Developing a Porcine Transdermal Absorption Model Using Normothermic Machine Perfusion.

Miles L Bolton[1, 2, 3], Emily L Milburn[1, 2, 4], Georgina E Chesworth[1, 2, 3],
William R Cowey[1, 2], Jessica A Ferguson[1, 2], Corban JT Bowers[1, 2], Reynard Spiess[5], Timothy R Entwistle[1, 2], John P Stone[1, 2, 6] and James E Fildes[1, 2, 7].

1Pebble Institute, Beech Court, Manchester, UK
2Pebble Biotechnology Laboratories, Beech Court, Manchester, UK
3School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom.
4School of Biosciences, Faculty of Science, The University of Sheffield, Sheffield, United Kingdom.
5Department of Chemistry, Faculty of Science and Engineering, The University of Manchester, Manchester, United Kingdom
6Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom.
7Healthcare Technologies Institute, School of Chemical Engineering, University of Birmingham, Birmingham, United Kingdom

Introduction & Aims

- The current gold standard for transdermal absorption research remains in vitro and small animal models, primarily rodents.
- Human and mouse skin differ significantly anatomically and physiologically, leading to poor translatability of potential therapeutics.
- Pigs represent a more translatable model but have ethical and economic restraints, limiting their use in vivo.
- To address this, we have developed an ex vivo normothermic machine perfusion (NMP) system, using surplus pig tissue from the food industry. This provides an ethical and cost-effective approach to drug development.

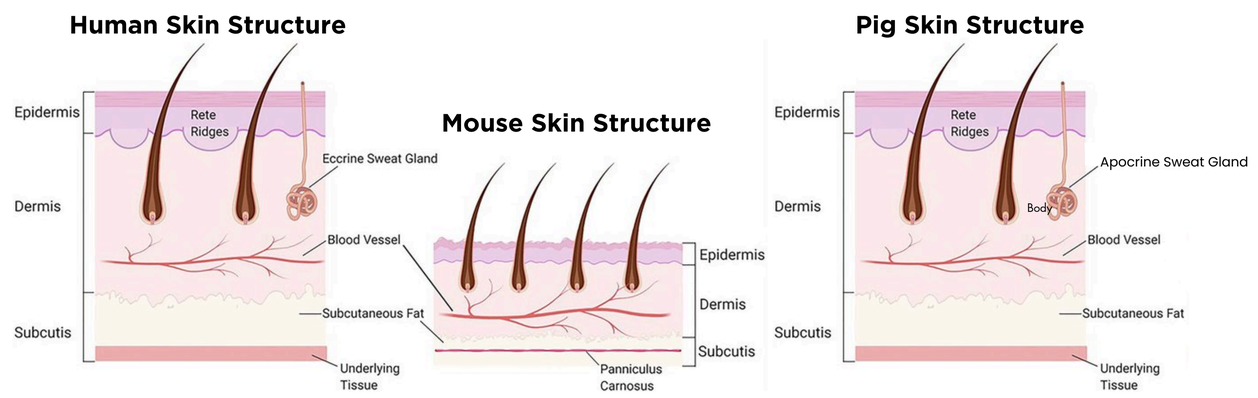

Figure 1 - Schematic demonstrating the structure of human, mouse and pig skin, respectively. Human and pig skin are anatomically similar with comparable epidermis thickness. Conversely, mouse skin has very different hair follicle density and a thinner epidermis.

