Investigating the mechanisms of human fibrosis across organ systems

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Project outline

Background: Fibrosis is a pathological process associated with a myriad of human diseases that cuts across organs systems; for example, lung, liver, heart, skin, mediastinum and bone marrow. Formation of fibrous tissue during disease can result in scar formation as a default wound healing process. This is an attempt to maintain tissue integrity following insult but also a barrier to healthy organ function and cell and tissue restoration. Following a heart attack for example, the formation of a non-contractile scar results in loss of function, pathological tissue remodelling, and ultimately leads to heart failure, a major cause of death both in the UK and worldwide. Similarly, in the liver excessive deposition of extracellular matrix induced by chronic inflammation can lead to cirrhosis, portal hypertension and liver cancer. Currently we lack novel methods to modulate fibrosis and scarring as an important prerequisite to conditioning the local injury environment and allowing regenerative strategies to prevent pathological progression of diseases such as heart failure and non-alcoholic steatohepatitis.

Hypothesis: A better understanding of both common and organ-specific fibrotic mechanisms and their interplay will provide significant insight towards the translation of anti-fibrotic therapeutic targets.

Description of work to be undertaken: A series of carefully designed, integrated cell-based assays combined with live imaging of primary human fibroblasts from distinct organs (heart and liver) will be developed to quantitatively assess parameters that influence fibrosis. A significant advance in this respect will be provided by the engineering of organ-specific human fibroblasts for live imaging of collagen dynamics through the insertion of a GFPtpz fluorophore into the alpha2(I)-procollagen N-terminal propeptide to generate a reporter-tagged collagen fusion (as has been carried out successfully in an in-house mouse model). The Col1-GFPtpz fibroblasts will be subjected to scratch wound assays, Boyden chamber migration and collagen gel experiments, to gather quantitative information on cell proliferation, migration and transdifferentiation (adoption of a myofibroblast fate), together with collagen deposition rates, density and alignment of fibres. The response to inflammatory, pro-fibrotic and anti-fibrotic mediators will be tested under three different treatments: (i) a pro-inflammatory cocktail; (ii) a reparative/pro-fibrotic cytokine cocktail; and (iii) anti-fibrotic treatment with neutralizing antibodies. Any differences in fibroblast behaviour associated with organ-of-origin (heart v liver) will be monitored, and real-time cell tracking will be used to profile how cells respond to various treatment conditions and whether there are variations in their behaviour depending on origin. The collagen network will be segmented and multi-parametric profiling used to quantify collagen dynamics. Comparison between

these data will provide novel insights into both distinct and overlapping mechanisms driving cardiac and hepatic fibrosis, and how they might be controlled to condition the injury environment and allow regenerative strategies to prevent disease progression. sc-RNA-Seq profiling will be used to identify signalling pathways that are activated to alter collagen dynamics in response to inflammatory/pro-fibrotic cues and validate candidates to identify either cross-organ or organ-specific nodal factors for genetic or pharmacological targeting to moderate the fibrotic response. An example of a phenotypic read-out here will be altered collagen dynamics, for example, from stable aligned fibres to a basket weave transient fibre network. The experimental data on fibroblast behaviour and collagen dynamics will be used to drive the development and calibration of mechanistic mathematical models. This will leverage an existing interdisciplinary collaboration whereby the NNRCO fellow will interact directly with modellers in the Baker/Waters groups. The models will assist in identifying the key cellular mechanisms driving the fibrotic response and be designed to predict the outcome of genetic or pharmacological intervention.

Expected value of the results: This fellowship will provide detailed spatial and temporal maps of human fibroblast cell behaviours and collagen dynamics during wound healing responses and will identify common and/or organ-specific fibrotic signatures that map onto distinct disease indications. This represents an essential first step towards understanding the mechanisms of fibrotic wound healing and how it might be targeted therapeutically, either between organs or in an organ-specific manner. The experimental data of this fellowship will be leveraged to support an existing collaboration that seeks to generate a predictive mathematical model of the fibrotic response. The longer-term goal is for the model to act as a triage for genetic/chemical screening of putative modulators of fibrosis and anti-inflammatory agents, prior to pre-clinical animal experimentation, and the development of anti-fibrotic therapeutics to treat disease.

