News & Views

NC3Rs Awards

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) has announced the winner of the 2011 3Rs prize. The annual prize is awarded to a UK-based scientist for Three Rs research published in a peer-review journal in the past three years. The 2011 entries were of such high quality that two 'Highly Commended' prizes were also awarded.[1]

The main prize went to Dr Ludovic Vallier, for work published in *The Journal of Clinical Investigation* which described a protocol for creating hepatocytes from induced pluripotent stem cells (iPSCs). Skin cells taken from patients suffering from hepatic metabolic disorders were used to generate iPSCs, from which liver cells could then be produced and used as *in vitro* models of inherited liver diseases.[2]

The 'Highly Commended' prizes were awarded to Dr Anna Williams and to Dr Stephen Pettitt, for work published in *Experimental Neurology* and *Nature Method*s, respectively. Dr Williams reported the development and characterisation of a brain and spinal cord *ex vivo* culture system for studying the mechanisms behind the loss and repair of the myelin sheath, a method that could be useful to screen therapies for multiple sclerosis.[3] Dr Pettitt described a technique for producing genetically modified mice, that has the potential to significantly reduce the number of animals required.[4]

[1] Anon. (2012). *Prizes awarded for new research improving animal welfare in the life sciences*. [Wellcome Trust, 12.03.12]. Available at: http://www.wellcome.ac.uk/News/2012/News/WTVM054637.htm (Accessed 20.04.12).

[2] Rashid, S.T., Corbineau, S., Hannan, N., Marciniak, S.J., Miranda, E., Alexander, G., Huang-Doran, I., Griffin, J., Ahrlund-Richter, L., Skepper, J., Semple, R., Weber, A., Lomas, D.A. & Vallier, V. (2010). Modelling inherited metabolic disorders of the liver using human induced pluripotent stem cells. *Journal of Clinical Investigation* **120**, 3127–3136.

[3] Zhang, H., Jarjour, A.A., Boyd, A. & Williams, A. (2011). Central nervous system remyelination in culture — a tool for multiple sclerosis research. *Experimental Neurology* **230**, 138–148.

[4] Pettitt, S.J., Liang, Q., Rairdan, X.Y., Moran, J.L., Prosser, H.M., Beier, D.R., Lloyd, K.C., Bradley, A. & Skarnes, W.C. (2009). Agouti C57BL/6N embryonic stem cells for mouse genetic resources. *Nature Methods* **6**, 493–496.

New Three Rs Projects Funded

The 3R Research Foundation Switzerland has awarded a total of CHF 382,000 to four new projects.[1] One of the projects, led by Professor Hunziker from the University of Berne, Switzerland, aims to develop a novel *in vitro* organ slice model for reproducing the repair of meniscal lesions in orthopaedic research. The model would use the meniscuses of cows slaughtered at a local abattoir and, if successful, would contribute to reducing the time and cost required for pre-clinical studies. A second grant was awarded to Professor He (Children's Hospital, Boston, MA, USA) for a project that proposes the use of microfluidic chambers to mimic nerve injury and regeneration *in vitro*, and in particular, for studying the influence of *PTEN* and *SOCS3* on axonal regeneration. Another of the projects funded, directed by Dr Thiel (Kantonsspital St Gallen, St Gallen, Switzerland), aims to render cultures of human airway epithelium amenable to genetic modification, which would permit detailed analyses of virus–host interactions at the molecular level, and would limit the need for genetically modified murine models of viral infection. Finally, a project led by Dr Kaufmann (University of Berne, Berne, Switzerland), aims to establish an *in vitro* system for the production of murine basophils. Since basophils are a rare population of leucocytes, this method could potentially avoid the sacrifice of large numbers of mice.

[1] Anon. (2012). *3R Research Foundation Switzerland*. Available at: http://www.forschung3r.ch/en/projects/index.html (Accessed 24.04.12).

Improving Housing Conditions for Laboratory Mice

Laboratory mice are routinely housed at temperatures below their optimum environmental temperature. The resulting thermal stress might influence their physiology and behaviour, impacting on the validity of the experimental data obtained.

