FESSH
Federation of European Societies for Surgery of the Hand

FESSH Interim Research Grant Progress Update

Title of project: Optimisation Of An Ex-Vivo Limb Perfusion Protocol For Composite Tissue Transplantation

Name of applicant: Kavit R Amin

Position: Clinical Fellow in Plastic Surgery

Academic degree: MBBS, BSc (Hons)

Institution: University of Manchester (UK)

Primary investigator (if other): James E Fildes
Principal investigator at the Manchester Collaborative Centre for Centre for Inflammation Research (MCCIR), NHS Principal Research Scientist at the Transplant Centre, Manchester Foundation Trust.

Date of award	December 2018
Date of report	2nd October 2019
Grant Awarded	€10,000

Summary of progress /findings

The first successful limb transplantation was reported over 20 years ago, yet no major imporvements to the graft preservation protocols have occurred. The donor limb is removed and the vessels flushed with cold preservation solution (most commonly UW, described over 60 years ago), and then submerged in ice. Despite cooling to minimise tissue injury, the metabolic requirements of tissues does not cease [1]. Ex-vivo normothermic perfusion offers a dynamic and physiological method of preservation, and has been successfully applied in our lab for heart, lung, kidney and more recently limb preservation.

The aims of this proposal were to:

(i) Optimise the preservation of limb allografts for transplantation. This will be carried out by comparing a bespoke physiological ex-vivo normothermic machine perfusion protocol (EVNP) with the gold standard of cold static storage (CSS).

(ii) Utilise EVNP as a platform to test and gain novel insight into reperfusion injury with a view to developing novel strategies that effectively mitigate tissue injury after revascularisation.

Progress to date

Forelimbs from landrace pig were obtained from an abattoir (schedule 1 procedures, Home Office Project Licence 40/3487). All experiments have now been carried out and currently the main focus is a more scientific evaluation of the samples obtained. As previously outlined, forelimbs from the same animal have been compared (n=10 forelimbs in total). For each experiment, limbs after preservation flush and handling as previously described in the grant were randomly assigned to 6hrs EVNP or 6hrs CSS. After this time period, limbs underwent 4hrs reperfusion (38°C) with whole blood on fresh circuits to mimic reperfusion injury. The data for the reperfusion part only will be discussed in this initial report but there is data specifically for the 6hr EVNP limb as well with its own readouts. This can be shown more clearly for the purposes of the final report.

Group testing out reperfusion studies in the early stages

Amendment of lab layout to enable paired comparisons of EVNP and CSS limbs

Analytical outputs include (Marked in green are completed, marked in blue are still under evaluation):

(i) Real-time haemodynamics will be recorded. Biochemical blood gas analysis (*pH, pCO2, pO2, Na, K, Cl, Ca, HCO3, glucose, and lactate*) will be routinely performed. Vascular integrity will be examined (*skin colour, temperature and capillary refill*).

~~(i)~~(ii) Infrared technology (FLIR, US) will provide information about global and regional tissue perfusion.

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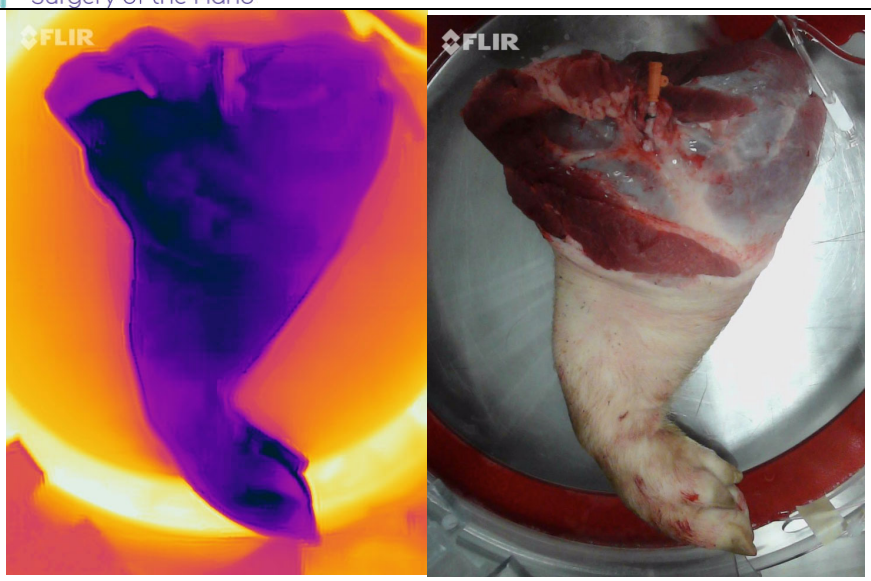


Image of limb prior to reperfusion on infrared and actual still images. Dark areas correspond with cold, non-perfused regions.

