POSTER PRESENTATIONS

Originally presented at:
IAT Congress 2019

Mouse to Man: an overview of the impact that mouse model research has had on the development of gene and stem cell therapies and the increasing use of personalised medicine

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The presenter

Steven Cubitt founded two companies, The Cube, followed by CCTech, which provide specialist services on a wide range of projects in the UK, Europe and globally. Steven has recently been engaged on several projects to provide strategic advice regarding future investment in the biomedical research facilities at leading universities.

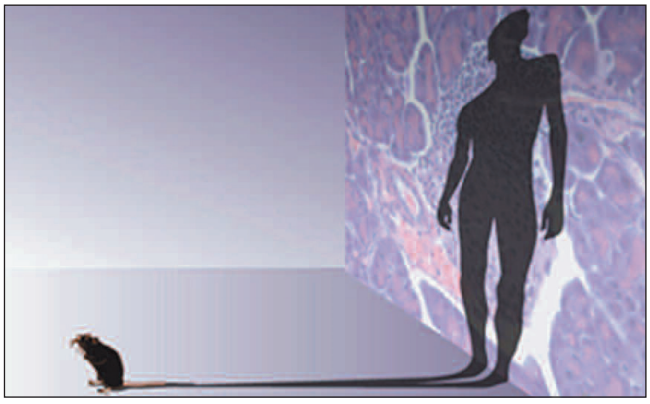
Gene therapies, stem cell therapies and personalised medicine

These are starting to transform treatments for a number of different debilitating diseases/conditions. A stem cell is a cell capable of becoming another more differentiated cell type in the body, such as a skin cell, a muscle cell or a nerve cell. Because of their ability to become different types of cells, stem cells offer us the greatest potential to treat degenerative conditions and illness that can affect us all – spinal cord damage, sports injuries, bone, cartilage and tendon damage, blood cancer, diabetes, multiple sclerosis, Parkinson's, Alzheimer's, arthritis, blindness, stroke and heart disease.

More than a decade of research on the biology of mouse stem cells has helped to pave the way for developing human stem cell lines and using them to treat disease.

The first impact of the mouse ES papers was enabling targeted gene knock outs in mice, a technology which has revolutionised mouse genetics and developmental biology.

It took seventeen more years for Jamie Thomson to isolate ES cells from human embryos, one of the great milestones in human biomedical research.

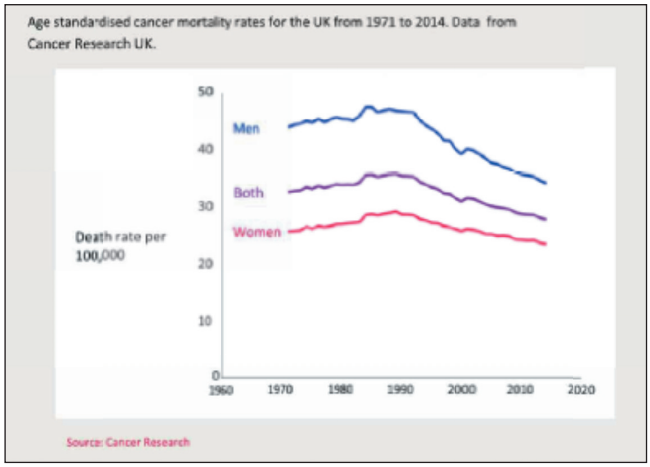


Cancer

In the UK society, for every £1 invested in cancer research in 1986, the investment gains between 24 – 28 pence every year, into the indefinite future.

The 1 year standardised survival rate in England increased

50% – 1971 65% – 2000 70% – 2010

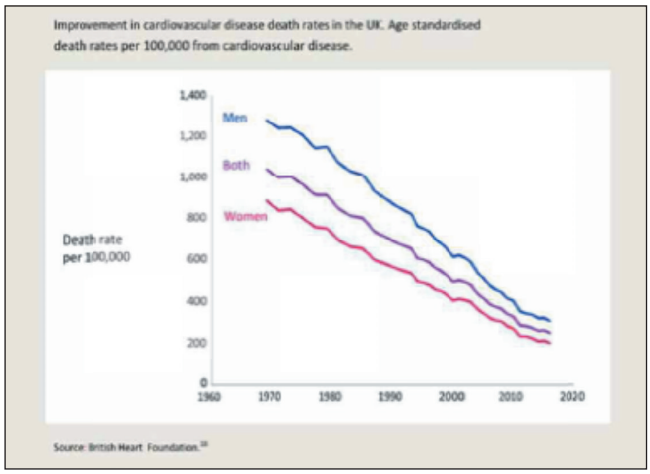


Cardiovascular diseases

Coronary heart disease, strokes and other circulatory diseases still account for more than a quarter of all deaths in the UK.

