Investigating the effect of extracellular pH on the mechano-response of cardiac fibroblasts

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Introduction

Cardiovascular diseases are the primary cause of death worldwide, causing 17.9 million fatalities per year. (World Health Organisation, 2022) Cardiac fibroblasts are a crucial component of the heart, as they oversee myocardial development and maintain homeostasis. When cardiac fibroblasts malfunction and differentiate into myofibroblasts, they trigger a condition called cardiac fibrosis. Cardiac fibrosis refers to the scarring of the heart muscle, which can lead to heart failure and cardiac dysfunction. There are no known treatments for cardiac fibrosis currently, emphasising the need to discover novel therapeutic options to treat this condition.

Studies have shown that as cardiac fibroblasts differentiate into myofibroblasts, the stiffness of the extracellular matrix increases due to an elevated deposition of collagen type I. (Hinderer and Schenke-Layland, 2019) Mechanical properties of cardiac tissue alter in response to changes in the chemical microenvironment during ischemic heart disease. One component known to vary is tissue pH, which falls from 7.4 in healthy tissue to 6.8 in diseased tissue. However, it remains to be determined how this pH decrease affects the ability of cardiac fibroblasts to adapt to changes in tissue stiffness.

Aims

The overall aim of this project was to investigate how mouse cardiac fibroblasts respond to shifts in extracellular pH, which leads towards discovering a method to revert the diseased tissue back into healthy tissue.

Method

Cardiac fibroblasts were extracted from mice and cultivated on six plates of type I coated polyacrylamide gels at stiffness levels of 8kPa and 50kPa, replicating healthy and diseased tissue. The cells were cultured in pH modified medium between pHs 6.3 and 7.3 for several days. Two plates were fixed in situ for confocal microscopy, while the remaining four plates were used for RNA extraction. The RNA was extracted from the cells and used to obtain cDNA via reverse transcriptase. The cDNA was subsequently diluted to 5ng/µl and combined with fibrotic markers to make a reaction mix for real-time PCR, using the SYBR Green assay. Gene expression of the fibrotic markers was quantified, with PPIA functioning as a housekeeping gene for comparative analysis. The two plates used for confocal microscopy were first stained with antibodies so that α-SMA, DNA and the F-actin cytoskeleton, could be visualised.

Outcomes of the project

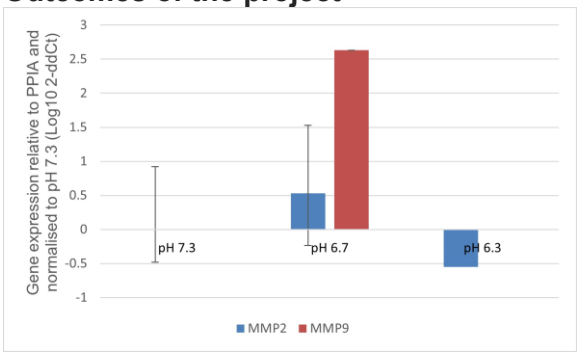

Figure 1. Gene expression of MMP2 and MMP9 relative to PPIA and normalised to pH 7.3. Once the Ct values were obtained from real-time PCR, they were converted into 2-ddCt results and logged for gene expression comparisons. Both MMP2 and MMP9 had an increased expression at pH 6.7 compared to the control of pH 7.3. MMP2 was expressed less at pH 6.3 than pH 7.3.

MMP2 and MMP9 are associated with collagen degradation, which rises during cardiac fibrosis. At the lower pH 6.7, both MMP2 and MMP9 have a higher expression compared to pH 7.3, indicating that they are activated during fibrosis to respond to the increased collagen levels. Conversely, MMP2 exhibits reduced expression at pH 6.3, suggesting that further pH reductions result in MMP2 having lower activity.

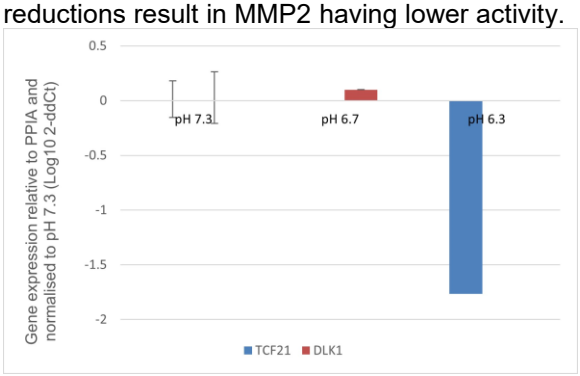

Figure 2. Gene expression of TCF21 and DLK1 relative to PPIA and normalised to pH 7.3. DLK1 was expressed higher at pH 6.7 than pH 7.3. TCF21 had a significantly lower expression at pH 6.3 compared to pH 7.3.

