Recombinant collagen peptide as 3D myocardial *in vitro* model

D. Mostert, I. Jorba, E.L. Klouda, N.A. Kurniawan, C.V.C. Bouten
Department of Biomedical Engineering & Institute for Complex Molecular Systems, Eindhoven University of Technology

materials-driven regeneration

Mimicking collagen *in vitro*

Collagen is the most abundant protein in many biological tissues, including the myocardial extracellular matrix (ECM), and does not only provide structural support, but also mediates many biological processes *in vivo*. For tissue engineering and disease modeling purposes human ECM is presently mimicked by animal-derived collagen. However, increasing concerns associated with animal derived materials, batch-to-batch variations, and the inability to tune the properties of collagen in a well-controlled manner result in a need for an alternative. Methacrylated Recombinant Collagen Peptide (RCP-MA, Fujifilm) is a novel collagen mimicking peptide based on human collagen I with the following characteristics:

- **Based on human collagen I**, containing no animal-derived components
- Highly **reproducible**
- **Enriched with arginine-glycine-aspartic acid (RGD) sequences** to control cell-adhesive properties
- Modified with methacrylic groups for **well-controlled crosslinking and hydrogel fabrication in a broad range of stiffness**

Figure 1: Material design for the recombinant collagen peptide based on human collagen I. Figure from Cellnest data sheet (Fujifilm).

Research Aim

In this project, we aim to **explore methacrylated recombinant collagen peptide (RCP-MA, Fujifilm) as a 3D hydrogel *in vitro* platform to mimic myocardial ECM**. Using this platform, we aim to assess how increasing stiffness and an increase in the number of cardiac fibroblasts, both happening upon myocardial injury, affect cardiomyocyte contractility.

Approach

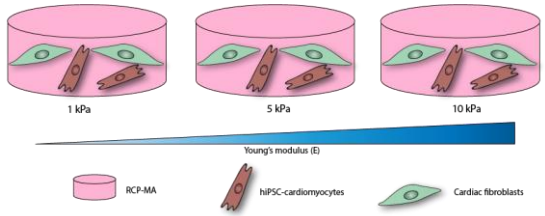

Figure 2: Graphical representation of experimental approach. Cardiomyocytes, derived from induced pluripotent stem cells (hiPSC-CMs), and human fetal cardiac fibroblasts (cFBs) are encapsulated as monoculture or co-culture inside three different types of RCP-MA hydrogels: 1) "soft" (~ 1 kPA), 2), "intermediate" (~ 5 kPa), and 3) "stiff" (~ 10 kPa). Chemical photocrosslinking is achieved using UV illumination at 5 mW/cm² and 365 nm in combination with lithium phenyl 2,4,6-trimethylbenzoylphosphinate (LAP). To assess the effect of cFB incorporation on hiPSC-CM contractility, either a monoculture of hiPSC-CMs was seeded or a co-culture representing the contractile (70% hiPSC-CM : 30% cFBs) or fibrotic (70% cFBs : 30% hiPSC-CMs) cellular environment of the myocardium.

Results

A: Tunability of RCP-MA stiffness

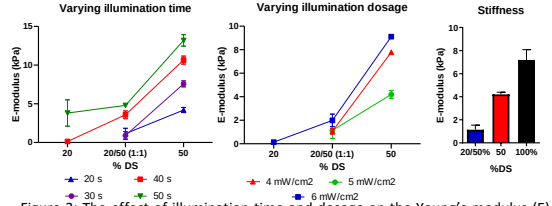

Figure 3: The effect of illumination time and dosage on the Young's modulus (E) of RCP-MA hydrogel constructs. Increasing the the degree of substitution (%DS, methacrylic groups), on the RCP-MA peptides results in increasing E-modulus. Moreover, increasing the time and dosage of UV illumination results in stiffer constructs in a well-controllable manner.

B: Cell viability and co-culture characterization

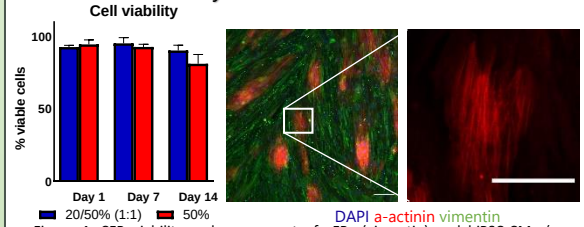

Figure 4: CFB viability and assessment of cFBs (vimentin) and hiPSC-CMs (a-actinin) phenotype encapsulated inside 20/50% RCP-MA assessed by means of immunofluoresence staining. Aligned sarcomere structures were found for hiPSC-CMs, demonstrating a maturing beating apparatus. Scale bar indicates 100 µm.

Conclusion

Our results propose RCP-MA as an attractive alternative to animal-derived collagen for cardiac disease modeling. Taken together, these results will shed light on the importance of stiffness and cardiac fibroblast presence in achieving the physiological behavior of cardiomyocytes *in vitro*.

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