Death rates have seen a 73% fall between 1974 and 2013.

A significant recent development is the speed at which these therapies are being transferred from mouse to man. These developments require new types of Good Manufacturing Practice (GMP) facilities which feed back into pre-clinical research needs. However, because human trials have given equivocal results, more animal research is still needed.



Summary

The Medicines and Healthcare Products Regulatory Agency (MHRA) licenses cell and gene therapy manufacturing facilities in the UK which include early-stage translational centres in the academic and public sector. 2018 marked the fourth consecutive year of increases in operational footprint within the UK's MHRA licensed GMP manufacturing facilities.

With new facilities scheduled to open in 2019. This network of facilities, operated by 21 organisations in the UK, comprises of 15 dedicated cell therapy sites, 6 dedicated gene therapy sites and 4 multifunctional sites.

Currently Life Sciences employs 240,000 people in the UK, with a turnover of £70 billion annually. With this current investment in advanced therapy medicinal product technology, the UK plans to maintain its significant lead role in this field.

Do Buccal swabs from Zebrafish give enough of a sample of DNA to be used as a viable non-invasive method of genotyping?

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Abstract

The Zebrafish *(Danio rerio)* has become a valuable research model within the laboratory. Due to the increased numbers used of transgenic Zebrafish, genotyping of the fish has become an everyday necessity to facilitate their use in order to identify the fish with the correct or necessary genes. Genotyping of the fish is usually performed at 6 months of age. By this time, the fish are classed as adults in size and are ready for breeding.

Currently the proven method of genotyping involves taking a tail fin clip from the fish to gain the sample. This involves surgically removing a piece of the tail fin under anaesthetic.

In previous studies buccal swabs have been taken from different species such as humans, mammals and larger species of fish. This involves taking a swab from the inside of the mouth, gathering cells which are used to extract the DNA. This study investigates if taking buccal swabs from Zebrafish gives enough of a sample of DNA to be a viable non-invasive method for genotyping.

This project was conducted within the principles of the 3Rs (Reduction, Refinement, and Replacement).

All fish were euthanised prior to sample taking to prevent risk of suffering.

Sample taking

The sample taking method for each fish needs to be repeatable. The method will change for each experimental group depending on the number of rotations of the swab but everything else will remain the same. For the purposes of this study I tested for the gene IPIP27A_F.

All equipment needs be set out beforehand ready for the sample taking.

Materials and Procedure

Remove one fish at a time from the MS222 anaesthetic solution, selecting each fish noting if it is male or female. Start the timer. Gently dab the fish on paper towel (TORK Hygiene Roll) to remove excess water and place the fish on sampling area, facing forwards towards you.

Using tweezers (Pake Dissecting Forceps Curved Fine Serrated Jaw 115m), gently open the mouth of the fish, just wide enough to be able to insert the swab (TAMIYA Craft Cotton Swab, Triangular, Extra small, 50PCS).

Insert the swab. Remove tweezers from the mouth, leaving the swab in place, allowing the mouth to close around the tip of the swab. Rotate the swab for the necessary amount of times depending upon the experimental group.

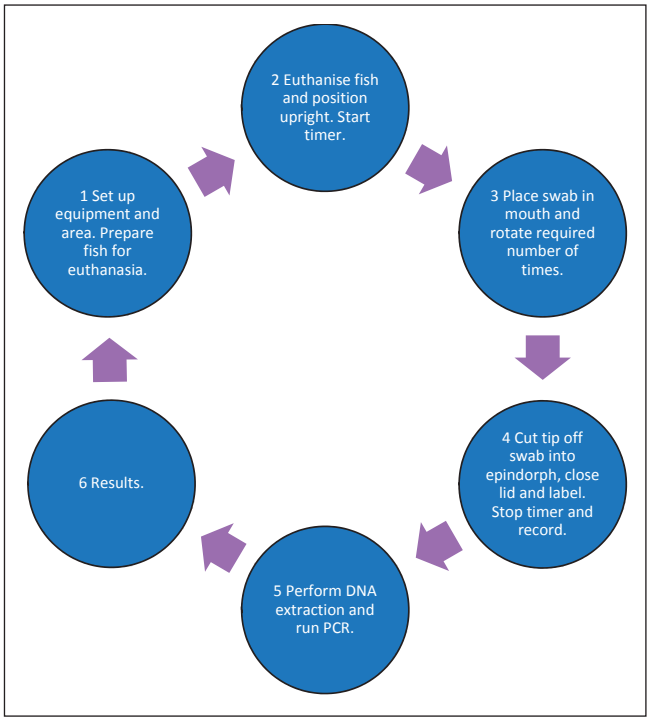

Figure 1. Basic Procedure

Remove swab from mouth, ensuring not to touch anything with the tip. Keeping hold, cut off the tip with the sample into the correct epindorph tube with scissors. Close the lid on the epindorph tube and stop the timer.

