News & Views

Drosophila as a Model for Ayurvedic Medicine

Ayurveda, the traditional system of medicine in India, is growing in popularity worldwide. Details on the procedural and mechanistic aspects of Ayurvedic therapy are absent from the scientific literature, partly due to a lack of adequate *in vivo* models in which formulations, rather than 'active components', can be tested. A report in PLoS One[1] proposes the fruit fly, *Drosophila melanogaster*, as a suitable *in vivo* model for testing Ayurvedic formulations according to modern scientific methods. The study employed a minimum of 200 flies, or larvae, for each assay, and tested two categories of formulations — a *Rasayana* (an herbal derivative) and a *Bhasma* (an organo-metallic derivative of mercury) — for their effects on longevity, development, fecundity, stress-tolerance, and heterogeneous nuclear ribonucleoprotein (hnRNP) levels.

According to the authors of the study,[2] the formulation-specific effects observed on several parameters of the life of the flies were in agreement with the recommended human usages in Ayurvedic practices. Thus, *Drosophila* appears to be a good model for examining the cellular and molecular basis of the effects of different Ayurvedic formulations.

[1] Dwivedi, V., Anandan, E.M., Mony, R.S., Muraleedharan, T.S., Valiathan, M.S., Mutsuddi, M. & Lakhotia, S.C. (2012). *In vivo* effects of traditional Ayurvedic formulations in *Drosophila melanogaster* model relate with therapeutic applications. *PloS One* **7**, e37113.
[2] Anon. (2012). *Ayurveda gets animal model* [Nature India, 15.05.2012]. Available at: http://www.nature.com/nindia/2012/120515/full/nindia.2012.73.html (Accessed 21.06.12).

Neonatal Tissue Culture Model for Vaccine Testing

The immune system of newborns does not respond well to many paediatric vaccines, rendering the babies susceptible to preventable infections. One of the limitations in the development of vaccines for neonates has been a lack of suitable models for determining age-specific human immune responses, that could help predict safety and efficacy in this particular group. To overcome this, Sanchez-Schmitz and colleagues assembled a human artificial immune system, the Neonatal Tissue Construct (NTC), by using blood from human umbilical veins as a source of endothelial cells and monocytes, human extracellular matrix, and 100% autologous intact plasma.[1] The system was tested with the live mycobacterial BCG vaccine, which is known to be safe and effective at birth. The NTC responded in the same way that newborns have in clinical trials — monocytes differentiated into mature dendritic cells (DCs) that were capable of inducing effector T-cell responses. In addition, while newborn and adult DC/T-cell co-cultures secreted IL-2 and TNF-α in response to BCG, only newborn cells demonstrated constitutive IL-4 production and a marked impairment in IFN-γ production.

This research was presented at the American Association of Immunologists' annual meeting, in Boston (MA, USA).[2]

[1] Hamzelou, J. (2012). Artificial immune system gives baby vaccines a booster. *New Scientist* **2866**, 14.
[2] Sanchez-Schmitz, G., Stevens, C., Baecher-Allan, C. & Levy, O. (2012). A novel human Neonatal Tissue Construct (NTC) models age-specific immune responses to Bacille Calmette-Guérin (BCG) vaccine. *Journal of Immunology* **188**, 166.27.

Organ On-a-chip

A gut-on-a-chip device that mimics the structure and physiology of the human intestine has been described in *Lab Chip*.[1] The *in vitro* model reproduces, in a controlled microfluidic environment, characteristics of the human intestine that are essential for its function, and represents a system that could be useful for studying intestinal diseases or for testing drugs.

The system is composed of two microfluidic channels, separated by a membrane coated with extracellular matrix, and lined with human intestinal epithelial (Caco-2) cells. To recreate the gut microenvironment, low shear stress was produced by liquid flowing through the channel, and peristaltic motions were mimicked by exerting cyclic strain. These conditions permitted the development of a columnar polarised epithelium, which grew into folds and formed a high-integrity barrier to small molecules. The resulting structure mimics the whole intestine more accurately than cells grown under static conditions. In addition, an intestinal bacterium, *Lactobacillus rhamnosus* GG, could be co-cultured on the lumi-

nal surface of the epithelium for periods exceeding a week. The presence of the bacterium did not affect cell viability, and, as previously observed in human studies, barrier function was improved.

