Development of a Dynamic Physiological Whole Blood System for Drug Evaluation

Georgina E Chesworth[1, 2, 3], Emily L Milburn[1, 2, 4], Miles L Bolton[1, 2, 3],
William R Cowey[1, 2], Jessica A Ferguson[1, 2], Corban JT Bowers[1, 2], John P Stone[1, 2, 5] , Timothy R Entwistle[1, 2] and James E Fildes[1, 2, 6].

[1] Pebble Institute, Beech Court, Manchester, United Kingdom.
[2] Pebble Biotechnology Laboratories, Beech Court, Manchester, United Kingdom.
[3] School of Biological Sciences, Faculty of Biology, Medicine & Health, The University of Manchester, United Kingdom.
[4] School of Biosciences, Faculty of Science, The University of Sheffield, United Kingdom.
[5] Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine & Health, University of Manchester, Manchester, United Kingdom.
[6] Healthcare Technologies Institute, School of Chemical Engineering, University of Birmingham, Birmingham, United Kingdom.

Introduction

In-vitro methods are invaluable in early phase drug evaluation, yet they often fall short in producing **clinically translatable** results. This is due to the inherent simplicity & inability to accurately recapitulate the in vivo environment. To address this we have developed a physiological alternative.

Pebble's **LIVING-BLOOD system** maintains blood components in a dynamic state that closely replicates human blood flow & cellular interactions, enhancing the **predictive accuracy** of how drugs will perform in clinical trial.

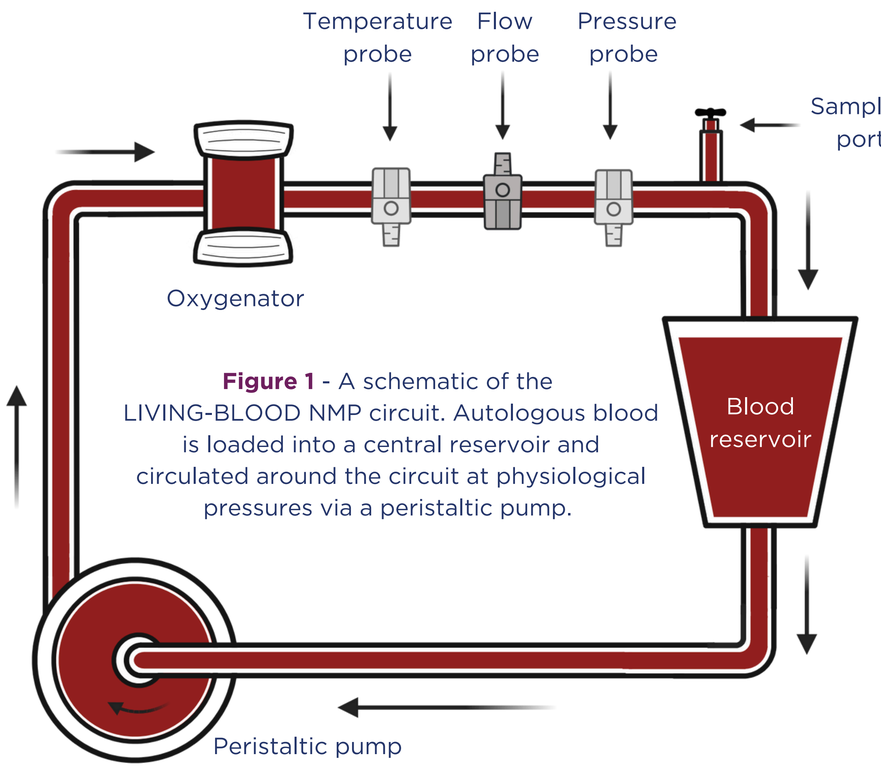

Figure 1 - A schematic of the LIVING-BLOOD NMP circuit. Autologous blood is loaded into a central reservoir and circulated around the circuit at physiological pressures via a peristaltic pump.

Methods

- Autologous whole blood was retrieved from landrace pigs via exsanguination, heparinised & filtered to remove clots; all biological material utilised was surplus to the food industry.
- Blood was combined with Pebble's proprietary perfusate & primed into a pre-built normothermic machine perfusion (NMP) circuit.
- The perfusate was circulated, according to physiological flow & pressures via a peristaltic pump, passing through a membrane oxygenator simulating gaseous exchange.
- Haemodynamics, biochemistry & co-oximetry were continuously monitored throughout the 6 hour perfusion, with serial perfusate samples taken & stored for later analysis.