Supervisor's recent relevant publications:

Ruth Baker

1. O. M. Matsiaka, **R. E. Baker**, E. T. Shah and M. J. Simpson. Mechanistic and experimental models of cell migration reveal the importance of intercellular interactions in cell invasion. *Biomedical Physics and Bioengineering Express* 5:045009 (2018).
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3. M. J. Simpson, **R. E. Baker**, S. T. Vittadello and O. J. Maclaren. Parameter identifiability analysis for spatiotemporal models of cell invasion. *Journal of the Royal Society Interface* 17:20200055 (2020).
4. T. P. Prescott, K. Zhu, M. Zhao and R. E. Baker. Quantifying the impact of electric fields on single-cell motility. *Biophysical Journal* 120(16):3363-3373 (2021).
5. S. Martina-Perez, H. Sailem and **R. E. Baker** (2022). Efficient Bayesian inference for mechanistic modelling with high-throughput data. *PLOS Computational Biology* 18(6): e1010191.

Paul Riley

1. C. Villa del Campo, […] and **P. R. Riley**. Regenerative potential of epicardium-derived extracellular vesicles mediated by conserved miRNA transfer. *Cardiovascular Research* 118(2):597-611 (2021).
2. F. C. Simões*, T. J. Cahill*, […] and **P. R. Riley**. Macrophages directly contribute collagen to scar formation during zebrafish heart regeneration and mouse heart repair. *Nature Communications* 11(1):600 (2020).
3. J. M. Vieira, S. Norman, […] and **P. R. Riley**. The cardiac lymphatic system stimulates resolution of inflammation following myocardial infarction. Journal of Clinical Investigation 128:3402-3412 (2018).

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5. N. Smart, S. Bollini, […] and P. R. Riley. De novo cardiomyocytes from within the activated adult heart after injury. Nature 474:640-644 (2011).

Maxwell Ruby

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2. **Ruby MA**, Massart J, Hunderosse DM, Schönke M, Correia JC, Louie SM, Ruas JL, Näslund E, Nomura DK, Zierath JR. Human Carboxylesterase 2 Reverses Obesity-Induced Diacylglycerol Accumulation and Glucose Intolerance. Cell Rep. 18:636-646, 2017.
3. Kirchner H, Sinha I, Gao H, **Ruby MA**, Schönke M, Lindvall JM, Barres R, Krook A, Näslund E, Dahlman-Wright K, Zierath JR. Altered DNA methylation of glycolytic and lipogenic genes in liver from obese and type 2 diabetic patients. Mol Metab. 5:171-183, 2016.
4. **Ruby MA**, Goldenson B, Orasanu G, Johnston TP, Plutzky J, Krauss RM. VLDL hydrolysis by LpL activates PPAR-α through generation of unbound fatty acids. J Lipid Res 51:2275-2281, 2010.
5. **Ruby MA***, Nomura DK*, Hudak CS, Mangravite LM, Chiu S, Casida JE, Krauss RM. Overactive endocannabinoid signaling impairs apolipoprotein E-mediated clearance of triglyceride-rich lipoproteins. Proc Natl Acad Sci USA 105:14561-14566, 2008 *contributed equally to this work

Sarah Waters

1. M. A. Ellis, M. P. Dalwadi, M. J. Ellis, H. M. Byrne and **S. L. Waters**. A systematically reduced mathematical model for organoid expansion. F*rontiers of Bioengineering and Biotechnology* 9:446 (2021).
2. **S. L. Waters**, L. J. Schumacher and A. J. El Haj. Regenerative medicine meets mathematical modelling: developing symbiotic relationships. *Nature Regenerative Medicine* 6:1-8 (2021).
3. E. F. Yeo, H. Markides, A. T. Schade, A. J. Studd, J. M. Oliver, **S. L. Waters** and A. J. El Haj. Experimental and mathematical modelling of magnetically labelled mesenchymal stromal cell delivery. *Journal of the Royal Society Interface* 18:20200558 (2021).
4. M. J. Chen, J. P. Whiteley, C. P. Please, A. Schwab, F. Ehlicke, **S. L. Waters** and H. M. Byrne. Inducing chondrogenesis in MSC/chondrocyte co-cultures using exogenous TGF-β: a mathematical model. *Journal of Theoretical Biology* 439:1-13 (2018).
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