Modelling and elucidating leukocyte-endothelial interactions in ex-vivo organ perfusion

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Introduction and background

- Ex-vivo lung perfusion (EVLP) provides a means of expanding the current donor pool available for transplant in the case of end-stage organ disease
- Previous research by our group has indicated the importance of IL-1β in determining the transplant success of perfused lungs and highlighted its mechanistic importance *in vitro*
- This project sought to establish a working *ex vivo* model of neutrophil tracking within the vasculature to validate these observations

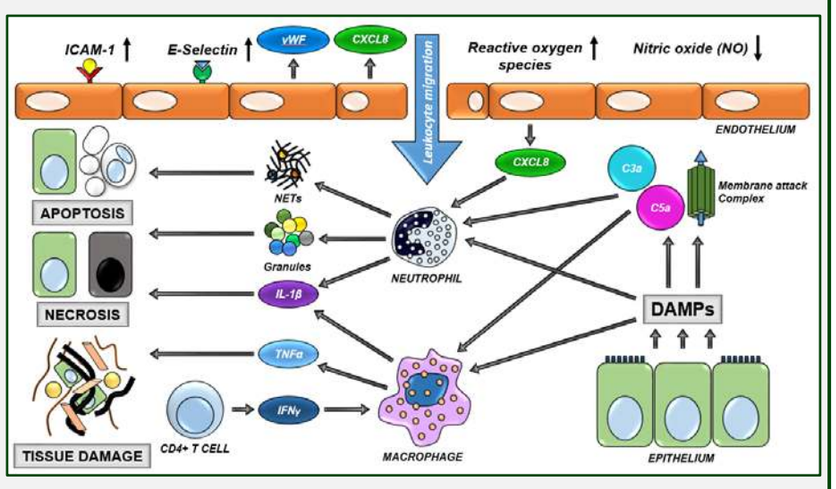
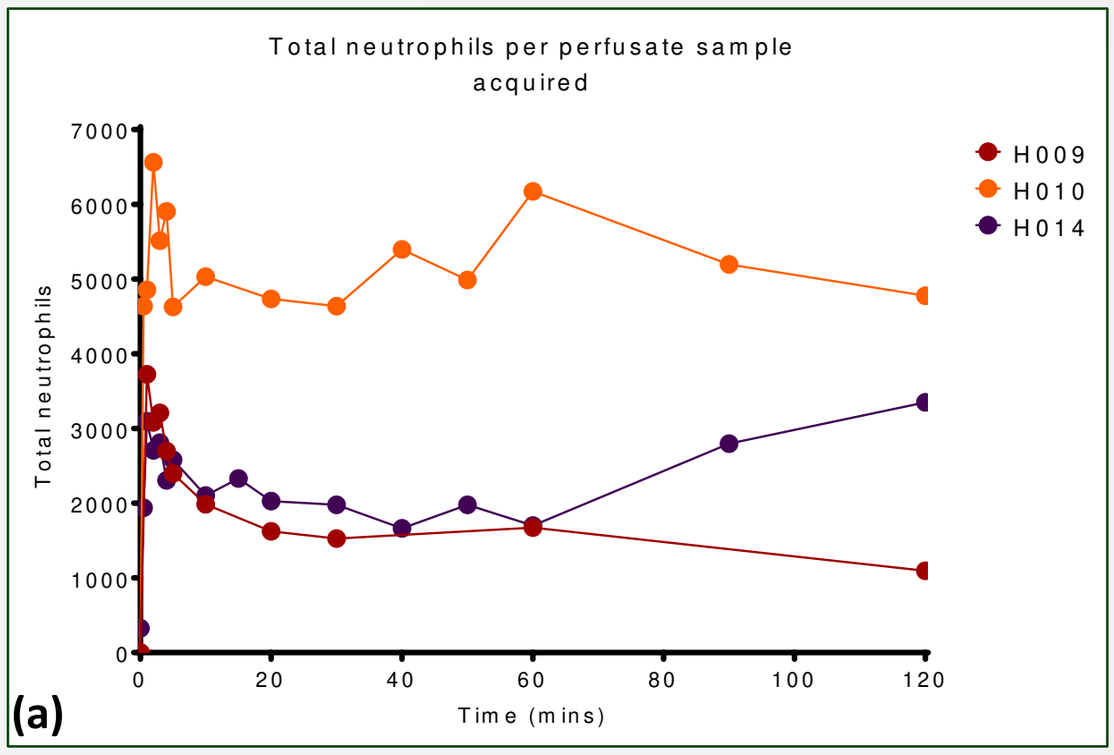


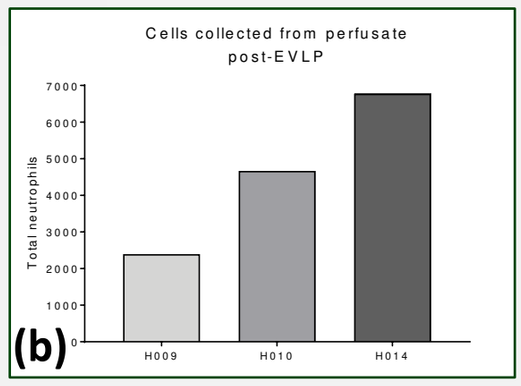
Fig. 1 – Ischaemia-reperfusion injury provokes endothelial dysfunction
This facilitates extravasation of circulating leukocytes out of the vasculature and into the tissues

