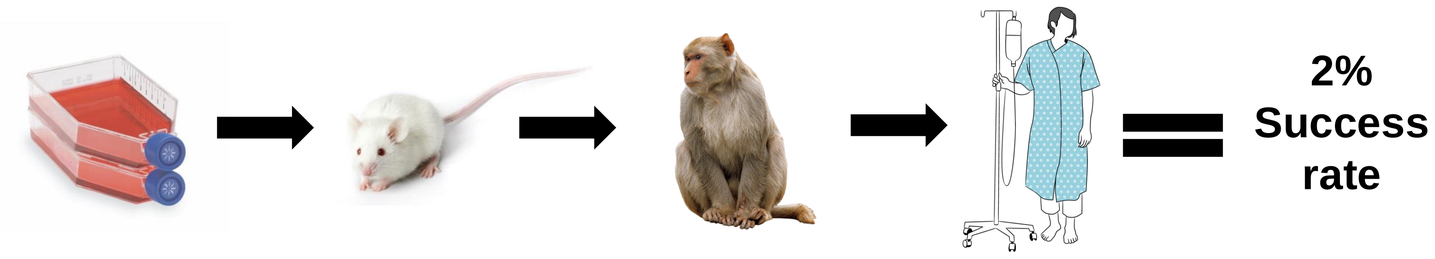
The Development of a Porcine Multi-Organ Liver-Kidney-Spleen Model to Advance Research in Tissue Engineering and Regenerative Medicine

Amy Stewart[1], Erin Armstrong[1], Corban Bowers[1], William Cowey[1], Kavit Amin[1], Timothy Entwistle[1], John Stone[1] and James Fildes[1,2]

[1]The Ex-Vivo Research Centre (EVRC) and Pebble Biotechnology Laboratories, Alderley Park, Macclesfield, Manchester, UK

[2]Healthcare Technologies Institute, University of Birmingham, Edgbaston, Birmingham, UK

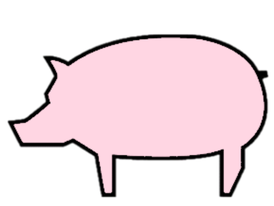
Introduction



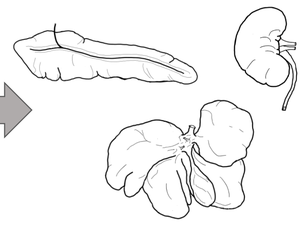
2% Success rate

- The traditional roadmap for the development of drugs and medical devices involves progression from in-vitro, rodent and large animal models, but successful translation in humans is rare.
- Next-generation approaches are required to replace the existing dogmatic drug and medical device development pathway.

Methods



Organs and blood were retrieved from food industry pigs following a schedule 1 kill. Briefly pigs were exsanguinated, and the blood collected.



Vessels were isolated, cannulated, and flushed with a preservation solution. Organs were stored on ice during transit.

Whole blood was cell saved to produce packed red blood cells which were mixed with a proprietary perfusion solution (Pebble Biotech).

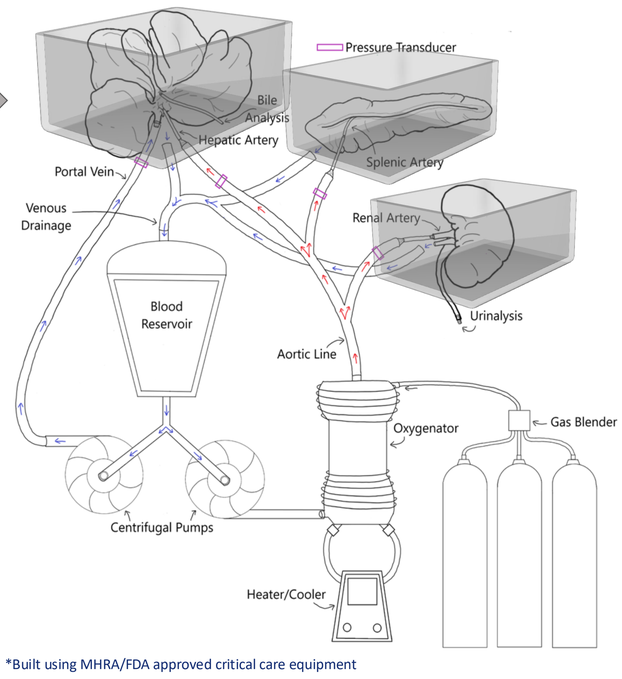
The LIVING-ORGAN system was built* and primed with perfusate (figure 1).

Organs were placed in an organ chamber, attached to the circuit via the cannulated vessels, and perfused for 24 hours.

Haemodynamics and blood biochemistry were continuously recorded.

