School of Postgraduate Studies  
Higher Degree Project Description Template

| Research theme     | Cardiovascular Disease  
|                   | Inflammation            |
| Supervisor        | Dr Sarah O’Neill        |
| Web page          | https://research1.rcsi.ie/pi/soneill/ |
| Title of PhD project | Platelet reactivity in a changing redox environment: implications for thrombus formation in disease states |

**Brief project description**  
In many disease states an imbalance between oxidants and anti-oxidants exists. Oxidative stress is manifested as this imbalance and is implicated in the pathogenesis and development of cardiovascular diseases. This precipitates an altered redox status in the vasculature contributing to endothelial dysfunction with subsequent effects on normal platelet function. The consequence is a loss of the production of the anti-thrombotic agents of nitric oxide (NO) and prostacyclin from endothelial cells. Together with this reduced bioavailability of NO and an increase in oxidants such as superoxide, inappropriate thrombus formation occurs resulting in myocardial infarction or stroke. The redox environment in the extracellular milieu is maintained through the existence of thiol/disulphide redox couples. These couples are referred to as ‘redox control nodes’ and include reduced and oxidised glutathione (GSH/GSSG) as well as reduced and oxidised cysteine (Cys/CySS). Taken together, these two redox couples comprise the predominant low molecular weight thiol/disulphide pool in human plasma. In normal platelet activation, redox changes do occur but the introduction of an imbalance in redox processes, in certain settings, contributes to disease states that are pro-thrombotic. Cysteines act as “redox switches”, sensing and reacting to changes in the surrounding environment, and therefore represent a key target for signalling cascades. The concept of redox regulation as a dynamic signalling system in both mammalian and bacterial cells has been emerging over the past number of years. In previous work, we established integrins have an endogenous thiol isomerase activity (1). Furthermore, we demonstrated that redox modulation of the platelet specific integrin αIIbβ3 involved an allosteric regulation of this enzymatic activity (2). Our confirmation that S-nitrosylation of αIIbβ3 can also modulate the activation state of the integrin in the intact platelet, revealed the interplay between redox and NO in regulating platelet function (3). However, the precise mechanisms in vivo resulting in redox modification of proteins are not clear. Therefore, in this proposal we will explore how the interplay of key redox regulators, such as glutathione, with NO, affects platelet function and impact on thrombus formation. Redox regulation of the platelet is crucial to the modulation of its function. Redox sites are present on the surface of the platelet in the form of thiols and disulphides. The redox state of such sites can impact on platelet function as it does in other biological processes such as integrin-mediated adhesion, HIV entry into the cell and receptor shedding. The regulation of platelets by redox and NO is, therefore, an important biomedical issue. A thorough understanding of these mechanisms and how they interact with other platelet signalling events is key for the development of novel therapeutic targets for the treatment of cardiovascular disease. This proposal for a PhD thesis, therefore, focuses on addressing the important issues of how the interplay of NO with redox regulators in the extracellular environment impacts on platelet function and the implications for thrombus formation in disease states.

**The objectives of this proposal are**
1) To assess the impact of an external redox environment on platelet function with the aim of evaluating the reactivity of the platelet and thrombus formation in such conditions.

2) To elucidate the mechanisms underlying the regulation of redox sensitive platelet proteins.

3) To examine the effects of an external redox environment on activated endothelial cells in the presence of platelets to elucidate the interplay of NO with this changing redox environment on platelet function.

References


Skills & techniques

Skills

- Project and time management
- The ability to communicate their research by
  - Oral communication
  - Written word
- Understanding the intricacies of experimental design
- Dealing with data and statistical analysis
- Proficiency in the critical assessment of current biomedical research
- Management of a small research consumable budget
- An understanding of health and safety issues in the laboratory

Techniques

- The isolation of platelets from human blood
- Light transmission platelet aggregometry
- Cell culture
- Flow cytometry
- Adhesion assays
- Western blot analysis
- Immunoprecipitation assays
- SiRNA assays
- Microscopy

It is anticipated the assessment of platelet function under conditions of redox stress could yield a novel method of predicting thrombotic events if brought to a microdevice level.