**Title:**

Local Anticoagulation via Gene Therapy: Towards Prevention of Cardioembolic Stroke

**Aim:**

Cardiac thromboembolism persists as the culprit behind most ischemic strokes; a majority arising from the left atrial appendage. In order to combat this disease and overcome limitations of systemic oral anticoagulants, we aimed to develop a novel anticoagulant viral vector to focally target cardiac regions of increased thrombotic potential, thereby presenting an alternative for long-term anticoagulation.

**Method:**

The anticoagulant phenotype of these vectors was functionally assessed through in vitro and ex vivo platforms. For in vitro testing, a novel, cell-based version of the calibrated automated thrombogram assay, coupled with the overall haemostatic potential assay were utilised to determine thrombokinetics in endothelial cell culture models. For ex vivo testing, freshly isolated and characterized porcine left atrial appendage endothelial cells were used. These cells were used to endothelialise microfluidic devices, which were stimulated with TNF-α prior to perfusion with human whole blood. Changes in fibrin, platelets and neutrophils were investigated in the presence and absence of our novel anticoagulant viral vector.

**Results:**

Experimental findings from our *in vitro* work showed a significant\* decrease in the endogenous thrombin potential of vector-transduced cells, coupled with a significant\* decrease in the velocity of thrombogenesis. Furthermore, transduced cells had a marked\* decrease in the peak rate of thrombin generation and a significant\* increase in the time taken to achieve peak thrombin generation. Experimental findings from our *ex vivo* work highlighted significant\* reductions in fibrin and platelets within the microfluidic model, as well as notable decreases in neutrophil populations.

\*: p < 0.0001

**Conclusion:**

These findings show the effects of our anticoagulant vectors in inhibiting thrombosis and help to guide the development of focal gene therapy strategies to target thrombosis at its point of origin in the prevention of cardioembolic-stroke