



New Developments in Cell-Based In-Vitro Testing & 3rd Annual Quasi-Vivo User Group Meeting

18th-20th May 2011 at Saarbrücken Castle



Provisional Program

Day 1: Wednesday 18th May

10:00-18.00 Registration

12:00-14.00 Setting-up Exhibitions and Posters

Conference starts 14.00

Welcome address by Dr Malcolm Wilkinson and Prof Claus-Michael Lehr

Session 1 : In-Vitro Perspectives to Biological Barriers - Chair Dr J Malcolm Wilkinson

14.15

Key Note 1: Claus-Michael Lehr (Professor & Head, Dept. of Drug Delivery, Helmholtz-Institute for Pharmaceutical Research)

In-vitro models of epithelial barriers in the context of drug delivery

14.50

Samuel Constant (Chief Operating Officer, Epithelix Sàrl)

Advances in the assessment of acute/long-term and repeated dose inhalation toxicity in-vitro

- 15.10 Dorothée Hallier-Vanuxeem (Development Manager, R&D, Cellial Technologies)
- Improved neurotoxicity testing: Ready-to-use in- vitro Blood-Brain Barrier model suitable for High-Throughput Screening***
- 15.30 Coffee/tea break**
15.45 **Key Note 2:** Ian Holyer (In-vitro Cell Biology, GSK)
- Barriers facing pharmaceutical drug development.***
- 16.20 Zoe Prytherch (In Vitro Toxicologist, Lung & Particle Research Group, Cardiff University)
- Alternatives for Lung Research: stuck between a rat and a hard place***
- 16.40 Simon Baker (Jack Birch Unit for Molecular Carcinogenesis, University of York)
- New pathways in the urothelium/bladder barrier as targets for drug discovery***
- 17.00 Closing session comments and questions**
17.15 Poster viewing session
18.00 Informal reception and drinks with host at Saarbrücken Castle

Day 2 - Thursday 19th May

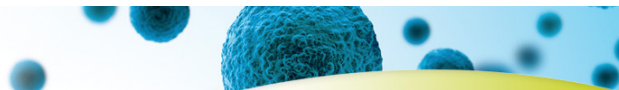
Session 2 : New Developments in Quasi-Vivo Systems – Chair, Professor David Tweats

- 8.50 Welcome introduction from the Chair
- 9.00 **Key Note 1:** Arti Ahluwalia (Professor of Bioengineering, Centre for Interdisciplinary Research "E. Piaggio" Pisa University) and Malcolm Wilkinson (CEO, Kirkstall, Sheffield)
- Review of developments in the MCMB/Quasi-Vivo systems***
- 9.40 Giancarlo Forte (Lead Researcher, Smart Biomaterial Group, Japan)
- Cardiac Muscle Engineering: Strategies to deliver stem cells to the damaged site***
- 10.00 Paolo di Nardo (Professor of Molecular and Cellular Cardiology, Dept of Internal Medicine "Tor Vegata", Rome)
- A Multilevel Strategy to Differentiate Progenitor Cells to Cardiomyocytes***
- 10.20 Coffee/tea break**
10.40 Arti Ahluwalia (Professor of Bioengineering, Centre for Interdisciplinary Research "E. Piaggio" Pisa University)
- An in-vitro model of glucose and lipid metabolism***

11.00 Helen Harrington (School of Molecular Medical Sciences, Nottingham University)

Utilising nanosensor incorporated scaffolds in development of a 3D lung model

11.40



InLiveTox update: Birgit Gaiser (School of Life Sciences, Heriot-Watt University)

ILT1 development and the study of Intestinal, Liver and Vascular Nanoparticle Toxicity

12.00 Closing session comments and questions

12.15 Lunch at Saarbrücken Castle

13.15 Poster and exhibitor viewing/networking

Session 3 : Organ Models In-Vitro – Chair, Professor Arti Ahluwalia

14.15 **Key Note 1:** Patrick Maurel (Research Director, Inserm U1040 Montpellier)

Review on in-vitro liver models

14.50 Sabrina Ehnert (Dept. of Traumatology, Munich, MRI)

Improvement of Hepatic Differentiation of Human Adipose-derived MSCs through Epigenetic Modification – Implication for in-vitro testing

15.10 Sheila MacNeil (Professor of Tissue Engineering, The Kroto Research Sheffield University)

3D skin models to study wound healing and bacterial infection

15.30 Coffee/tea break

15.50 Kelig Pernelle (Inserm U991, Rennes)

Tissue culture and regenerative medicine

16.10 Christiane Guillouzo (Institut National de la Santé et de la Recherche Médicale, Inserm U991, Rennes)

In-vitro testing of safety and toxicology using microsystems and HCS strategies

16.30 **Key Note 2:** Andrew Bennett (Director of FRAME Alternatives Laboratory, Nottingham University)

Studying liver disease models in-vitro

17.10 **Closing session comments and questions**

19.00: **Conference Dinner Reception (pre-dinner drinks)**

19.30: **Conference Dinner at the Schloss Halberg**

Day 3 - Friday 20th May

Session 4: New advances in in-vitro cell Culture – Chair, Professor Sheila MacNeil

8.50 Welcome introduction from the Chair

9.00 **Key Note 1:** Stefan Przyborski (Professor of Cell Technology & Chief Scientific Officer Reinnervate Ltd., Durham University)

Development and application of technology for routine three-dimensional cell culture

9.40 John Haycock (Director - Centre for Biomaterials & Tissue Engineering, Sheffield University)

In-vitro testing - the development of methods for detecting acute skin inflammation.

