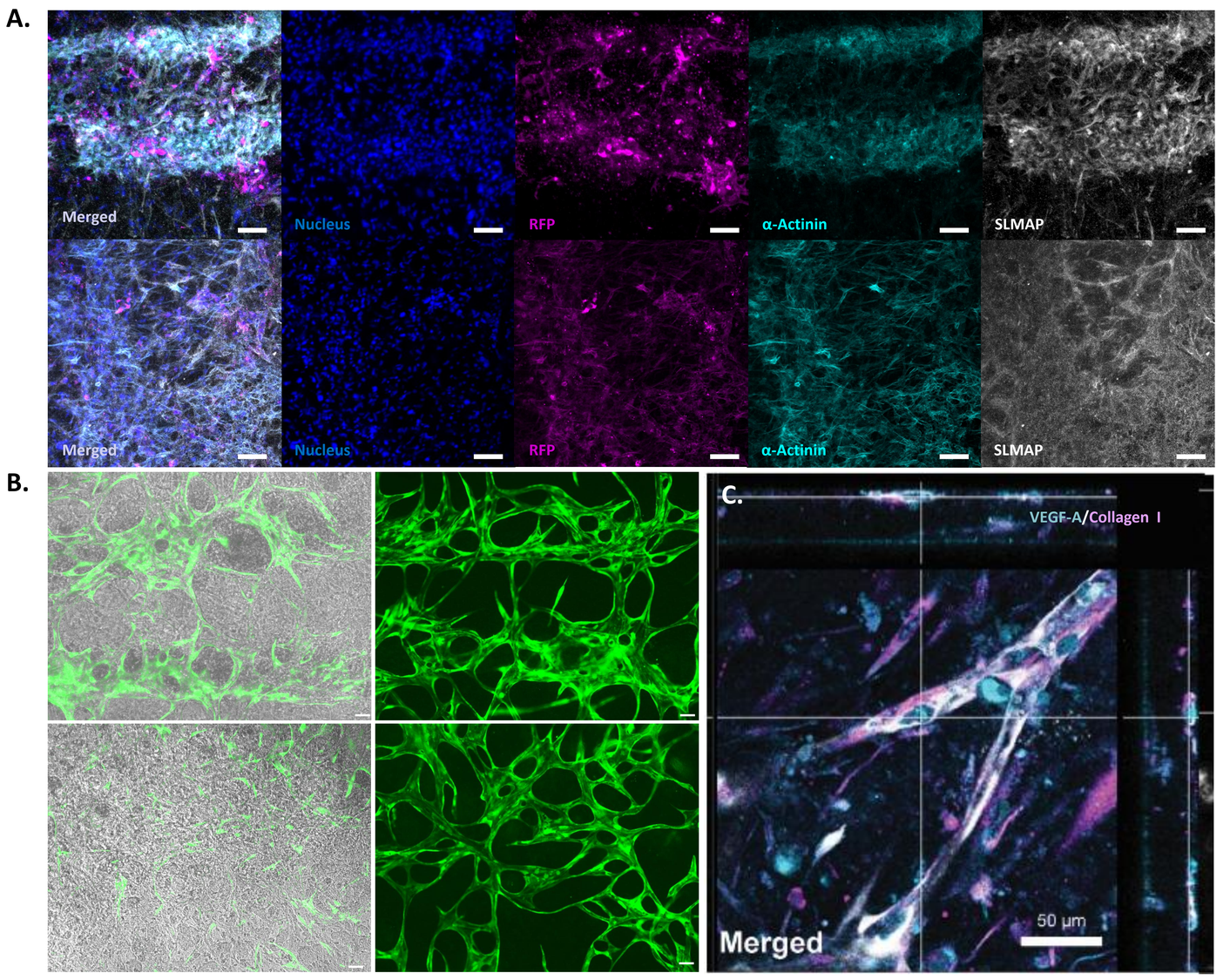


Figure 3. Structural organization and vascular features in aligned cardiac tissues. (A) Immunostaining of aligned (top) and unaligned (bottom) microtissues showing cardiomyocytes (α-actinin, SLMAP), endothelial cells (RFP), and nuclei (DAPI).
(B) GFP+ endothelial networks in aligned (top) vs unaligned (bottom) conditions.
(C) VEGF and Collagen I staining indicating vascular structure in co-culture microtissues (Scale bar: 50 µm).

Conclusion

- Acoustic alignment enables controlled spatial organization of cardiac cells in 3D microtissues.
- Multicellular co-culture supports improved tissue structure compared to cardiomyocyte-only systems.
- Aligned constructs maintain high cell viability after fabrication. This approach provides a platform toward more physiologically relevant *in vitro* cardiac models

References

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E: n.khoshdelrad@zmbh.uni-heidelberg.de