[1] Kim, H.J., Huh, D., Hamilton, G. & Ingber, D.E. (2012). Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip* **12**, 2165–2174.

Non-invasive Genotyping of Animals

In order to determine the genotype of transgenic animals, genomic DNA has to be extracted from cells. The most common method for obtaining tissue for this purpose is through biopsy of certain tissues, for example, the tail, ear or toe, and the procedure may cause pain and distress to the animals involved. Although DNA samples can also be obtained from sloughed intestinal epithelial cells present in stool samples, the necessary DNA extraction methods are expensive or require the use of toxic organic solvents. In addition, they also suffer from poor reliability, as stool material contains PCR inhibitors and an excess of bacterial DNA. To promote a wider adoption of this source of cells — which is recommended by many IACUC guidelines because it is non-invasive — a cost-effective, non-toxic and reliable method has been described by Chen et al.[1] This novel method uses a particular reagent, AquaStool, to prevent PCR contaminants from binding to faecal DNA, thus allowing their removal by centrifugation. This procedure can be used on animals of any age and physiopathological condition, and has the potential for improving the welfare of transgenic animals.

For genotyping animals with systemic expression of vital fluorophore markers, another non-invasive protocol has been described in *BioTechniques*.[2] This method is based on the quantitative measurement of the fluorescence intensity in hair samples, and, since it does not require the removal of hair roots, it should cause only minimal stress to the animals. The processing and evaluation of the hair samples can be completed in less than an hour, and the hair samples can be preserved for long-term storage at ambient temperatures. In addition, multiple samplings and analyses are possible, allowing the effects of environmental conditions or ageing on transgene expression to be investigated.

[1] Chen, Z., Mantha, R.R., Chen, J.S., Slivano, O.J. & Takahashi, H. (2012). Non-invasive genotyping of transgenic animals using fecal DNA. *Lab Animal* **12**, 10–19.
[2] Garrels, W., Cleve, N., Niemann, H. & Kues, W.A.

(2012). Rapid non-invasive genotyping of reporter transgenic mammals. *BioTechniques Rapid Dispatches* **May**. Available at: http://www.biotechniques. com/multimedia/archive/00180/BTN_A_000113874 _O_180319a.pdf (Accessed 26.06.12).

Endothelial Cells Show Differences

Endothelial cells are important in the pathogenesis of many types of infections, and are widely used in *in vitro* experiments aimed at understanding, for example, how microbes interact with the cells to cause bloodstream infections.

A particular type of endothelial cells, human umbilical vein endothelial cells (HUVECs), is often used for *in vitro* studies of infections by *Candida albicans* and *Staphylococcus aureus*, but HUVECs have a relatively short lifespan in culture, and exhibit significant donor-to-donor variation, and their availability can be limited by medical and ethical issues. To overcome these difficulties, immortalised endothelial cells have been developed and used instead of primary cells — HMEC-1, an immortalised cell line from dermal human microvascular endothelial cells, is such an example. However, when the interactions of *C. albicans* and *S. aureus* with HUVECs and HMEC-1 cells were compared, both pathogens interacted with HMEC-1 cells in a significantly different manner as compared to HUVECs: the yeast showed significantly reduced adherence to and invasion of HMEC-1 cells; HMEC-1 cells were less susceptible to damage induced by *C. albicans*, but more susceptible to damage caused by *S. aureus*; and HMEC-1 cells secreted low amounts of IL-8 in response to infection with either organism, whereas infection of HUVECs induced substantial IL-8 secretion.