Laboratory mice are usually housed at 20–24°C, while their preferred temperature is close to 30°C. Since their metabolic demands are higher at lower temperatures, mice eat about 60% more at 20–24°C than at 30°C, to satisfy their increased energy requirements. A down-

side to increasing ambient temperature in animal housing is that warmer temperatures increase aggression, but offering a range of temperatures within a cage is currently impractical. In the wild, mice build nests to cope with variations in temperature, but, in the laboratory, they have restricted access to nesting material. A paper published in *PLoS One* reports the use of the behavioural titration technique, involving the housing of mice in cages at different temperatures, for determining the amount of nesting material the animals require for any potential thermal discomfort to be overcome. According to the results of the study, for common laboratory strains (naïve CD-1, BALB/c and C57BL/6) housed under normal laboratory temperatures, the animals should have access to between 6–10g of nesting material.[1]

[1] Gaskill, B.N., Gordon, C.J., Pajor, E.A., Lucas, J.R., Davis, J.K. & Garner, J.P. (2012). Heat or insulation: Behavioral titration of mouse preference for warmth or access to a nest. *PLoS One* **7**, e32799.

Flaws in Laboratory-controlled Circadian Rhythms

Fruit flies have been routinely used as the model system for studying eukaryotic circadian clocks, but key laboratory-based assumptions about circadian behaviour do not appear to be supported by observations made in the wild.[1]

In a laboratory setting, flies are exposed to light–dark cycles and are more active before 'light on' and 'lights off' signals (i.e. dawn and dusk). Hence, it was accepted that morning and evening circadian oscillators controlled their behaviour. However, when observed in the wild, flies have a different activity pattern: they are most active during the day and have a burst of activity in the middle of the day (as opposed to a 'siesta' in the laboratory). Surprisingly, flies that have mutated circadian clocks and that show altered behaviour in the laboratory, do not show abnormal rhythms in the wild.

This study suggests that fly behaviour in the wild is quite different from that seen under controlled laboratory conditions, and that some features are impossible to observe in such a simplified environment.[2] By adjusting the temperature and light conditions in the laboratory to more realistically reproduce the flies' outdoor environment, natural circadian rhythms could be observed under these controlled conditions.

[1] Vanin, S., Bhutani, S, Montelli, S., Menegazzi, P., Green, E.W., Pegoraro, M., Sandrelli, F., Costa, R. & Kyriacou, C.P. (2012). Unexpected features of *Drosophila* circadian behavioural rhythms under natural conditions. *Nature, London* **484**, 371–375.

[2] Scudellari, M. (2012). *Lab studies lie about the clock*. [The Scientist, 04.04.12]. Available at: http://the-scientist.com/2012/04/04/lab-studies-lie-about-the-clock/ (Accessed 20.04.12).

In Vitro Model of Bladder Infection

An *in vitro* human bladder cell infection model, which has potential to replace animal experiments, has been developed by Andersen and colleagues.[1]

Uropathogenic *Escherichia coli* (UPEC) are able to infect bladder epithelial cells (BECs) on the bladder luminal surface. Studies on mouse models suggest that a particular chain of events might take place: bacteria colonise BECs and proliferate intracellularly; then, elongated, filamentous bacteria escape and, on the bladder epithelium, revert to their rod-shape; finally, these rod-shaped bacteria infect and multiply in neighbouring BECs.

By using a flow chamber-based cell culture system to mimic the conditions that bacteria experience *in vivo*, it was possible to induce an UPEC infection *in vitro*. Bacteria successfully colonised cells on the flow chamber–BEC layer. Exposure to a flow of urine, which UPEC face when re-emerging on the bladder luminal surface, led to the outgrowth of filamentous bacteria that could revert to a rod-shape capable of colonising other BECs. This novel *in vitro* model permits late-stage events of the uropathogenic cascade that had only been studied on animals to be investigated in a human BEC system.

[1] Andersen, T.E., Khandige, S., Madelung, M., Brewer, J., Kolmos, H.J. & Møller-Jensen, J. (2012). *Escherichia coli* uropathogenesis *in vitro*: Invasion, cellular escape, and secondary infection analyzed in a human bladder cell infection model. *Infection & Immunity* **80**, 1858–1867.