To see this system in action, scan the QR code:

Circuit Design: Perfusate was stored in a central reservoir which feeds into two parallel centrifugal pumps (figure 1). The first of these transits blood through an oxygenator (supplying 95% O2, 5% CO2) where it is also heated to normothermia via a heater/cooler unit. This supplies arterial blood to the organs which free drains out of the veins back into the central reservoir.
The second pump bypasses the oxygenator, supplying deoxygenated venous blood to the portal vein.



*Built using MHRA/FDA approved critical care equipment

Figure 1: Schematic showing liver-kidney-spleen perfusion circuit

Results

Haemodynamics, blood biochemistry and co-oximetry (not shown) remained physiologically stable. Bile and urine production began immediately and were maintained throughout, demonstrating restoration of organ function. Macroscopic tissue preservation was excellent following 24 hours of perfusion.

✓ Blood flow restored to entirety of organ vasculature.

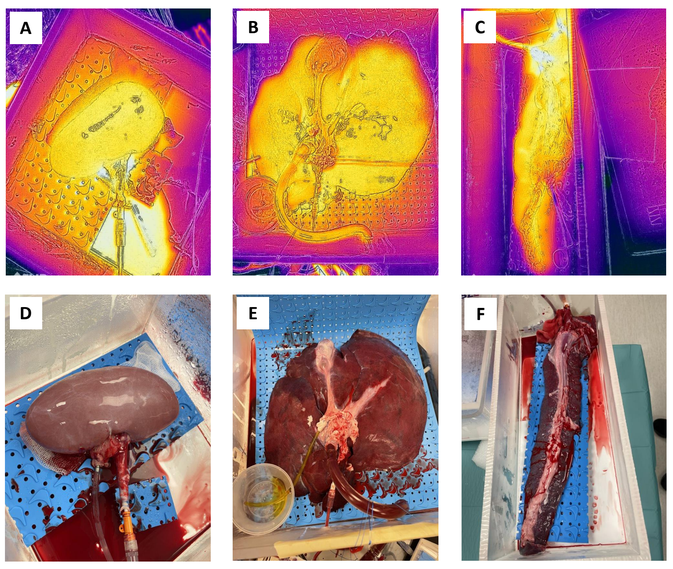


Figure 2: A-C. Representative infrared imaging demonstrating homogenous perfusion across the tissues. **D-F.** Representative images showing healthy macroscopic appearance of organs.

✓ Physiological organ function is restored.

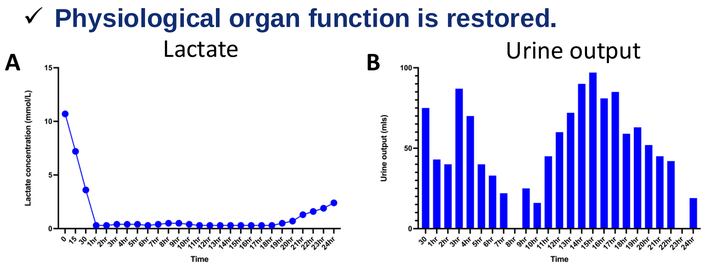


Figure 3: A Lactate concentration is reduced as a result of liver and kidney conversion. **B** Continuous urine output is indicative of sustained renal function.

✓ Stable haemodynamics are maintained within physiological parameters.

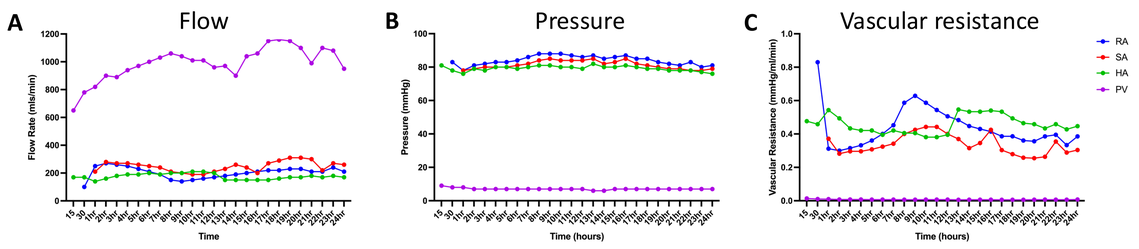


Figure 4: A Mean arterial blood flow, **B** pressure and **C** vascular resistance for each organ remain within physiological parameters. RA Renal Artery (Blue). SA Splenic Artery (red). HA Hepatic artery (green). PV Portal vein (purple).

✓ Active metabolism regulates blood biochemistry at physiological concentrations.