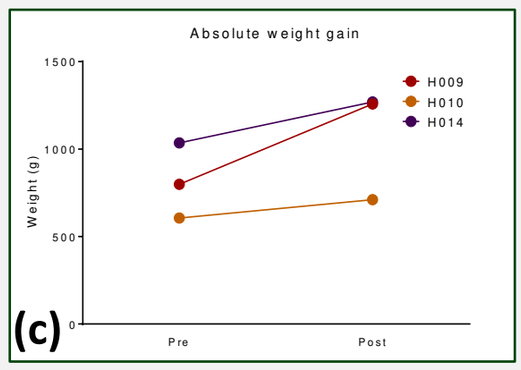
Project aims

1. Utilise developed model of ischaemia-reperfusion injury (IRI) using EVLP to assess the effect of IL-1β neutrophil adhesion during reperfusion injury. This will initially use whole EVLP before moving across into paired split-EVLP
2. Analyse perfusate and tissue samples to observe the correlation of lung function with endothelial and neutrophil cell activation

Results







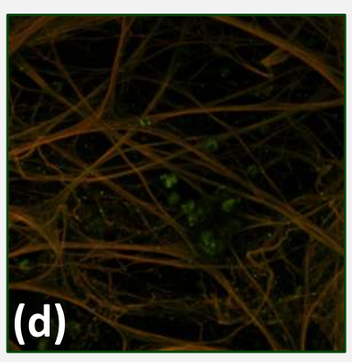
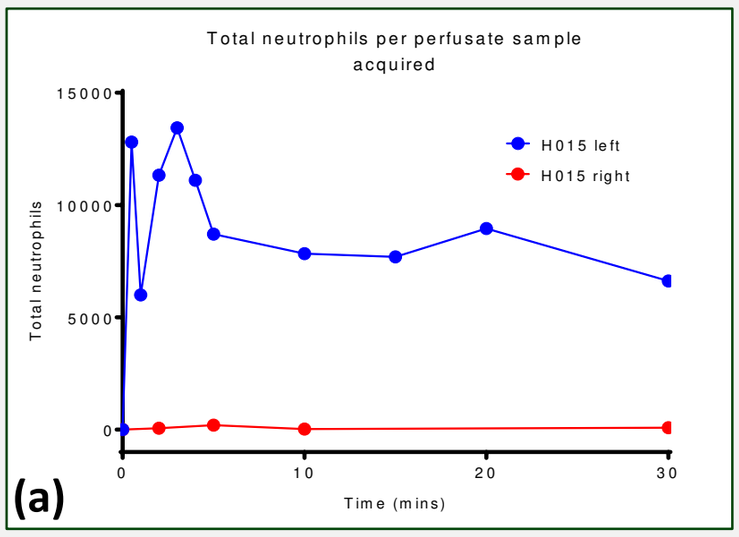


Fig. 3 – Establishment of neutrophil tracking model in EVLP
- $9x10^6$ CFSE-labelled neutrophils infused into perfusion circuit at 'T0.' Detectable in perfusate at regularly acquired time points of perfusion *ex vivo* (a) and in perfusate filtered post-perfusion (b). A higher number of cells suggests reduced infiltration into the tissues in conjunction with a greater degree of weight gain (c).
- Neutrophils (green) imaged in PFA-fixed tissue post-perfusion (d), indicating infiltration into the tissue during EVLP. Alveolar walls are also visible (orange). Imaged via dual-photon microscope (Zeiss LSM 880)



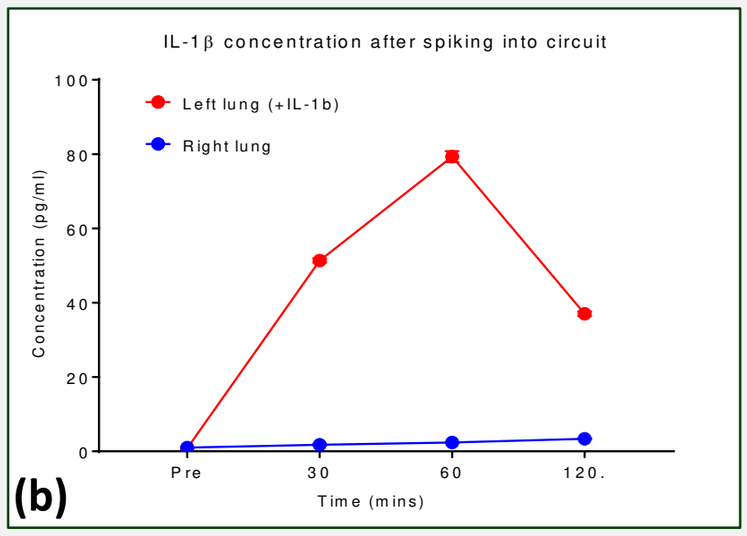
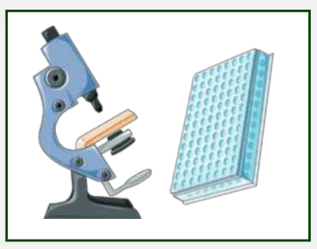


Fig. 4 – Simultaneous dual single lung perfusions
- Neutrophils infused into one lung of a pair perfused simultaneously (a) indicates CFSE+ neutrophil detection whilst absent in the lung with no infusion
- IL-1β is detectable in perfusate once infused but not to the same level in control lung (b)

Methods



Analysis of perfusate and tissue samples/imaging

Quantification of CFSE+ neutrophils in perfusates

Fig. 2 – Dual EVLP circuits. Study used whole EVLP (*n=3*) and dual single lung perfusions (*n=2*)

Discussion

- A simultaneous paired split-EVLP model has been developed and optimised – enabling direct comparison of intervention with control
- CFSE-labelled neutrophils are detectable in perfusate and in imaged tissue post-perfusion

Future work

- Utilise model to test effect(s) of adding IL-1β into perfusion circuit on lung physiology and function, as assessed by the outputs measured as part of this work

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Blood and Transplant Research Unit in Organ Donation and Transplantation

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