Blood–Retinal Barrier Model

Diabetic macular oedema is a retinopathy that can occur as a complication of diabetes. While changes to the properties of the blood–retinal barrier (BRB) are known to play a part in the pathology, the molecular mechanisms underlying BRB dysfunction are still unknown. To overcome these shortcomings, Wisniewska-Kruk *et al.* have recently developed and characterised a novel *in vitro* BRB model.[1]

The model was based on primary bovine retinal endothelial cells (BRECs), which maintained

BRB properties, as demonstrated by the expression levels of the endothelial junction proteins, occludin, claudin-5, VE-cadherin and ZO-1, and the specific pumps, glucose transporter-1 (GLUT1) and efflux transporter P-glycoprotein (MDR1). In order to assess the influence of other cell types, namely, pericytes and astrocytes, on the barrier properties of the BRB, different co-culture models were compared. Co-cultures of BRECs with bovine retinal pericytes and rat glial cells showed the highest trans-endothelial resistance, and decreased permeability after vascular endothelial growth factor (VEGF) stimulation. To reproduce the *in vivo* situation, BRECs were exposed to VEGF, resulting in the VEGF-dependent cellular alterations that occur in diabetic macular oedema, such as increased expression of plasmalemma vesicle-associated protein in endothelial cells. In addition, endothelial junction proteins showed reduced expression levels and an abnormal organisation — the numbers of intercellular gaps in BRECs monolayers were elevated, and the mRNA levels of GLUT-1 and MDR1 were down-regulated.

[1] Wisniewska-Kruk, J., Hoeben, K.A., Vogels, I.M., Gaillard, P.J., Van Noorden, C.J., Schlingemann, R.O. & Klaassen, I. (2012). A novel co-culture model of the blood–retinal barrier based on primary retinal endothelial cells, pericytes and astrocytes. *Experimental Eye Research* **96**, 181–190.

Advances in 3-D Tumour Model Systems

Advances in three-dimensional (3-D) tumour models, combined with microfluidic systems, are permitting drug delivery and resistance studies to be performed *in vitro*. A 3-D microscale perfusion-based two-chamber tissue model was developed to test the cytotoxicity of anticancer drugs in combination with hepatic metabolism. To achieve this purpose, liver cells and brain cancer cells were grown in each of the chambers, which were connected in tandem, and contained a scaffold made from biodegradable poly(lactic acid) by using a solvent-free method. In the presence of the liver cells, the drug, temozolomide, was less toxic to the cancer cells. Furthermore, cells grown as 3-D cultures were found to be more resistant to the effects of the drug than those grown in two dimensions. By using liver cells with different cytochrome P450 subtypes, metabolism-dependent cytotoxicity against cancer cells was seen for the anti-cancer drug, ifosfamide.[1]

However, for some *in vitro* culture systems, non-uniform distribution of cells is still a problem, and the 3-D structures might end up with regions of varying cell density. This, in turn, can lead to differences between molecular diffusion in the *in vitro* cell compartments as compared to solid tumours. A new technique for developing *in vitro* 3-D tumour models, based on a microfluidic device containing three parallel channels separated by narrowly spaced posts, might avoid this shortcoming. The system was tested by loading melanoma cells into the central channel at high density, then perfusion-culturing them overnight. Afterwards, the viability, density and diffusion properties of the resulting 3-D structures were assessed. This tumour model shows encouraging potential for use in *in vitro* studies of transport and cellular uptake.[2]

[1] Ma, L., Barker, J., Zhou, C., Li, W., Zhang, J., Lin, B., Foltz, G., Küblbeck, J. & Honkakoski, P. (2012). Towards personalized medicine with a three-dimensional micro-scale perfusion-based two-chamber tissue model system. *Biomaterials* **33**, 4353–4361.

[2] Elliott, N.T. & Yuan, F. (2012). A microfluidic system for investigation of extravascular transport and cellular uptake of drugs in tumors. *Biotechnology & Bioengineering* **109**, 1326–1335.

Human *In Vitro* Angiogenesis Model

A recently developed tissue-engineered *in vitro* angiogenesis model includes pericytes and endothelial cells, an interaction that is essential *in vivo* for the maturation and stabilisation of blood microvessels. The study, which was described in the *Journal of Cellular Physiology*, used a human endothelialised reconstructed connective tissue model that promoted the formation of a three-dimensional branching network of capillary-like tubes (CLT) with closed lumens, to determine whether pericytes were spontaneously recruited around the CLT. By using green fluorescent protein-positive fibroblasts and endothelial cells, the authors showed that pericytes were recruited from the population of fibroblasts in the model, and that the system promoted the spontaneous formation of a network of capillaries and the recruitment of pericytes derived from fibroblasts.[1]

[1] Berthod, F., Symes, J., Tremblay, N., Medin, J.A. & Auger, F.A. (2012). Spontaneous fibroblast-derived pericyte recruitment in a human tissue-engineered angiogenesis model *in vitro*. *Journal of Cellular Physiology* **227**, 2130–2137.