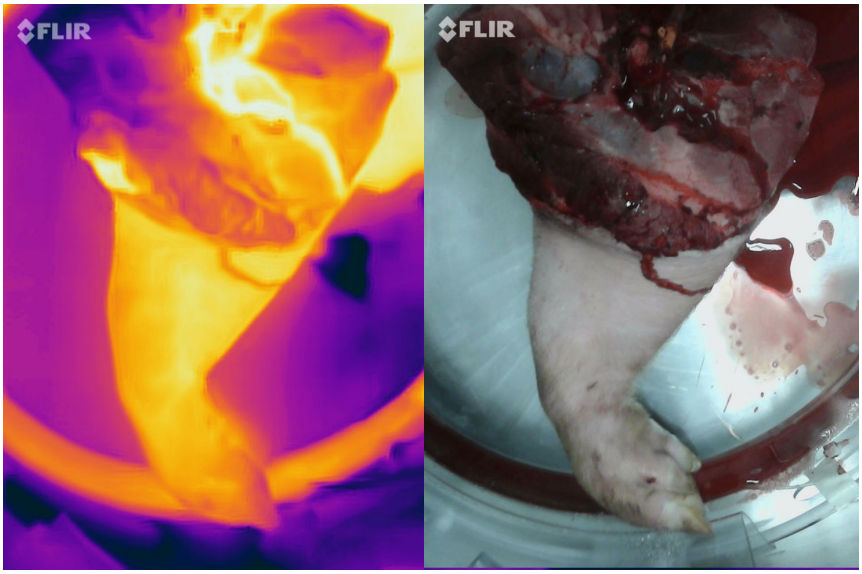


Image of limb after reperfusion on infrared still images at 5mins. Lightened enhanced areas correspond with warmth and tissue perfusion. Venous blood can be seen arising from the muscle and skin edge.

(iii) Myocyte viability will be assessed by comparing pre and post-perfusion biopsies using *ATP Bioluminescence* Assays via fluorescent light generated from luciferase.

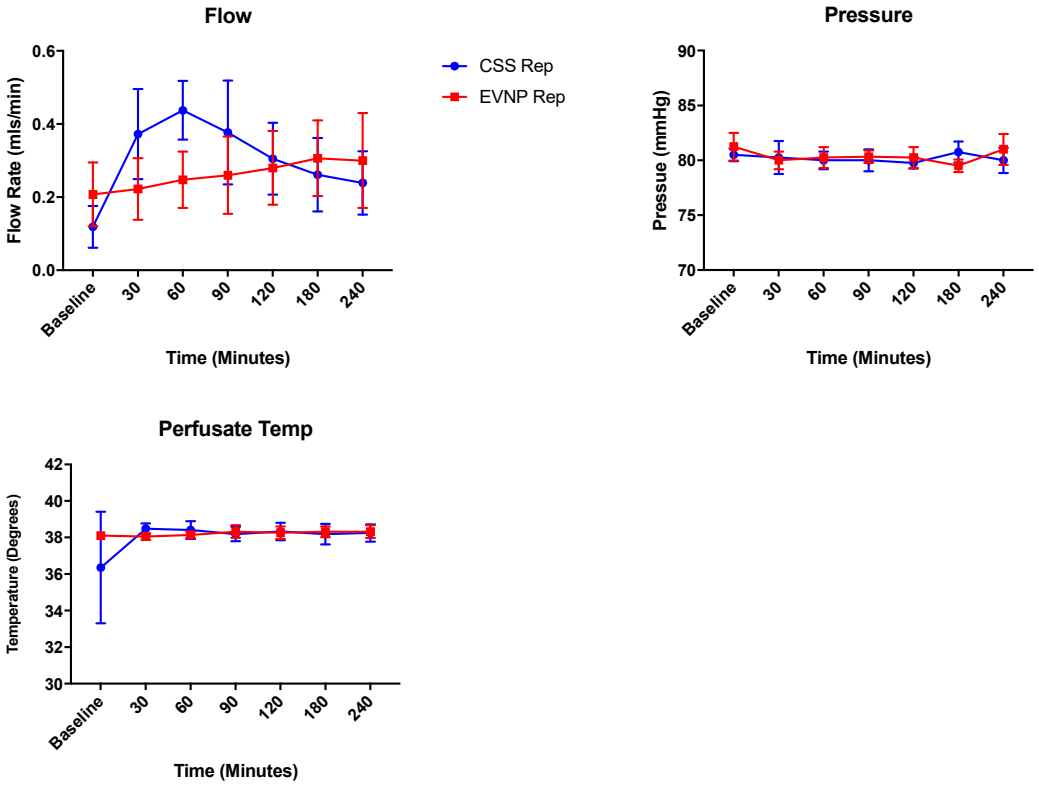
(iv) Cell-free DNA will be quantified as a marker of cell death.