Dispose of the holding end of swab into the bin.

Now an inspection of the fish is carried out, looking for any signs of damage to the mouth.

Record all results on the recording sheets and take any pictures.

The fish can now be disposed of in the yellow disposable bag. Reset the timer and wipe down the tweezers ready for the next fish.

DNA Extraction and PCR

First add 50 milimolar (mmol) of Sodium Hydroxide to the sample inside the epindorf tube, repeating for all samples. Vortex each sample for 5 seconds, then heat all samples to 95°C for 20 mins in a thermal cylinder. After this add 5 mM solution Tris buffer (PH8 1 molar (M) solution) to each sample. The samples can now be stored at 4°C ready for PCR.

For PCR begin with using 0.2ml PCR tubes, adding 2µL of the DNA sample mixed with 8.5 ul H2O, 1µL of primer IPIP27A_F, 1µL of primer IPIP27A_R, and 12.5 µL GoTag® green master mix (Promega). Run this on the following PCR cycle using a Techne 3Prime Thermal Cycler for:

Run Order	Cycles	Temperature (Degrees Celsius)	Time (seconds)
1	1	95	120
2	30	95	30
		60	30
		72	30
3	1	72	120
4	1	4	Hold

Table 1. PCR cycle used.

Results

The results gained from Set 1 were very promising. Each sample gave a positive result when compared to the control sample. In total 12 samples were taken, 2 samples for a range of rotations (1 male and 1 female for each) including 0, 2, 4, 6, 8 and 10. This helps to indicate firstly if a DNA positive result can be given and secondly if there are any differences to be found between the number of rotations.

The first male sample of 0 rotations is not as clear as the rest of the samples. Due to this being the first sample which had been taken, it may be the technique that was the cause. It is not possible to rule out that this may also be a result of a lack of DNA being taken due to a low number of rotations. The signal from the results becomes very strong on the female sample of 2 rotations and remains strong throughout the rest of the samples.

Pictures of the fish taken post sampling will help to evaluate if there were any visible signs of damage being caused. From the pictures, no signs of mouth damage are visible.

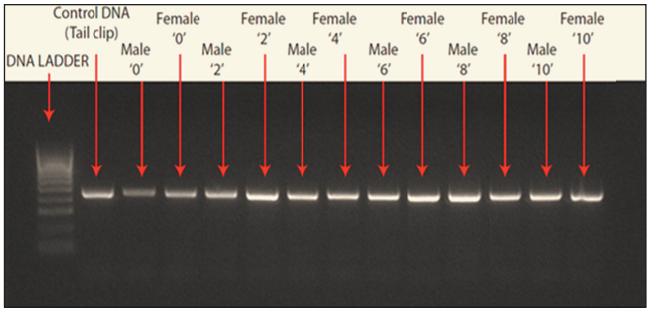

Figure 2. UV transilluminator image of results for Set 1

TRIAL RUN	STRAIN	D.O.B	SEX	NUMBER OF ROTATIONS	TIME TAKEN (seconds)	SIGNS OF DAMAGE
1	WT	29.1.2015	M	0	56.4	NO
2	WT	29.1.2015	F	0	19.7	NO
1	WT	29.1.2015	M	2	24.4	NO
2	WT	29.1.2015	F	2	15.3	NO
1	WT	29.1.2015	M	4	27.2	NO
2	WT	29.1.2015	F	4	16.5	NO
1	WT	29.1.2015	M	6	35.2	NO
2	WT	29.1.2015	F	6	20.9	NO
1	WT	29.1.2015	M	8	27.8	NO
2	WT	29.1.2015	F	8	22.9	NO
1	WT	29.1.2015	M	10	29.3	NO
2	WT	29.1.2015	F	10	22.1	NO

Table 2. Record sheet for set 1

Further investigations

This method of genotyping for Zebrafish needs to be further investigated. The results gained from this project show that it is possible to gain a positive result using this method but it has been limited to using only euthanised animals, being tested using a single gene. For the future, investigations into other genes will be needed to ensure it is functional across the Zebrafish genome. Investigations using live animals will be

necessary to ensure that there is no damage to the mouth area or any other lasting harm has occurred and that the fish are able to function fully after the procedure.