Therefore, according to the authors of the paper,[1] "*C. albicans* and *S. aureus* interact with HMEC-1 cells in a substantially different manner than with HUVECs, and data obtained with one type of endothelial cell cannot necessarily be extrapolated to other types".

[1] Seidl, K., Solis, N.V., Bayer, A.S., Hady, W.A., Ellison, S., Klashman, M.C., Xiong, Y.Q. & Filler, S.G. (2012). Divergent responses of different endothelial cell types to infection with *Candida albicans* and *Staphylococcus aureus*. *PloS One* **7**, e39633.

Brain Tumour Biomarkers Identified

A team from The University of Nottingham (Nottingham, UK) has identified genetic markers that hold promise as new diagnostic tools

for a rare and aggressive type of brain tumour.[1]

The study was led by Professor Richard Grundy at the University's Children's Brain Tumour Research Centre, and focused on central nervous system primitive neuro-ectodermal tumours (CNS PNET), for which the molecular characteristics and the best treatment options are unknown. Despite the need for more effective therapy, little research has been done on this type of cancer, because it is quite rare. The researchers obtained 142 CNS PNET samples from 20 institutions in nine countries. Based on the gene expression of two markers, LIN28 and OLIG2, three molecular subgroups were identified: primitive neural (group 1), oligoneural (group 2), and mesenchymal lineage (group 3). When age, survival and metastases were considered, group 1 tumours tended to affect the youngest patients and had the poorest survival rates; patients with group 3 tumours had the most metastases at diagnosis.

According to Professor Grundy,[2] "an international effort was needed to bring sufficient numbers of cases together to make the breakthrough we needed to better understand this disease or indeed diseases identified in our study. The next step is to translate this knowledge into improving treatments."

Professor Grundy was a speaker at a FRAME-organised scientific meeting, and the proceedings of this meeting are freely available at: http://www.frame.org.uk/atla_issue.php?iss_id=117

[1] Picard, D., Miller, S., Hawkins, C.E., Bouffet, E., Rogers, H.A., Chan, T.S., Kim, S.K., Ra, Y.S., Fangusaro, J., Korshunov, A., Toledano, H., Nakamura, H., Hayden, J.T., Chan, J., Lafay-Cousin, L., Hu, P., Fan, X., Muraszko, K.M., Pomeroy, S.L., Lau, C.C., Ng, H.K., Jones, C., Van Meter, T., Clifford, S.C., Eberhart, C., Gajjar, A., Pfister, S.M., Grundy, R.G. & Huang, A. (2012). Markers of survival and metastatic potential in childhood CNS primitive neuro-ectodermal brain tumours: An integrative genomic analysis. *Lancet Oncology*. [Epub ahead of print.]

[2] Thorne, E. (2012). *Genetic markers hope for new brain tumour treatments*. [The University of Nottingham Press Releases, 15.06.12]. Available at: http://www.nottingham.ac.uk/news/pressreleases/2012/june/genetic-markers-hope-for-new-brain-tumour-treatments.aspx (Accessed 26.06.12).

Computer Model to Predict Side-effects

A computational method has been used to predict the side-effects of medications that have been approved for human use.[1] The technique employed, called the similarity ensemble approach, relies on a drug's chemical structure to determine the molecules it might bind to in the body. For reference, the researchers used a database of drug-like molecules and the human proteins that they are known to interact with.

The team, from the University of California and Novartis, selected 656 marketed drugs and 73 targets for adverse drug reactions. Overall, among the more than 47,000 possible drug–target pairs, 1,600 potential interactions were predicted. Of these, over 400 were previously known, and over 350 more were recorded in proprietary databases. Approximately 700 of the remaining predictions were tested experimentally: 151 were confirmed and 65 were ambiguous. To determine the clinical value of the findings, the newly-confirmed interactions were compared with data on more than 2,700 drugs, including their targets and any known side-effects. Of the 151 interactions, 82 were found to be significantly associated with at least one adverse effect.[2] This approach could assist in the identification of problems early during drug development and might help researchers to find new uses for approved drugs.