~~(ii)~~**(v)** Skin and muscle biopsies will be collected pre and post-perfusion.. Architectural integrity (H&E) and will undergo independent assessment by transplant histopathologists to grade

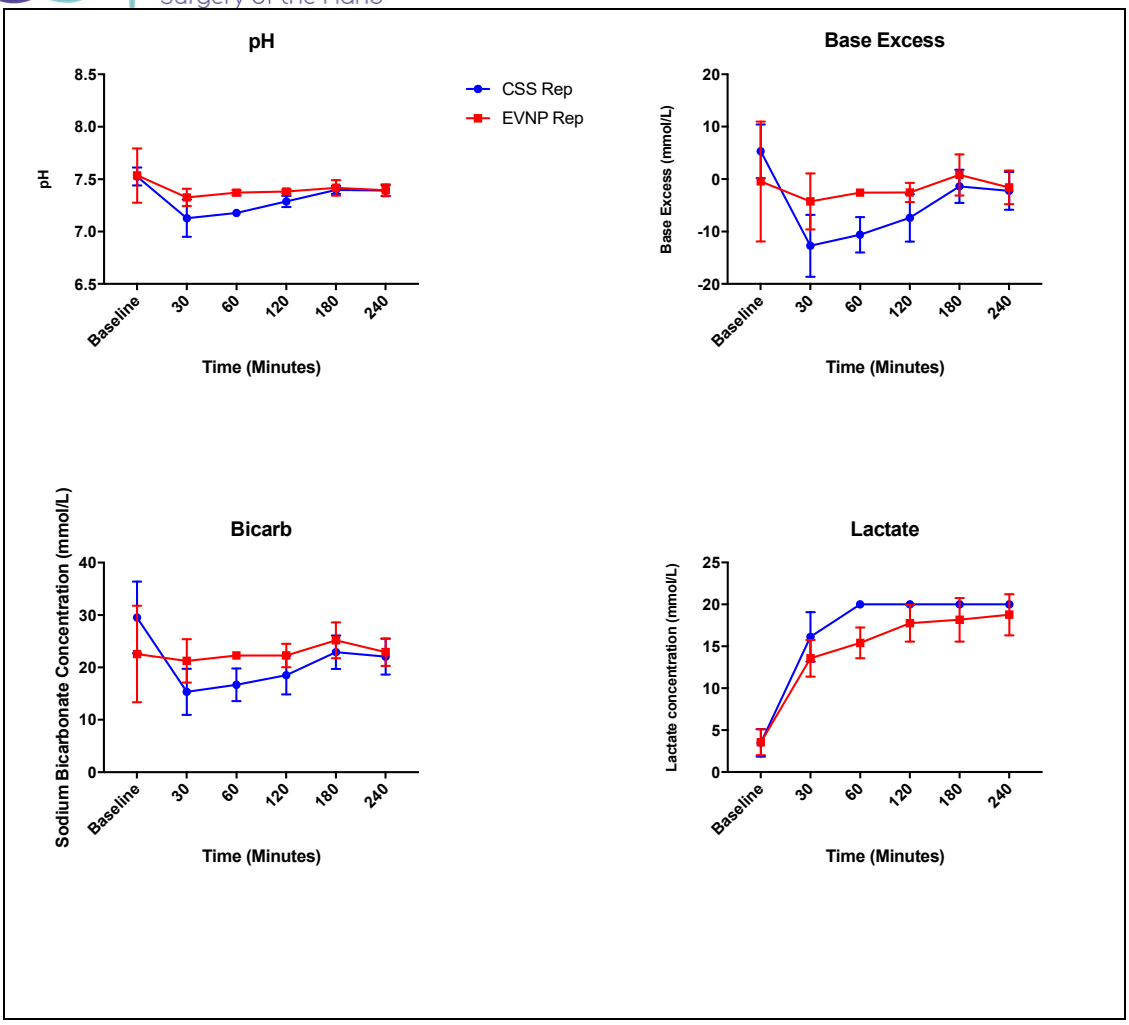
tissues to clinical transplant standards.