The length of time (seconds), taken to extract each sample is something to look at reducing. A possible cause may be due to using tweezers to open the mouth of the fish first which is no longer necessary, as the mouth will open when touched by the swab.

Acknowledgements

Special thanks to Graham Morrissey (Director, BSF), Adam Hurlstone and Anthony Jackson.

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Alternative training method using a mouse simulator in intravenous lateral tail vein procedures

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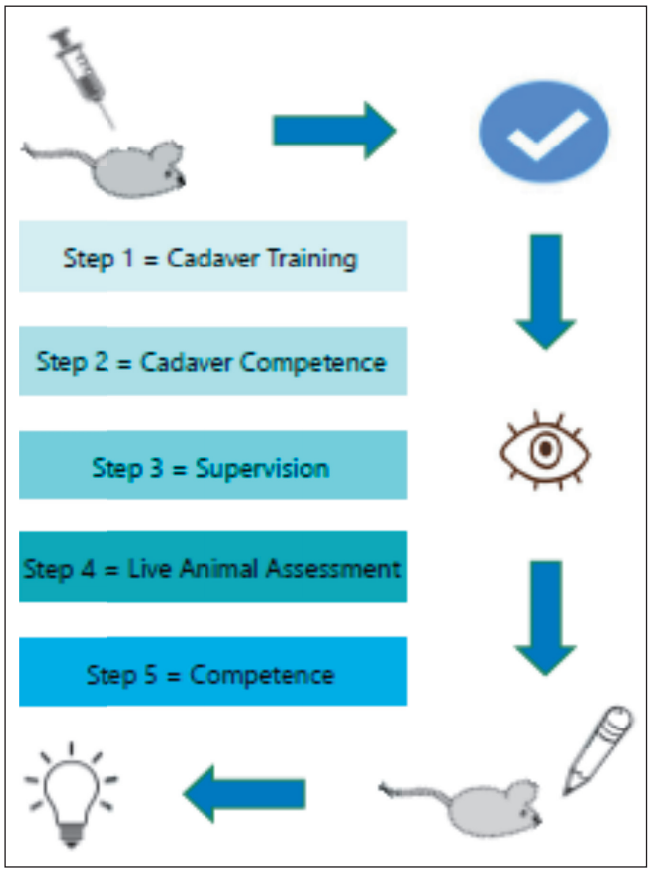
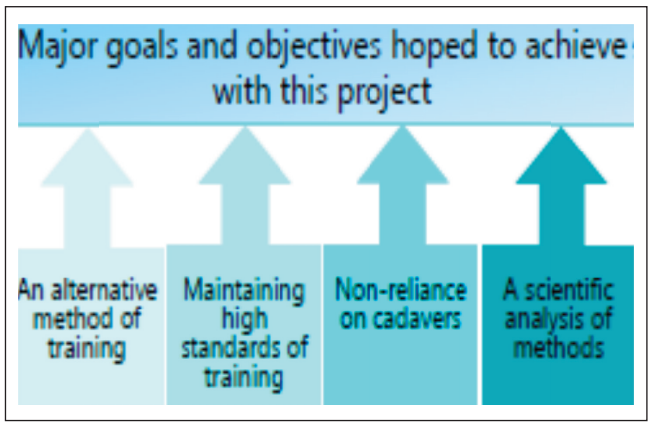
Introduction

The purpose of this study was to reduce or replace mouse cadavers for use in procedural training.

- Our small, in-house breeding colony was greatly reduced as a refinement due to unethical wastage.
- Our five-step training programme incorporates the use of cadavers in training towards competence.
- An alternative method was necessary to maintain high standards and for future planning should our in-house breeding colony dissolve.



Project Goals



Method

For this study we took 2 groups of 10 participants (a combination of researchers and technical staff).

This was a randomised, controlled trial with blinding at the assessment stage.

All participants had mixed experience in various technical procedures such as oral gavage, intraperitoneal injections, etc., but none had ever carried out intravenous injections of any kind.

An independent statistician was responsible for randomly allocating the participants into the two groups (cadaver and simulator).

The simulator used was a 'Mimicky Mouse' model purchased independently. This model has 2 lateral tail veins leading to a reservoir. An intravenous tail injection into these veins can be simulated using sterile water.

Training was carried out following a standard operating procedure written specifically to mimic the same procedure for both methods.

Training on both cadavers and simulators was carried out by the same trainer who determined when participants were ready for a competence assessment.

The assessor for competence was blinded to which method the participant had trained with.