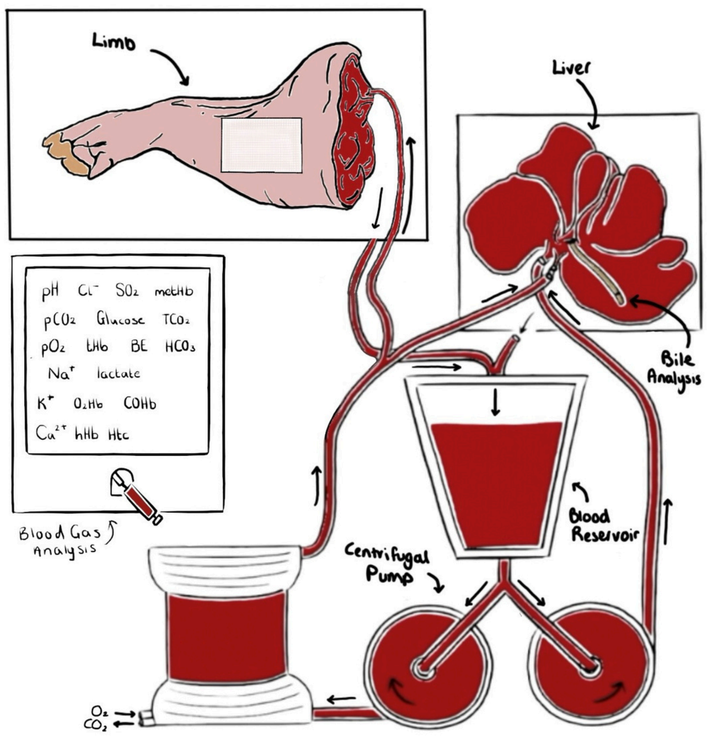

Figure 2 - Schematic overview of the NMP circuit. Perfusate is recirculated via a centrifugal pump through an oxygenator and into the arteries of the tissues. A separate pump provides deoxygenated blood to the venous side of the circuit. A blood gas analyser ensures biochemistry remains within physiological parameters.

Methods

- Porcine forelimbs, livers and autologous whole blood were retrieved following standard clinical transplant protocols.
- An NMP circuit was prepared with Pebble's proprietary perfusate and supplemented with packed red blood cells.
- Perfusate was recirculated via a centrifugal pump, passing through an oxygenator and then entering the limb and liver via the brachial artery and hepatic artery cannulas, respectively. A separate pump recirculated deoxygenated perfusate into the liver via a portal vein cannula. This restored full function and metabolism.
- Haemodynamics and blood biochemistry were continually monitored throughout. A 25-mg nicotine patch was applied to the limb surface to assess transdermal absorption.
- Serial perfusate samples were collected and stored for later analysis of nicotine and it's metabolite, cotinine, via high performance liquid chromatography with mass spectrometry (HPLC-MS).
- The perfusion was performed for a total of 12 hours.

Results

✓ Haemodynamics are maintained within physiological range

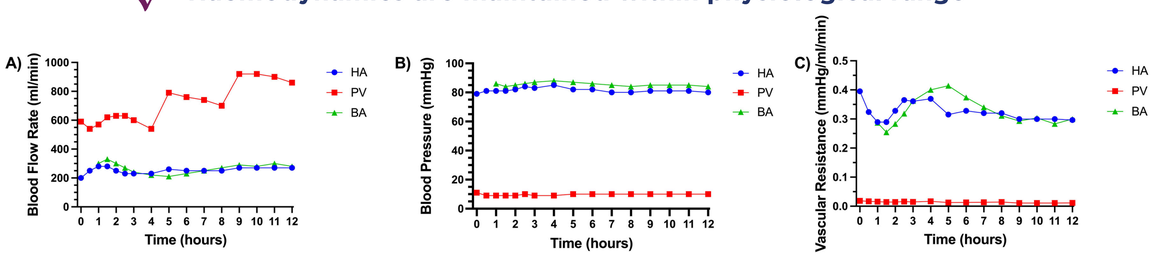

Figure 3- Haemodynamics remain stable across the perfusion. A) Blood flow through the hepatic artery (HA), portal vein (PV) and brachial artery (BA), B) Blood pressure through each vessel and C) Intra-vascular resistance through each vessel

✓ Electrolytes remain stable

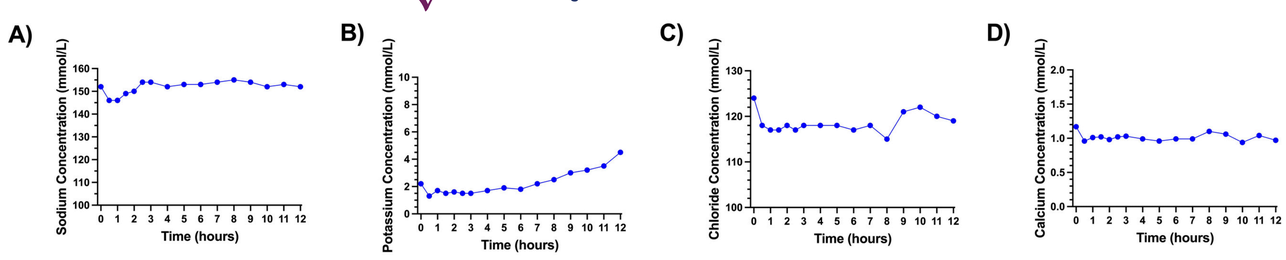

Figure 4- All electrolytes were maintained within physiological range. A) Sodium concentration varied little B) Despite an incremental increase, potassium concentration remained within a standard clinical range C) Chloride concentration was physiological throughout with small fluctuations and D) Calcium concentration was stable.