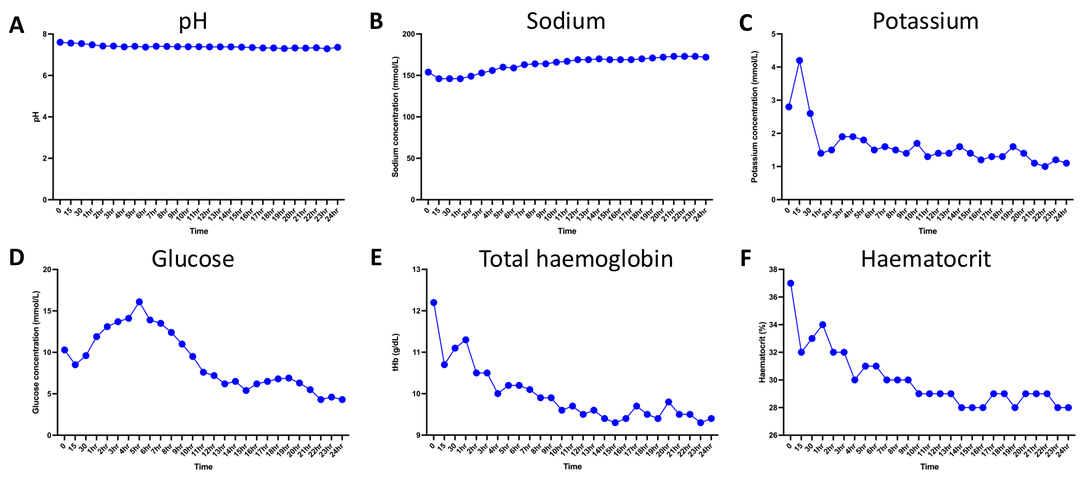


Figure 5: A Stable pH = acid-base equilibrium and absence of metabolic acidosis. **B** Stable sodium concentration as ion balance controlled. **C** Stable potassium demonstrate organ heath and absence of haemolysis. **D** Glucose concentration reduced by metabolic activity and renal/hepatic gluconeogenesis. **E** Total haemoglobin (tHb) reduced due to RBC processing by the spleen. **F** Haematocrit levels reduced due to recycling by liver and spleen.

✓ Healthy tissue observed following 24 hour perfusion.

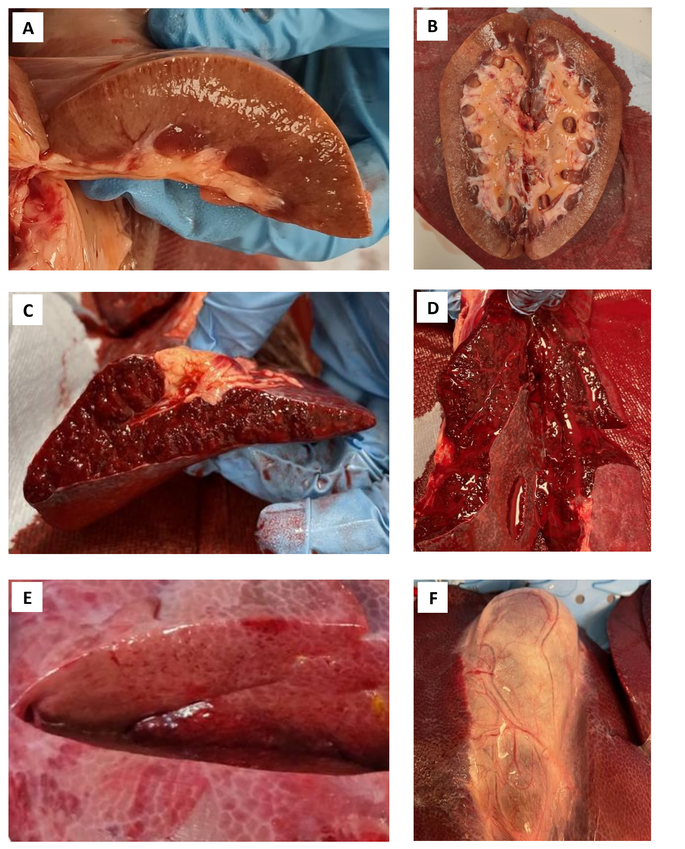


Figure 6: Representative images from macroscopic dissection of organs following 24 hour perfusion. A-B Kidney. **C-D** Spleen. **E** Liver **F** Gall bladder.

Conclusions and future perspectives

We have developed a next-generation isolated LIVING-ORGAN system capable of accelerating the development of drugs and medical devices.

- **Highly comparable** to the in-vivo environment, allowing physiological evaluation of organs.
- **Drug and medical device agnostic**, applicable from early proof of concept to new drug submission.
- **Cost effective** - On average 90% cheaper than large animal models.
- **Higher throughput** – Time-to-start is days rather than months, without the need for regulatory approval.
- **Data rich** – Producing clinically relevant data: drug biodistribution, tropism, uptake, organ specific toxicity, immunogenicity and bioavailability.
- **Applicable to acute phase modelling** – Currently limited to 24 hours. Future work will focus on extending this to several days.

Pebble Biotechnology Laboratories

If you would like to use our model, please get in touch:

PBL, Alderley Park,
Cheshire, SK10 4TG, UK
contact@pebble.bio
www.pebble.bio