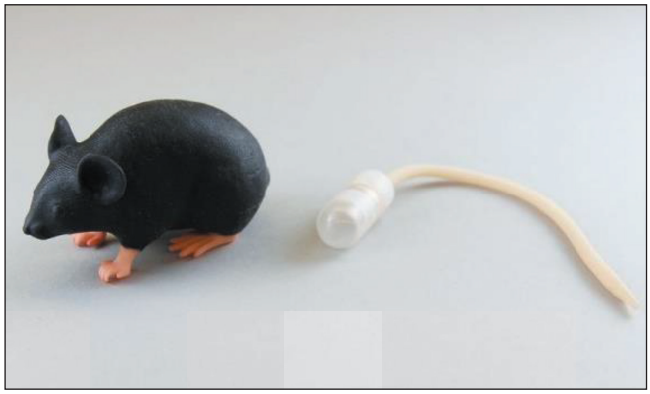


Figure 1. 'Mimicky Mouse' simulator.

Results

Competence was assessed by using a S1K method via injection into the lateral tail vein.

- this method allows for a clear assessment of competence
- it is a straight pass or fail
- results showed there appeared to be no significant difference between the simulator and cadaver[2]
- overall pass rates were lower than expected
- chi square p-value = 0.64

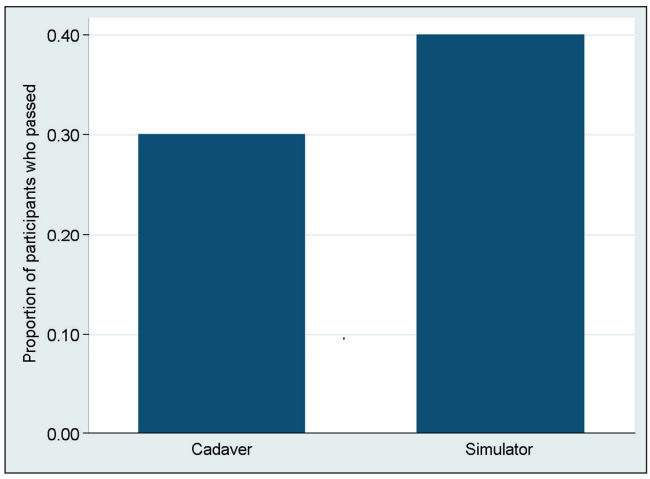


Chart 1. Comparison of pass rates for cadaver v simulator.

Conclusions

Much larger numbers would be required to ensure an equivalence level of success.

- It does demonstrate however that the simulator can be used as an alternative method of training with a level of success.
- Quality of the training was not reduced.
- This is a useful tool for facilities with no cadaver availability.
- The study supports the 3Rs objective to directly replace or avoid the use of animals (cadavers).
- Opens up the possibility of training in various procedures using a simulator.
- The market is currently limited in good quality simulators for research training.
- Organisations collaborating in training research could achieve statistical significance for various methods of training quality with simulators using these methods

Acknowledgements

I would like to thank:

All participants that took part in this study.

My colleagues in the BSF for supporting this study particularly James Gates who carried out the training.

The Wellcome Trust for funding.

LSHTM for allowing me to carry out this study.

References

1 www.procedureswithcare.org.uk/intravenous-injection-in-the-mouse/https://www.nc3rs.org.uk/the-3rs

2 **Douglas C Vines, David E Green, Gen Kido, Harald Keller** (2011) Evaluation of mouse tail-vein injections both qualitatively and quantitively on small animal PET tail scans, *J. NMT, vol 39, no 4, 264-270.*

3 https://www.vet-tech.co.uk/laboratory-supplies/training-simulator/mimicky-mouse-training-simulator.aspx

Surgery refinements improve success rates in rat bile collection

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Introduction

Rats are typically the rodent species of choice for investigating the absorption, distribution, metabolism and excretion of new chemical entities. In the absence of a gall bladder, they are ideal for researching the time course of excretion and metabolism of novel xenobiotics in bile.

This poster compares the model success rates using 3 different housing regimes during recovery from surgery.

The experimental study phase of all 3 methods remained unchanged – animals were tethered and singly housed in glass metabolism cages in order to separately collect bile and excreta and obtain an excretion balance for the duration of the study (up to 96 hours).

Only animals deemed to be healthy and with a good bile flow were used on study. Bodyweights and clinical signs including assessment of urine, faeces and bile output were recorded during the recovery period to monitor the health of the animals for animal welfare.

Methods

BILE AND DUODENUM CANNULATION SUCCESS RATES

Year		Model
1997-2000	Method 1	Dual Cannulation – Direct Catheters
2011-2016	Method 2	Dual Cannulation – Direct Catheters
2016-2019	Method 3	Dual Cannulation – PinPort™

Rat strains used in this poster were Harlan Hanover Wistar, Charles River Sprague Dawley and Hanover Wistar.