Results

✔ Arterial blood gas analysis revealed a comprehensive biochemical profile. Blood biochemistry remained stable.

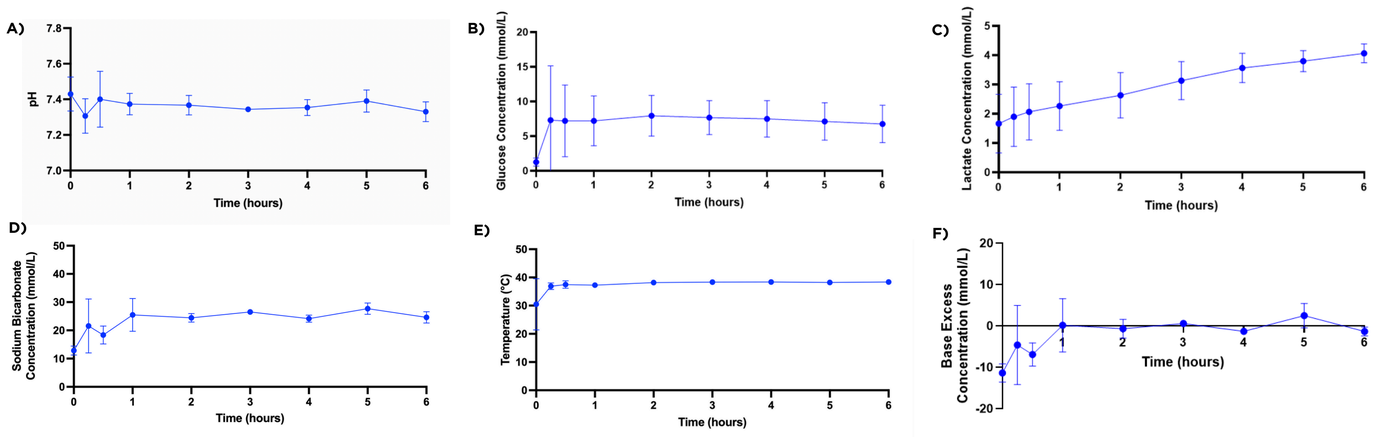

Figure 3 - Stable metabolic profile. A) pH overall was maintained within physiological parameters despite slight acidosis within the first hour. B) Glucose was maintained within normal parameters. C) Lactate concentration remained stable although a slight increase was observed. D) Sodium bicarbonate fluctuated, but remained stable overall. E) Temperature remained at normothermia. F) Base Excess increased in the first hour, but remained physiological for the remainder of the perfusion.

✔ Co-oximetry reflected physiological levels

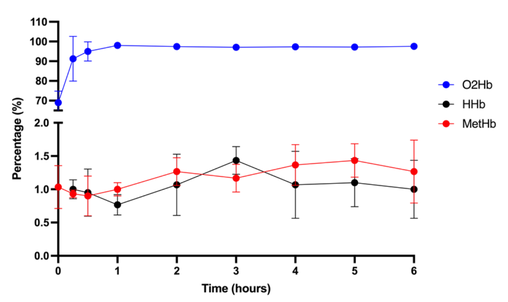

Figure 3 - Co-oximetry percentages were recorded. A) O_2Hb was unchanged from 1 hour. B) HHb varied slightly over time. C) MetHb remained within normal range.

✔ pCO_2 and pO2 were maintained at physiological pressures.

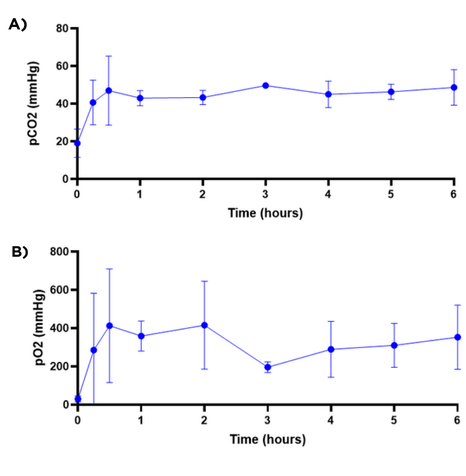

Figure 4 - pO_2 and pCO_2 were maintained. A) pCO_2 was controlled to a physiological pressure. B) pO_2 remained constant despite a notable decrease after 3 hours.