TCF21 regulates the development of cardiac fibroblasts, but its expression decreases as the cardiac fibroblasts differentiate into myofibroblasts at lower pH levels. DLK1 showed a higher expression at pH 6.7, implying that it is expressed more in myofibroblasts. However, contrasting studies suggest DLK1 inhibits cardiac fibroblasts from differentiating into myofibroblasts. (Troncone et al., 2017)

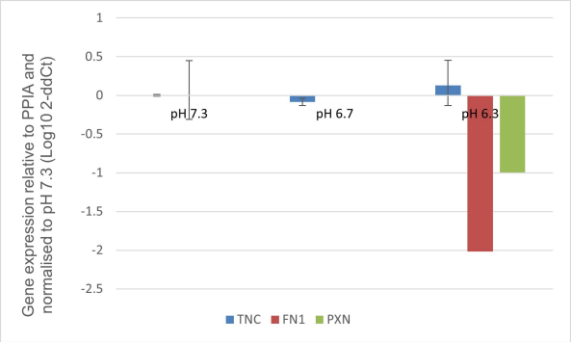

Figure 3. Gene expression of TNC, FN1 and PXN relative to PPIA and normalised to pH 7.3. Compared to the control of pH 7.3, TNC had a lower expression at pH 6.7 and higher expression at pH 6.3. Both FN1 and PXN had lower expressions at pH 6.3. PXN was not expressed at pH 7.3 so was normalised to pH 6.7.

TNC is a regulator for controlling inflammation and tissue repair, so is expressed highly in myofibroblasts. Although expression for TNC was lower for pH 6.7 compared to the control, it increased for pH 6.3. FN1 is crucial for collagen deposition and contributes to the differentiation of cardiac fibroblasts to myofibroblasts. Despite its higher expression in myofibroblasts, the data found that FN1 decreased in expression at pH 6.3. PXN functions as a focal adhesion adaptor protein responsible for recruiting signalling proteins and was only exhibited at lower pH levels. This indicates PXN is upregulated in myofibroblasts, implicating its involvement in one of the signalling pathways during cardiac fibrosis.

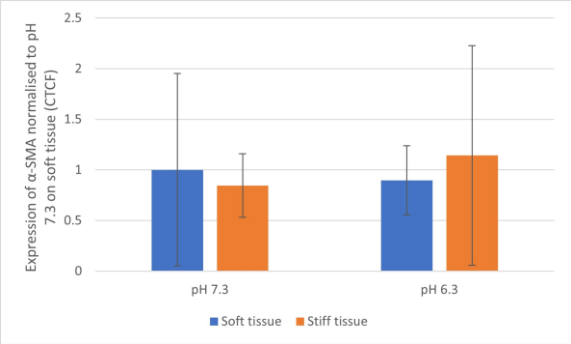

Figure 4. Expression of α-SMA from cells cultured on soft (8kPa) and stiff (50kPa) plates in pH 6.3 and 7.3 modified media. The plates were stained with antibodies and viewed under a confocal microscope under the TXRED filter. The CTCF value was calculated for each well and used to compare the expression of α-SMA, with the results normalised to pH 7.3 on the soft gel.

The protein α-SMA is found in myofibroblasts but not cardiac fibroblasts. The expression was highest in cells cultured on stiff gel at pH 6.3, mimicking diseased tissue. Surprisingly, α-SMA was also expressed highly in cells cultured on soft gel at pH 7.3, which emulates healthy tissue. Despite this, the data does indicate that pH influences the stiffness response.

Impacts of the work

The group will continue to work on understanding the mechano-biology of cardiac fibroblasts. The expression of additional fibrotic markers will be investigated at different pHs in order to develop a further understanding of how extracellular pH impacts cardiac fibroblasts. The outcomes from this project will contribute towards the ongoing pursuit of a treatment for cardiac fibrosis. Furthermore, research from this studentship contributes to the broader interests of the molecular bioscience community, aligning with the Biochemical Society's strategy. Over the last two decades, mechanotransduction has been a topic of interest to researchers and is involved in numerous biological responses. Less is known of how chemical pathways influence mechanotransduction, called mechano-chemical signalling. We investigated how both extracellular pH and substrate stiffness influence the mechano-response of cardiac fibroblasts, utilising a combination of qPCR and confocal microscopy to assess cellular changes and measure gene expression. This enables further research into how different chemical pathways affect mechanotransduction. The results of this experiment are applicable beyond cardiac biology because factors such as ageing and disease can alter the pH and mechanical properties of numerous tissues.

Subject-specific and transferable skills gained and contribution to future career plans

Working on this project has provided me with invaluable work experience and allowed me to learn and develop different skills. Among the new laboratory techniques that I learnt were RNA extraction, RNA reverse transcription, qPCR, preparing pH modified culture media and confocal microscopy, which has significantly prepared me for my final year project at university. The experience has helped me to develop my critical thinking and data analysis abilities, particularly in the context of interpreting real-time PCR results and drawing meaningful conclusions. Working in a group has boosted my confidence and taught me how to effectively communicate within a research team. Additionally, I learnt how to manage my time effectively and how to maintain organisation when independently culturing cells in the lab. I really enjoyed the experience gained from this studentship and this has reinforced my desire to pursue a PhD in biochemistry.

Acknowledgements

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References

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