The same surgical technique was used for all 3 methods. Catheters were placed in the bile duct to collect bile; a second catheter was secured into the duodenum where artificial bile salts were infused. Both catheters were exteriorised via a tail cuff.[1]

Method 1 – Animals were tethered and singly housed in glass metabowls from surgery until the end of study (not performed at this establishment).[2]

Method 2 – Animals were allowed to recover from surgery, singly housed in a standard cage whilst being tethered. They were transferred to a glass metabowl the day before dosing.[3]

Method 3 – A modified tail cuff was developed which enabled both catheters to be passed through the tail cuff and connected to the dual PinPort™. The U-shaped loop was connected to the dual port permitting the animal to recirculate its own bile back into the duodenum during the recovery period. The port was protected by a cap screwed onto the tail cuff. This modification meant that animals did not need to be tethered and could be group housed with standard environmental enrichment in standard caging. It also enabled the recovery period to be extended and therefore to allow the animals more time to recuperate from the surgical procedure.

Following surgery, the animals were returned to group housing and their health and welfare monitored during the recovery period (Figure 1).[4]

When bile was to be collected for analysis, the U-shaped loop was removed from the port within the tail cuff adapter (Figure 2) and a dual tether fixed onto the dual port. The exteriorised catheters from the tether were connected to a dual stainless steel swivel device. Animals were then singly housed in metabolism cages to enable bile, urine and faeces to be collected for periods of up to 96 hours (Figure 3). Artificial bile salts were infused through the duodenal cannula to replace the bile collected, animals were continuously tethered for the duration of the study.

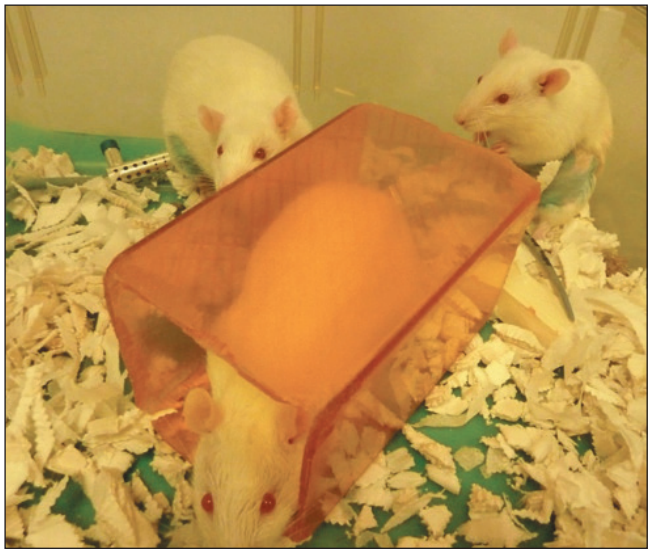

Figure 1. Group-housed animals that are untethered with the PinPort™ cannula transferring the animal's own bile between the bile duct and duodenum.