Dilated Cardiomyopathy Studied *In Vitro*

An *in vitro* stem cell culture system has been developed for studying dilated cardiomyopathy

(DCM), an important form of heart disease.[1] DCM is characterised by ventricular dilation, systolic dysfunction and progressive heart failure, that can lead to a requirement for heart transplantation.

Although the origin of the disease is not always clear, it can be caused by inherited genetic mutations. To create the *in vitro* model, skin cells were taken from DCM patients known to carry a point mutation in the gene encoding the sarcomeric protein, cardiac troponin T; cells from unaffected family members were subjected to the same treatment and used as the controls. Induced pluripotent stem cell (iPSC) technology was employed to convert skin cells to stem cells, which were then encouraged to become heart muscle cells. Compared to the control cardiomyocytes, cells derived from DCM patients showed structural alterations and were more prone to mechanical stress. They were also less responsive to Ca^{2+} signalling, and had reduced contractibility. When the diseased cells were treated with β-blockers, some signs of recovery were visible. In addition, overexpression of a protein (sarcoplasmic reticulum Ca^{2+} adenosine triphosphatase; Serca2a) that is currently undergoing clinical trials as a possible gene therapy for DCM, improved the function of DCM cells.

According to the senior author of the study,[2] Professor Wu, primary human cardiac cells are difficult to obtain and cannot be grown for long periods in the laboratory. Cardiac drugs are tested *in vitro* for any potential toxic effects on cell lines derived from hamster ovaries or human embryonic kidney cells. Although such cells have been induced to reproduce the electrophysiology of human myocytes, a reliable source of human healthy and diseased heart cells would be a great asset for use in investigating disease mechanisms and testing potential therapies.

[1] Sun, N., Yazawa, M., Liu, J., Han, L., Sanchez-Freire, V., Abilez, O.J., Navarrete, E.G., Hu, S., Wang, L., Lee, A., Pavlovic, A., Lin, S., Chen, R., Hajjar, R.J., Snyder, M.P., Dolmetsch, R.E., Butte, M.J., Ashley, E.A., Longaker, M.T., Robbins, R.C. & Wu, J.C. (2012). Patient-specific induced pluripotent stem cells as a model for familial dilated cardiomyopathy. *Science Translation Medicine* **4**, 130ra47.

[2] Conger, K. (2012). *Scientists show that lab-made heart cells can be ideal model for disease research, drug testing*. [Inside Stanford Medicine, 18.04.12]. Available at: http://med.stanford.edu/ism/2012/april/heartbeat.html (Accessed 19.04.12).

Model of Bacterial Intestinal Infection

A vertical diffusion chamber (VDC) model system that reflects the low oxygen conditions of the intestinal lumen, has been successfully employed to mimic *Campylobacter jejuni* intestinal infection *in vitro*.[1]

Intestinal epithelial cell (IEC) lines have been used for studying *C. jejuni* infection, but the experimental conditions have not been accurate enough, and the *in vitro* infection models have not reflected clinical symptoms of the infection. These shortcomings might be due to the atmospheric conditions under which the experiments are conducted, as *C. jejuni* is a microaerophilic bacterium and the intestinal lumen is a low-oxygen environment. The model system described by Mills *et al.* relies on a VDC to create microaerobic conditions at the apical surface and aerobic conditions at the basolateral surface of cultured IECs, thereby more-closely mimicking the conditions in the human intestine. Compared to an aerobic environment, there was increased bacterial interaction with the intestinal cells and a corresponding host immune response. By testing a non-motile *C. jejuni* mutant in the model, motility was shown to be an important factor during the initial stages of the infection.

[1] Mills, D.C., Gundogdu, O., Elmi, A., Bajaj-Elliott, M., Taylor, P.W., Wren, B.W. & Dorrell, N. (2012). Increase in *Campylobacter jejuni* invasion of intestinal epithelial cells under low-oxygen coculture conditions that reflect the *in vivo* environment. *Infection & Immunity* **80**, 1690–1698.