Project Details	
Project Code	MRCIIAR24Br RichardsonR
Title	Investigating the role of extracellular vesicles in influencing inflammation during tissue regeneration in zebrafish
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Zebrafish have a remarkable ability to regenerate damaged tissues. Tissue repair and regeneration requires communication between multiple different cell types including immune cells, fibroblasts and endothelial cells. Extracellular vesicles (EVs) deliver molecular messages between cells and our data suggests they promote regeneration. This project will determine how EVs might be harnessed to facilitate optimal repair of damaged tissues via influencing inflammation.
Description	Small (30-1000nm) lipid bound extracellular vesicles (EVs) are showing promise as biomarkers and potential therapeutic avenues for multiple diseases including cancer and cardiovascular disease. EVs act as protective vehicles for molecular messages and facilitate communication between cells across extracellular space. EV numbers are elevated following a myocardial infarction and it has been suggested that they can play roles in immunomodulation and neovascularisation during repair (1,2). However, due to the complexity of labelling and tracking these small vesicles there is much to be learnt about EV function in vivo. A previous PhD student in the lab has established a zebrafish model that allows us to identify and isolate endogenous, cell type specific EVs, revealing transfer between cardiovascular cell types and providing the unique opportunity to determine EV function during endogenous regeneration (3). Proteomics of cardiac EVs extracted after injury reveals enrichment of inflammatory and wound healing mediator cargos (including complement proteins, granulin and fibronectin), both early after injury and at the onset of regeneration (unpublished data). This preliminary data suggests that EVs may play a role in modulating inflammatory cells and pathways, processes we have previously shown to be important for cardiac regeneration (4). The overall aim of this project is to further understand the role of this transfer of EV cargos during cardiac repair and regeneration. We hypothesise that cargo transfer to immune cells increases after injury and can have immunomodulatory effects, promoting regeneration. This project will provide excellent training opportunities to develop in vivo skills in a regenerative model, flow cytometry, molecular and 'omics analyses of tissue samples as well as sophisticated confocal and super resolution microscopy. Objectives: 1 – Prep period and first 3 months of project. Perform comparative analyses with data from mouse (5) and human (6) cardiac EVs to identify which is the most promising regeneration associated cargo (Opportunity for the student to steer the project). 2 – Year 1. Determine the cell types producing and receiving these cargo carrying EVs via flow cytometry and imaging of the heart. Our proteomics data is from total cardiac EVs but by using our strategy to transgenically label cell types and their EVs (3) we can determine the cells that are sending and receiving these cargos. Candidates we can already assess include neutrophils, macrophages, endothelial cells and cardiomyocytes. 3 – Year 2. Confirm and extend the evaluation of cardiac EV cargo by assessing RNAs carried by EVs with guidance from

	the 2nd supervisor and industrial partner. 4 – Years 2-4. Characterise cardiac injury repair and regeneration in zebrafish lacking proteins carried by EVs. For example, complement components (e.g. C3 and C5) are enriched in our proteomics dataset but the role of this inflammatory pathway hasn't been studied during regeneration. Mutants for these targets can be generated via CRISPR/Cas9 technology (Opportunity for the student to steer the project). References 1. Akbar N, et al., (2017). JCI Insight. 2(17): e93344 2. Beltrami C, et al., (2017). Mol Ther. 25(3): 679-693. 3. Scott A, Sueiro Ballesteros L, Bradshaw M, Tsuji C, Power A, Lorriman J, Love J, Paul D, Herman A, Emanueli C, Richardson RJ. (2021) In Vivo Characterization of Endogenous Cardiovascular Extracellular Vesicles in Larval and Adult Zebrafish. ATVB. 41(9): 2454-2468 4. Bevan L, Lim ZW, Venkatesh B, Riley PR, Martin P, Richardson RJ. (2020) Specific macrophage populations promote both cardiac scar deposition and subsequent resolution in adult zebrafish. Cardiovasc Res. 116(7):1357-1371. 5. Claridge B, et al., (2021). Proteomics. 21(13-14):e2100026 6. Leitolis A, et al., (2019). Int J Mol Sci. 20(6):1279
Supervisory Team	
Lead Supervisor	
Name	Dr Rebecca Richardson
Affiliation	Bristol
College/Faculty	Faculty of Life Sciences
Department/School	School of Physiology, Pharmacology and Neuroscience
Email Address	rebecca.richardson@bristol.ac.uk
Co-Supervisor 1	
Name	Dr Aled Clayton
Affiliation	Cardiff
College/Faculty	Division of Cancer & Genetics
Department/School	School of Medicine
Co-Supervisor 2	
Name	
Affiliation	
College/Faculty	
Department/School	
Co-Supervisor 3	
Name	
Affiliation	
College/Faculty	
Department/School	