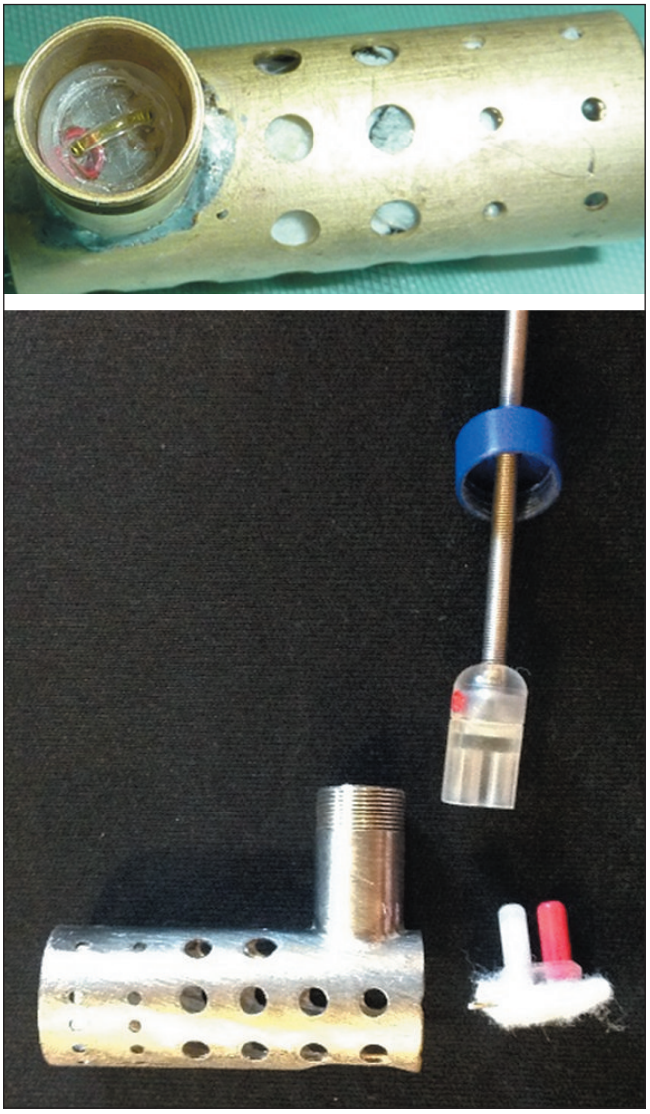

Figure 2. The PinPort™ tail cuff in-situ during the recirculation phase.

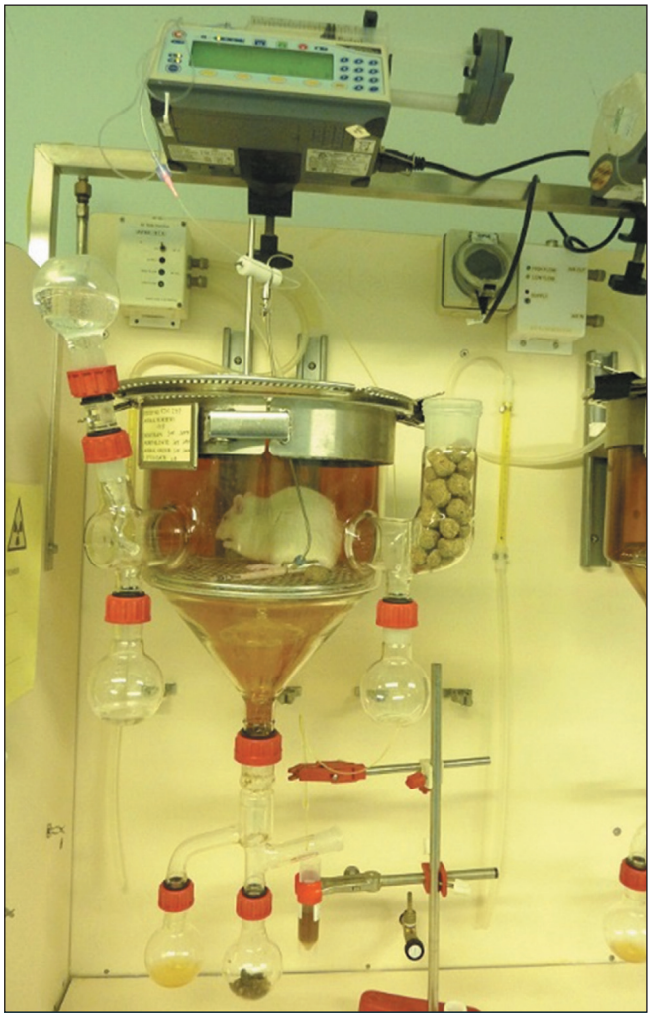

Figure 3. Tethered rat in a metabolism cage during the sample collection period.

Results

The surgery success rate for method 1 was not recorded, surgery success rates for methods 2 and 3 were >99%.

Table 1 shows the percentage of animals suitable for starting the study and the percentage of animals completing the study.

Tethering/housing conditions and associated success rates are shown in Tables 2 and 3.

Comparison of procedural issues between method 2 and method 3: Table 4.

The dropout rate during the surgery/recovery period for method 2 was 13%, against 10.1% for method 3.

The recovery period has been extended from 3 days (method 2) to 6 days (method 3).

Extending the length of the recovery/study period to 10 days for method 3 increases amount of time for problems to occur.

BILE DUCT (BILE AND DUODENUM) CANNULATION SUCCESS RATES

Year		Model	No. of Animals Prepared	% Suitable for Study	% Achieving End of Study
1997-2000	Method 1	Dual Cannulation – Direct Catheters	266	74	Not Recorded
2011-2016	Method 2	Dual Cannulation – Direct Catheters	392	87	82
2016-2019	Method 3	Dual Cannulation – PinPort™	288	90	77

Table 1. Bile Duct (Bile and Duodenum) Cannulation Success Rates

Number of days	Single Housed in Glass Metabowls	Continuously Tethered in Standard Cage	Group Housed with Env. Enrichment	Study – Single Housed in Glass Metabowls
Method 1	5	0	0	3
Method 2	0	3	0	3
Method 3	0	0	6	4