[1] Lounkine, E., Keiser, M.J., Whitebread, S., Mikhailov, D., Hamon, J., Jenkins, J.L., Lavan, P., Weber, E., Doak, A.K., Côté, S., Shoichet, B.K. & Urban, L. (2012). Large-scale prediction and testing of drug activity on side-effect targets. *Nature, London* **486**, 361–367.

[2] Wein, H. (2012). *Computer method predicts drug side effects*. [NIH Research Matters, 25.06.12]. Available at: http://www.nih.gov/researchmatters/june2012/06252012sideeffects.htm (Accessed 26.06.12).

Immune Differences Between Mice and Humans

Evolutionary changes in gene expression are thought to be behind many phenotypic differences between species. Taking this into consideration, the mouse and human transcriptional responses to a microbial insult have been compared.

Cultures of primary human and mouse macrophages were exposed to lipopolysaccharide (LPS), a Toll-like receptor agonist, and their gene expression pattern was monitored. The researchers used a platform that allowed cross-species interrogation, coupled with deep sequencing of mRNA 5′ ends, and found that extensive divergence in LPS-regulated gene expression existed between humans and mice. In fact, 24% of orthologous genes were considered to be 'divergently regulated' and were enriched for genes encoding cell surface receptors (e.g. TLR6, IL-7Rα) and inflammatory cytokines/chemokines (e.g. CCL20, CXCL13).

These differences can have functional conse-quences — for example, LPS promoted subse-quent TLR6 responses in mouse, but not in human, macrophages. An intriguing finding was that the genes most susceptible to divergent regulation tended to have complex and highly conserved promoters, as these can probably produce a wide variety of responses to environ-mental or evolutionary pressures, without themselves changing.

[1] Schroder, K., Irvine, K.M., Taylor, M.S., Bokil, N.J., Cao, K-A., Masterman, K-A., Labzin, L.I., Semple, C.A., Kapetanovic, R., Fairbairn, L., Akalin, A., Faulkner, G.J., Baillie, J.K., Gongora, M., Daub, C.O., Kawaji, H., McLachlan, G.J., Goldman, N., Grimmond, S.M., Carnici, P., Suzuki, H., Hayashizaki, Y., Lenhard, B., Hume, D.A. & Sweet, M.J. (2012). Conservation and divergence in Toll-like receptor 4-regulated gene expression in primary human *versus* mouse macrophages. *Proceedings of the National Academy of Sciences of the USA* **109**, E944–E953.
[2] Anon. (2012). *Of mice and men*. [A-IMBN Research, 27.06.12]. Available at: http://www.natureasia.com/A-IMBN/article.php?id=631 (Accessed 27.06.12).

Cholesterol Research with Zebrafish

Researchers have used zebrafish to shed light on the cellular mechanisms involved in cho-lesterol processing. The link between dietary fat and cholesterol uptake has been difficult to dissect, partly because the intestine is a complex system that includes a variety of cell types, mucus and symbiotic microorgan-isms.

According to Dr James Walters, who pre-sented the work at the International Zebrafish Development and Genetics Conference (Madi-son, WI, USA), larval zebrafish "provide a bio-logically complete and scientifically accessible system for studying the workings of the gut".[1] As the larvae are optically clear, it is possible to follow fat transport and processing through their body wall into the intestine. In order to view how lipids are absorbed and processed by the intestinal cells, fluorescently tagged cho-lesterol, or fatty acids, were added to the water before the larval fish were fed a diet high in lipids, or high in protein and low in lipids. The researchers found that cholesterol was only absorbed when the fish ate a high-lipid diet, not a low-lipid diet. The fats and cholesterol were packaged into separate and clearly visi-ble compartments within the cells. In addi-tion, some long-chain fatty acids, particularly oleic acid, appeared to be very effective at pro-moting cholesterol uptake, acting to drive a

cholesterol uptake protein to the surface of intestinal cells. Current research is exploring the use of this system for drug testing.