✓ Blood biochemistry is within standard clinical parameters

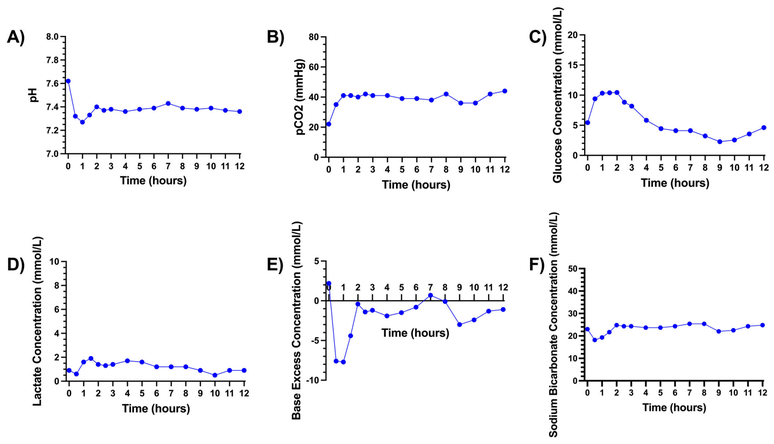

Figure 5 - Stable metabolic profile - A) pH was maintained with no metabolic acidosis B) pCO_2 was stable C) glucose was consumed with active glycolysis D) lactate concentration remained low demonstrating cori cycle processing E) Base remained within range following initial supplementation and F) After initial sodium bicarbonate supplementation the concentration remained stable.

✓ Nicotine is transdermally absorbed

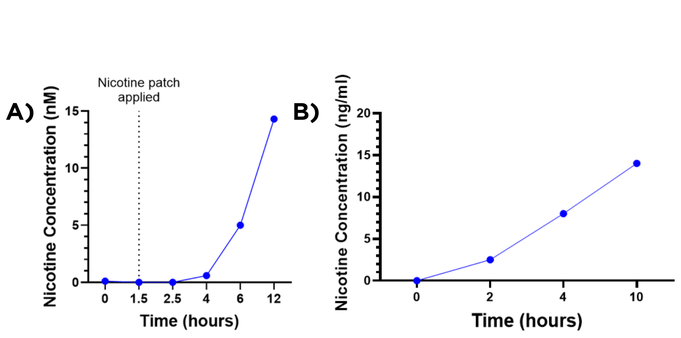

Figure 6 - HPLC-MS drug concentration analysis. A) After application of the nicotine patch, there was an increasing trajectory in perfusate nicotine concentration B) In vivo human data showing mean plasma nicotine concentration after application of a 25-mg nicotine patch.

✓ The liver metabolises Nicotine

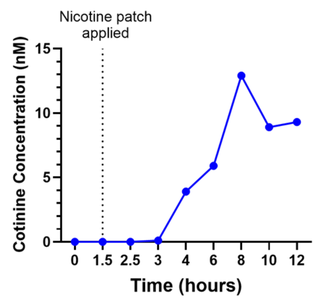

Figure 7 - Cotinine concentration, a metabolite of nicotine, also increased after patch application peaking after 8 hours of perfusion.

✓ Tissue is homogenously perfused

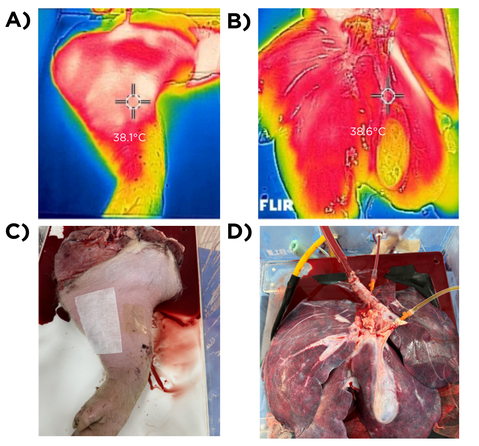

Figure 8 - A-B: Infrared images showing homogenous perfusion across the vasculature of A) limb B) liver. C-D: Macroscopic images of C) the limb appearing globally pink and D) the liver appearing healthy during the perfusion.

Conclusions and Future Perspectives

- Transdermal absorption of nicotine was successful with active metabolism by the liver forming cotinine, confirming the suitability of this platform as a superior model for testing drug efficacy and toxicity.
- This system has the potential to increase the number of therapies that successfully transition through the preclinical pathway and into clinical trials for direct patient benefit.
- Further experiments will be performed with commercially available therapies to confirm the validity of this model.

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