Table 2. Bile Duct (Bile and Duodenum) Cannulation Success Rates

	No. Animals Surgically Prepared		
Method 1	266	Continuously Tethered, Single Housed in Glass Metabowls	
		5 Day Recovery (76% Success Rate)	Not Recorded
Method 2	392 (3)	Continuously Tethered in Home Cage (No Environmental Enrichment)	Single Housed in Glass Metabowls
		3 Day Recovery (87% Success Rate)	Average 72 Hour Study (94% Success Rate)
Method 3	288 (1)	Standard Caging, Group Housing, Standard Environmental Enrichment	Single Housed in Glass Metabowls
		6 Days Recovery (90% Success Rate)	Average 96 Hour Study (85% Success Rate)

Table 3. Success Rates in Tethering/Housing Condition Post-Surgery

	2011-2016 - Direct Catheters		2016-2019 - PinPort ™	
	During Recovery	During Study	During Recovery	During Study
Died during surgery	3 (0.8)		1 (0.3)	
Incorrect port connection				1 (0.4)
Snapped catheter	4 (1.0)	1 (0.3)	3 (1.0)	3 (1.2)
Bile leaking from tail cuff (inc. catheter detached from port)			6 (2.1)	3 (1.2)
Tether/catheter chewed	16 (4.1)	7 (2.1)	1 (1.0)	1 (0.4)
No bile flow	10 (2.6)	0		20 (7.7)
Poor condition/weight loss or both	16 (4.1)	10 (2.9)	16 (5.6)	8 (3.1)
Found dead	1 (0.3)	1 (0.3)	2 (0.7)	
Unknown	1 (0.3)			
Mis-dosed		2 (0.6)		2 (0.8)
Total	51 (13.0)	21 (6.2)	29 (10.1)	38 (14.8)

Table 4. Surgery and Study-Associated Issues - Number of Animals (%)

The overall dropout during surgery, recovery and study periods for method 2 (over 6 days) is 12% per day, compared to method 3 (over 10 days) 6.7% per day.

The incidence of poor condition/bodyweight loss after surgery and the number of snapped catheters were similar for methods 2 and 3.

The number of losses attributed to the permanent tethering of animals in method 2 were higher due to the difficulty of performing repairs to chewed tethers although animal losses due to no bile flow was lower in method 2 compared to method 3. Note that in method 2 no connectors were used. All losses were euthanised in accordance with humane end points.

During the development of method 3, a number of animals were lost due to the catheter becoming detached from the port during tethering, this reduced over time as experience with the method was gained. It has been observed that bile from animals was still flowing when the connector was removed from catheter at necropsy.

However it is clear that the welfare improvements in method 3 are enormous as evidenced by the improved bodyweight gain in PinPort™ animals (method 3) when compared to the other methods[4].

The surgical success (measured by the number of animals producing acceptable bile flow after a recovery period) was comparable to the continuously tethered model (method 2).

Conclusions

Surgical success rate, reduction in bodyweight losses, comparable animal health observation, and acceptable bile flow show that the PinPort™ model hugely improves animal health and welfare without infringing scientific integrity[4].

References

1 **R. Remie, J.W. Rensema, G.H.J. Van Wunnik and J.J.Van Dongen:** Permanent double bile fistula (with intact enterohepatic circulation). In: Manual of microsurgery on the laboratory rat, (eds. R. Remie, J.W. Rensema, G.H.J. Van Wunnik and J.J.Van Dongen), Elsevier Science Publishers B.V. (Biomedical Division) Amsterdam, New York, Oxford. Ch. 10, pp 201-213 (1990).

2 **H. Van Wijk, P. Donachie, D.L. Mann, H. McMahon and D. Robb (2001)** A novel bile duct cannulation method with tail cuff exteriorization allowing continuous infusion and enterohepatic recirculation in the unrestrained rat. Laboratory Animals (2001) 35, 325-333.

3 **N. Burden, J. Kendrick, L. Knight, V. McGregor, H. Murphy, M. Punler, H. van Wijk** (2017). Maximizing the success of bile duct cannulation studies in rats: recommendations for best practice. Laboratory Animals, 51(5): 457-464.

4 **H. van Wijk[1], L. Crossman[1], G. Adjin-Tettey[1], D. Haida[1], J. Kendrick[1], E. Newell[1], S. Korte[2] and L. Wright[1]:** In Courtesy of the 3Rs: How to Avoid Single Housing of Bile Duct Cannulated Rats in ADME Studies, Using a Modified Tail Cuff. [1]Covance Laboratories Ltd, Harrogate, UK; [2]Covance Preclinical Services GmbH, Münster, Germany Presented at SOT San Antonio, The Toxicologist, Vol:150 (1):493 (PS, No 3104).