[1] Genetics Society of America (2012). *Zebrafish research shows how dietary fat regulates choles-terol absorption*. [ScienceDaily, 23.06.12]. Available at http://www.sciencedaily.com/releases/2012/06/120623094417.htm (Accessed 25.06.12).

Selected *ATLA* Papers Available on InterNICHE Database

As reported in the March issue of *ATLA*,[1] InterNICHE has developed a new website on alternatives to animal experiments in medical, veterinary medical and biological science educ-ation and training (http://www.interniche.org/alternatives).

One of the website's database resources is the free-access InterNICHE *Studies Database* (http://www.interniche.org/studies), which aims to improve access to information that can support curricular transformation and replacement of animal experiments. It includes references, abstracts and other details for over 750 pub-lished studies, searchable by discipline, author and keyword. Its contents reflect major themes such as technological innovation, experience of implementation, and assessment and compara-tive studies. Since each study has been researched and included for its relevance to the pedagogical, ethical and economic issues pre-sented by the use of animals, alternatives and technology in education and training, this resource can help identify specific alternative tools and approaches to enhance practical classes. A section for comments and the inclu-sion of some studies in favour of harmful ani-mal use aims to encourage constructive critique and debate.

We are pleased to announce that full-text con-tent of selected papers from the *ATLA* archive will shortly be available for downloading from the database.

[1] Anon. (2012). InterNICHE launches new website. *ATLA* **40**, 9–13.

Translation of *The Principles* into Japanese

Russell and Burch's book, *The Principles of Humane Experimental Technique*, has been translated into Japanese by Noriyuki Kasai, Professor Emeritus and Guest Professor,

Tohoku University Centre for Laboratory Animal Research and The Institute for Animal Experimentation, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan. The initial translation took Professor Kasai five months, but it was interrupted by the earthquake which hit Japan in 2011, since Tohoku University and its animal facility were close to the centre of the disaster.

The book is published by Adthree Publishing Company Ltd, 4-27-37, Higashinakano, Nakano-ku, Tokyo 164-0003, Japan, and can be obtained from www.Amazon.co.jp for 3,000 Yen.

Creating 3-D Chips with Human Cells and Tissues

The US National Institutes of Health (NIH) has awarded 17 grants aimed at creating 3-D chips with living cells and tissues that accurately model the structure and function of human organs such as the lung, liver and heart.[1]

Once developed, the tissue chips will be tested with compounds known to be toxic or non-toxic in humans, and data from these tests will help to predict the safety of potential drugs in a faster and more cost-effective way.

According to the NIH press release, "More than 30% of promising medications have failed in human clinical trials, because they are determined to be toxic despite promising preclinical studies in animal models. Tissue chips, which are a newer human cell-based approach, may enable scientists to predict more accurately how

effective a therapeutic candidate would be in clinical studies. Tissue chips merge techniques from the computer industry with modern tissue engineering by combining miniature models of living organ tissues on a transparent microchip. Ranging in size from a quarter to a house key, the chips are lined with living cells and contain features designed to replicate the complex biological functions of specific organs."

[1] Anon. (2012). *NIH funds development of tissue chips to help predict drug safety.* [NIH News, 24.07.12]. Available at: http://www.nih.gov/news/health/jul2012/ncats-24.htm (Accessed 27.07.12).

NC3Rs Funding Update

The NC3Rs has announced the award of 21 new grants totalling £5.1 million.[1] This is the largest single allocation of funding ever made for Three Rs research in the UK, and has been made possible by additional funding to the NC3Rs from the Medical Research Council and the BBSRC. The awards include 14 project grants and seven pilot study awards.

This year saw a joint NC3Rs/BBSRC initiative on new ways of measuring and assessing animal welfare, and seven of the 17 awards, totalling more £2.3 million, resulted from that call. Further information available at: www.nc3rs.org.uk

[1] Anon. (2012). *Research Funding Update. Record Amount Awarded for 3Rs Research.* London, UK: NC3Rs.