✔ Physiological haematocrit & haemoglobin levels were observed.

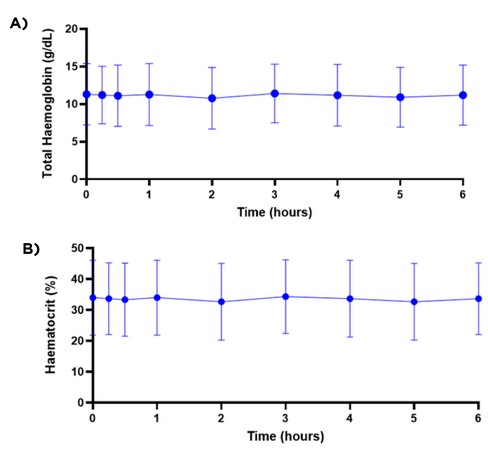

Figure 5 - Haematology data was controlled with little change. A) Average total haemoglobin was constant throughout the perfusion. B) There was limited fluctuation in the average haematocrit percentage.

✔ Electrolyte balance remained stable overall.

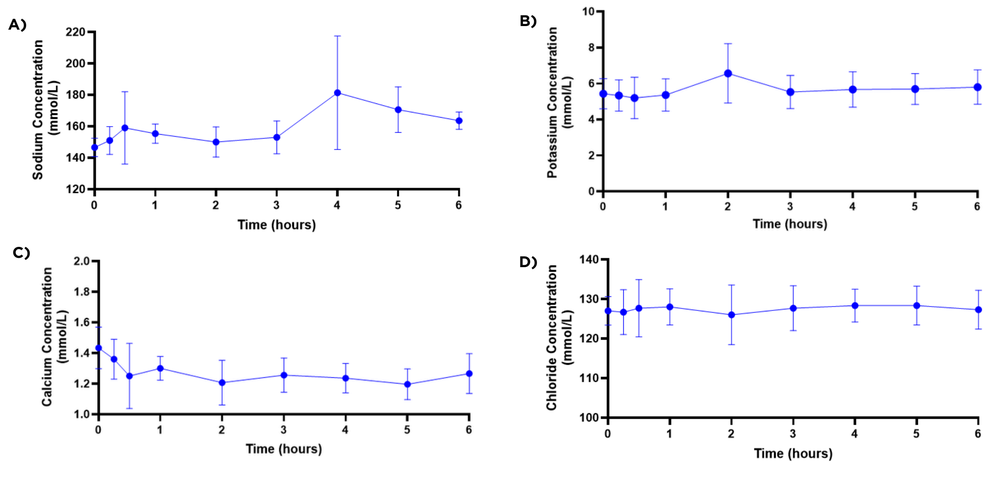

Figure 6 - The electrolyte balance was stable overall. A) Sodium concentration remained within a standard range with a slight increase after 4 hours. B) Potassium concentration remained unchanged from baseline. C) After an initial decrease, calcium concentration was sustained at a standard level. D) Chloride concentration was stable throughout the perfusion.

Conclusions and Future Perspectives

Our LIVING-BLOOD system recapitulates the intravascular environment in a way that is unachievable with existing in-vitro approaches. This may allow for a more comprehensive assessment of a drug's toxicity potential & any associated risks, facilitating the development of **safer** & **more effective** therapeutic interventions. Future experiments will involve using **human blood** sourced from NHSBT in order to assess therapeutic interactions & toxicity with over **4,000** blood components.

Enhanced predictive accuracy of toxicity testing

Potential to support custom immune profiling for personalised toxicity analysis

No regulatory approvals required

Complete studies in weeks at a fraction of in-vivo costs

PBL PEBBLE INSTITUTE
Pebble Biotechnology Laboratories, Beech Court,
122 Hollin Lane, Manchester, SK9 4LD
email: contact@pebble.bio
www.pebble.bio