Naturally occurring COL1 fragments induce COL3 formation in 3D PEG hydrogel model with human ventricular cardiac fibroblasts

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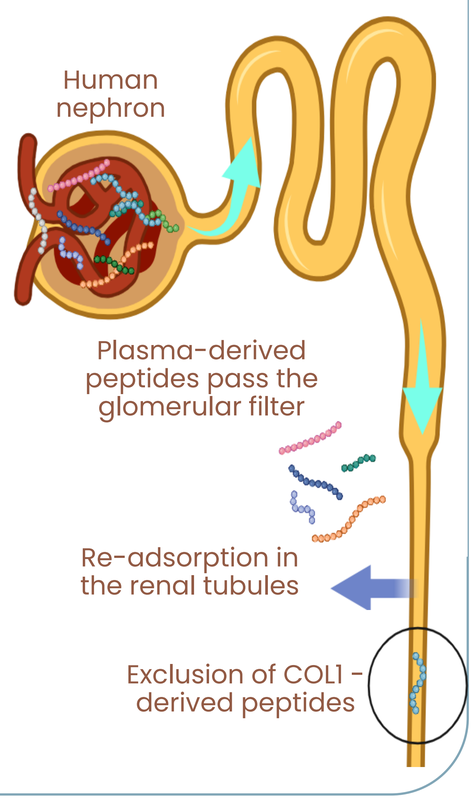
BACKGROUND

- Cardiac fibrosis is a common feature of many cardiovascular diseases and is characterized by activation of cardiac fibroblasts.
- COL3 is one of the ECM proteins affected early during the myocardial fibrotic process and contributes to increased tissue stiffness[1].
- Certain COL1 peptide sequence motifs have been observed to stimulate collagen formation or degradation[2].
- Previous research evaluating the blood and urinary peptidome in healthy individuals using capillary electrophoresis coupled with mass spectrometry demonstrated little overlap and a lack of correlation in peptide abundance between blood and urine. Blood-derived peptides may therefore pass the glomerular filter and are actively re-absorbed in the tubules.
- Exception to this were COL1-derived peptides, possibly due to an unwanted biological function[3]. Based on this work, the top nine most abundant in urine peptides were synthesised.

HYPOTHESIS & AIM

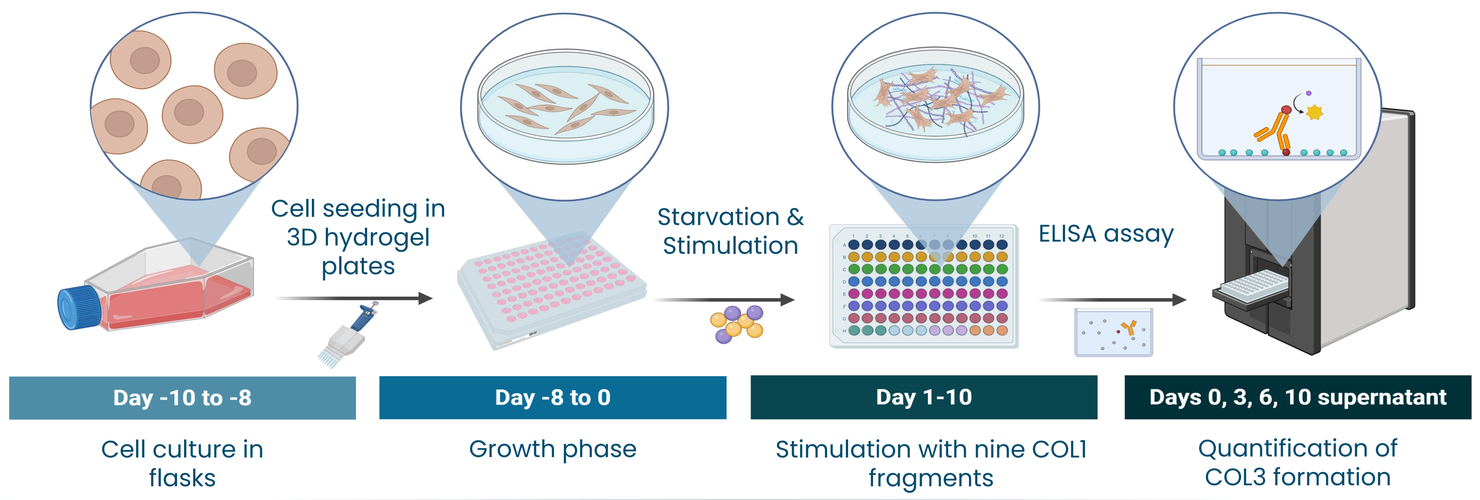
Naturally occurring COL1-derived peptides may possess biological activity and promote COL3 formation by cardiac fibroblasts, thereby contributing to collagen deposition during cardiac fibrosis.

To investigate this hypothesis, the present project examines the effects of nine of the most abundant naturally occurring urinary COL1-derived peptides on COL3 turnover in a 3D in vitro cardiac fibroblast model.