Primary outcome measure:

The primary outcome measure of the project has been met in that stability in haemodynamics after reperfusion was seen more readily in the EVNP group compared to the CSS group.



Further, it was also seen that anaerobic (lactate) and cell injury markers (potassium) and acid-base balance are lower and more stable in the EVNP group after reperfusion. These are the same markers used by groups internationally performing limb perfusion.



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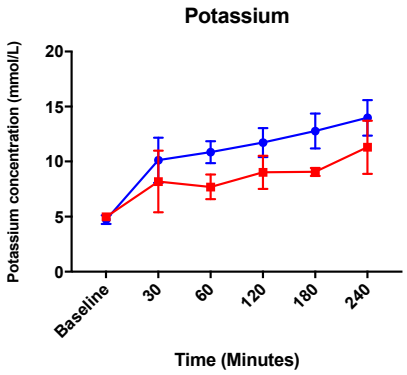
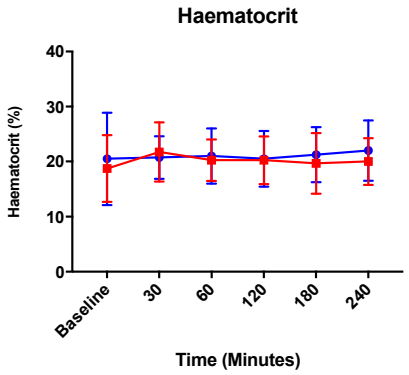
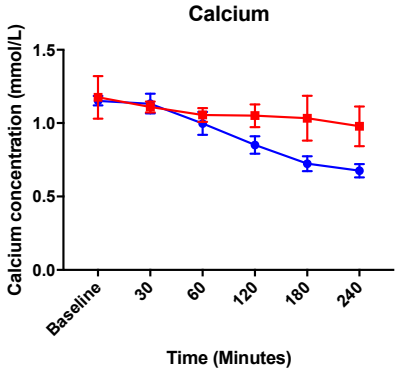
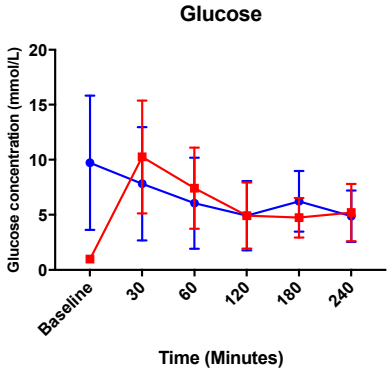






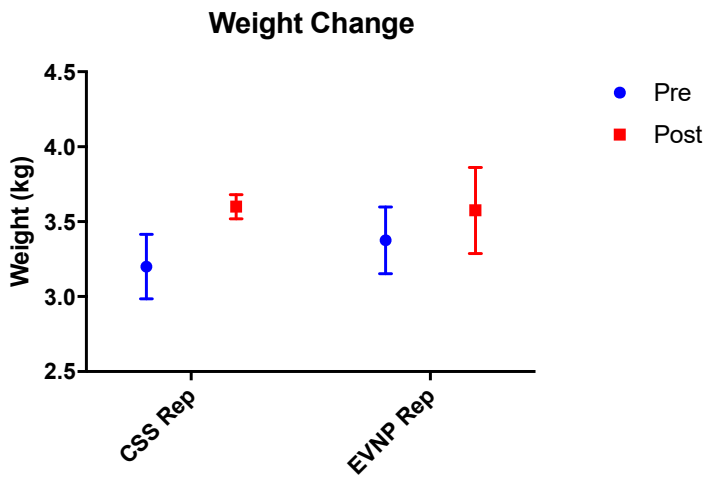


Secondary outcome measure:

Infrared imaging demonstrated more homogenous reperfusion within the EVNP limb. It is expected that this will result in less cell injury markers within the perfusate as well as less injury demonstrated by architectural integrity on histological analysis.

Change in weight:

Weight can be considered a marker of oedema. More specifically this is seen as detrimental to the health of tissues and indeed implies tissue injury later that can manifest as fibrosis.



Presentations based on work performed in this study

Pending and will be presented in Basel 2020

Publications based on work performed in this study

Pending but we are convinced that this novel study will be published. FESSH will be acknowledged of course upon receipt of publication.