Biochemical society report

Introduction to the Project's Aims and Objectives:

The scope of our project evolved from a broad exploration of ideas to a more specific focus due to the mounting evidence pointing to a significant association between the detrimental effect of iron accumulation on endothelial cell function and cardiovascular and neurodegenerative diseases. The hosting lab has shown that a transmembrane protein called Neuropilin-1 (NRP1) suppresses iron-dependent oxidative stress and senescence in endothelial cells. Thus, our primary aims were two: firstly, to gain further evidence on the role of exogenous iron in endothelial cell senescence in view to understand the contribution of iron overload in endothelial cells to the development of cardiovascular disease and neurodegeneration. This direction was guided by previous research that highlighted elevated iron levels as a common feature in both cardiovascular and neurodegenerative diseases, by a recent study from the hosting lab showing that NRP1 has osteoprotective and anti-inflammatory function, and by preliminary data showing that iron modulates NRP1 expression. Secondly, we sought to investigate the mechanism on how iron modulates NRP1 expression. This investigation aimed to shed light on whether Neuropilin-1 could serve as biomarker or a potential therapeutic target for diseases linked to iron accumulation.

Summary of the Work Undertaken:

Over the course of my six-week internship in the laboratory, I acquired a wealth of valuable techniques and skills that will be pivotal for my future endeavours, especially as I aspire to pursue my own research. Coming from a background with limited prior laboratory experience, I leave this internship equipped with both knowledge and practical expertise. My learning journey encompassed several key areas:

1. Culturing and Splitting Primary Endothelial Cells: I gained proficiency in culturing and accurately splitting primary endothelial cells. This encompassed tasks such as the precise handling of cell cultures media preparation, dilution calculations, preparation of cell lysates for protein and RNA studies, and immunostaining. Importantly, these skills will allow me to confidently work with any cell type and to perform cell-based in vitro assays in future projects.
2. Experimental Planning and Design:

Collaborating with Dr Raimondi, I actively participated in the planning and design of our experiments. We introduced dose-dependent treatments by subjecting a group of cultured cells to iron treatment in growth media while maintaining another group in control, untreated growth media.

3. Immunostaining Techniques: I performed immunostaining procedures, whereby cells were labelled with specific antibodies to detect Neuropilin-1 and other proteins used as controls. This allowed us to assess the impact of iron levels on treated cells.
4. Reverse Transcription and qPCR: I acquired proficiency in RNA purification, reverse transcription to generate cDNA and in operating a Real Time qPCR operation. This included interpreting quantitative data and deriving meaningful conclusion from the results.

Description of Results/Outcome of the Project:

Our research showed compelling results with significant meaning. The most noteworthy discovery was that cells subjected to iron treatment exhibited heightened expression levels of NRP1. This finding raises intriguing questions about the potential for therapeutic interventions targeting Neuropilin-1 to mitigate the incidence of cardiovascular and neurodegenerative diseases.

During my internship, I not only achieved significant milestones but also confronted challenges and learned from my experiences. One such challenge was the realization of the toxic nature of DMSO when towing the cells, which should be quickly removed or diluted with sufficient media to avoid cellular stress or cell death.

Impact of the Work/Results:

The work we conducted is important for the vascular field as it can open for new research into Neuropilin-1 and new in vitro and in vivo testing for ways it can affect human cells and what diseases it can be associated with. The potential societal benefit of our research is substantial. As the global burden of dementia and cardiovascular diseases continues to grow, identifying modifiable risk factors becomes paramount. Our work suggests that targeting iron accumulation in endothelial cells may offer a novel therapeutic approach and that NRP1 could be a biomarker and a therapeutic target. If successful, this could translate into interventions that reduce the incidence and severity of both cardiovascular diseases and neurodegenerative disorders. Ultimately, our findings have the potential to enhance the quality of life for individuals at risk of these debilitating conditions.

Contribution of the Skills/Studentship to Future Career Plans/Goals:

The laboratory skills I acquired during my internship hold significant value for my future research aspirations, particularly in senescence and immunology. Proficiency in cell handling, immunostaining, PCR and cell culture techniques equips me with essential tools for my Ph.D. studies, facilitating in-depth exploration of cellular processes. These skills are complemented by transferable attributes, such as attention to detail and effective time management, which enhance my ability to contribute meaningfully to scientific research and to multitasking, collaborate effectively, and address complex challenges in a dynamic research landscape. This research has expanded my horizons, and I am eager to continue my journey in scientific inquiry and contribute to the pursuit of solutions for complex health issues.

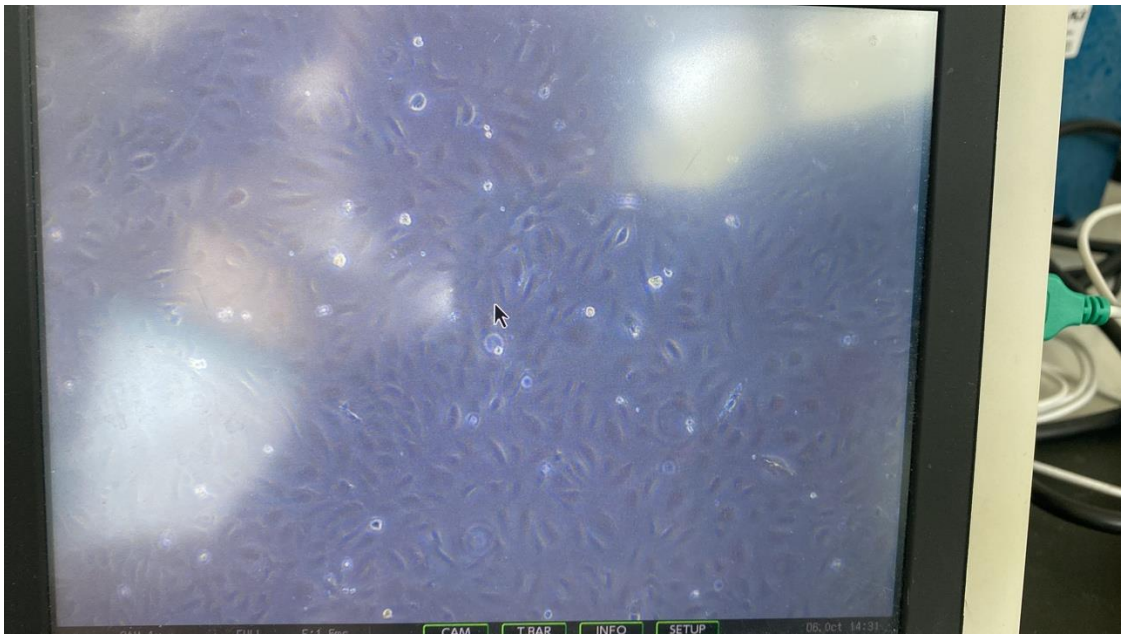

Figure 1: one of my attempts in culturing HUVEC cells