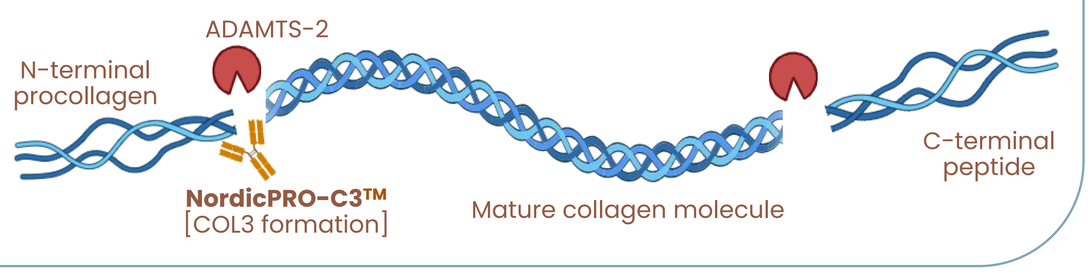


METHODS

Human ventricular cardiac fibroblasts (HCF-v) were cultured in PEG hydrogels for 10 days and stimulated with nine naturally occurring COL-1-derived peptides and/or a cytokine cocktail. COL3 formation was quantified in culture supernatants before stimulation on days 3, 6, and 10 using the NordicPRO-C3™ competitive enzyme-linked immunosorbent assay (ELISA). The data were analysed using a linear mixed effects model with calculation of estimated marginal means.

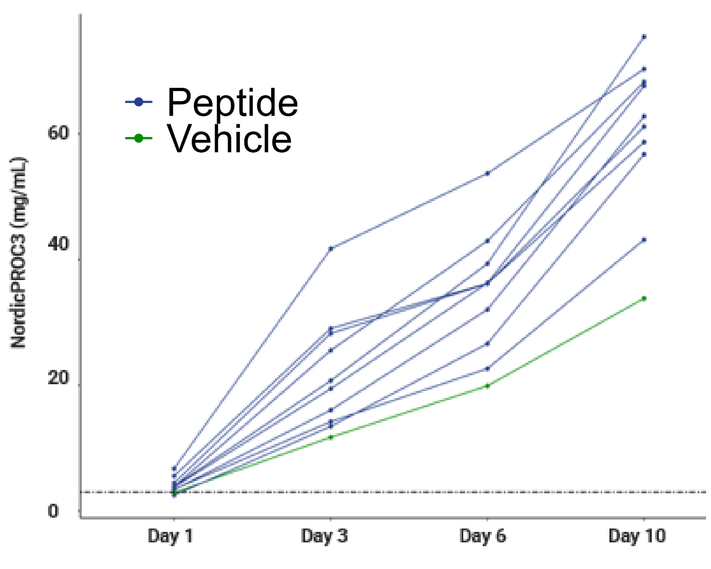


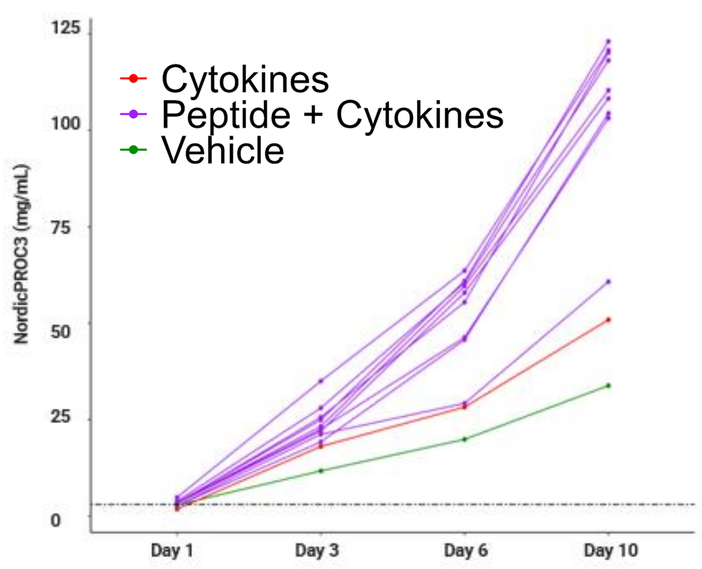
The NordicPRO-C3™ biomarker detects the formation of COL3 by the ADAMTS-2-generated N-terminal pro-peptide of COL3.



RESULTS

Consistent over-time increase of COL3 formation by HCF-v throughout the 10-day stimulation period when stimulated with COL1-derived peptides in a 3D *in-vitro* model





- The nine COL1-derived peptides (blue) increased COL3 formation throughout the 10-day stimulation period compared with the vehicle (green).
- COL3 formation increased further when peptides were combined with cytokine stimulation (purple), compared with both the vehicle control (green) and the cytokine stimulation alone (red).

CONCLUSION

Naturally occurring COL1-derived peptides induced a pro-fibrotic response in cardiac fibroblasts, resulting in increased COL3 synthesis. These findings suggest that COL1 fragments may contribute to cardiac ECM remodeling and warrant further investigation in fibrosis-related cardiomyopathies.

1: Chaher, N. et al. *Non-invasive in vivo imaging of changes in Collagen III turnover in myocardial fibrosis*. npj Imaging 2024 2:1 2, 1–15 (2024)
2: *Devos H et al, A Naturally Occurring Urinary Collagen Type I Alpha 1-Derived Peptide Inhibits Collagen Type I-Induced Endothelial Cell Migration at Physiological Concentrations*. Int J Mol Sci. 2025 Aug 2;26(15):7480. doi: 10.3390/ijms26157480. PMID: 40806611; PMCID: PMC12347341.
3: Magalhães, P. et al. *Comparison of Urine and Plasma Peptidome Indicates Selectivity in Renal Peptide Handling*. Proteomics Clin Appl 12, (2018)

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