10.00 David Davies and Clive Schofield (Parker Hannifin)

Engineering Your Success: small companies working with Parker on biotech product development

10.20 **Coffee/tea break**

10.40 **Key Note 2:** Lewis Smith (Professor, Centre for Translational Therapeutics, University of Leicester)

The uses and abuses of in-vitro studies in drug development

Galenos Network and EuroPhD program:

11.10 **Introduction - Prof Claus-Michael Lehr**

Student papers

Tais Gratieri, PhD, (Brasil and Switzerland)

Vivekanand Bhardwaj, PhD, (India and UK)

12.00 **Closing comments round up and final summary:
Dr Malcolm Wilkinson**

12.15 Meeting Close

Poster presentations

Poster number	Title	presenter
1	Assessing the free brain/free plasma ratio <i>in vitro</i> in early drug discovery	Romeo Cecchelli <i>Cellial Technologies, France</i>
2	ZnO and TiO ₂ nanoparticles passage through an <i>in vitro</i> model of intestinal barrier.	Isabella De Angelis <i>Istituto Superiore di Sanità Rome</i>
3	Model Development of <i>In Vitro</i> intestinal toxicokinetics of the Cyanotoxin MICROCYSTIN LR	Valerie Fessard <i>Toxicology of contaminants, France</i>
4	Nanoparticle toxicity in a human hepatocyte cell line	Birgit Gaiser <i>Heriot-Watt University, Edinburgh, UK</i>
5	ZnO toxicity is not a nanoparticle specific effect but largely due to a substantial release of toxic zinc ions	Tina Bürki-Thurnherr <i>Swiss Federal Laboratories, Switzerland</i>
6	Comparison of physiological endpoints for developmental neurotoxicity in different cell-based <i>in vitro</i> systems.	Greg Podrygajlo <i>University of Freiburg, Germany</i>
7	Improving <i>in vitro</i> methods by developing and using defined culture media	Jan van der Valk <i>3Rs-Centre Utrecht Life Sciences, The Netherlands</i>
8	Detection of cell-physiological changes induced by poly-unsaturated fatty-acids (PUFA) with phenotypic microarrays	Ralph Weyandt <i>SGS INSTITUT FRESENIUS GmbH</i>
9	Accumulation and translocation of nanomaterials across the human placenta	Cordula Hirsch <i>Swiss Federal Laboratories, Switzerland</i>
10	Assessing the toxicological impact of a panel of engineered nanoparticles for risk assessment purposes	Ali Kermanizadeh <i>Heriot-Watt University, Edinburgh, UK</i>
11	The InLiveTox system – A novel microfluidic <i>in vitro</i> test system	Julia Susewind <i>Saarland University, Germany</i>
12	Complex Characterization of Drug Formulation by means of combined Dissolution and Permeation Measurement	Sandra Gantzsch <i>Saarland University, Germany</i>
13	Development of a Tissue Engineered 3D Immunocompetent Model of the Human Upper Respiratory Tract	Paul Cato <i>University of Nottingham, UK</i>
14	Modifications of Quasi-Vivo® to allow non-invasive imaging of long-term tissue engineering of human skin	Sheila MacNeil <i>Sheffield University, UK</i>
15	Real time and <i>in situ</i> control of environmental parameters in the Quasi-Vivo® Bioreactor	Serena Giusti <i>Pisa University, Italy</i>
16	Phenotypic Cardiomyocyte in the Squeeze Pressure Bioreactor	Federico Vozzi <i>Pisa University, Italy</i>

- | | | |
|----|---|--|
| 17 | Quasi-Vivo® Connected
culture for comparison of allometric scaling in vitro models | Nadia Ucciferri
<i>Pisa University, Italy</i> |
| 18 | Two possible solutions for
Allometric Scaling of a hepatocyte-endothelial connected culture
model | Tommaso Sbrana
<i>Pisa University, Italy</i> |
| 19 | Chondrocyte cultures in the Quasi-Vivo® | Daniele Mazzei
<i>Pisa University, Italy</i> |

Abstracts: Oral presentations

**Advances in the assessment of acute/long-term and repeated dose
Inhalation toxicity *in vitro***

Samuel Constant, Song Huang, Mireille Caulfuty, Rosy Bonfante, Rebecca Frauenfelder, Mélany Monachino, Jean-Paul Derouette and Ludovic Wiszniewski

Epithelix Sàrl, 14 Chemin des aulx, CH-1228 Plan-les-Ouates, Geneva, Switzerland

Most of the *in vitro* cell models for long term testing of chemicals suffer of at least two shortcomings: 1. The failure of reproducing the *in vivo* physiological characteristics. 2. A limited shelf-life. Epithelix has developed a standardized Air-liquid Interface *in vitro* cell model of the human airway epithelium (MucilAir™) which is free of these limitations.

MucilAir™ maintains the fully differentiated, morphologically and functionally, characteristics of the native tissues for more than one year. The typical characteristics of the airway epithelium are observed (e.g. tight junctions, cilia beating, ciliated cells, basal cells, mucous cells, cytokine/chemokine/metalloproteinase release, active ion transport and CypP450s activity) [1]. Epithelia from several pathologies can be reconstructed (e.g. Asthma, COPD, CF, etc.) [2].

Due to its unique long shelf-life, this model is used for studying the human respiratory diseases, and for testing the long-term/chronic effects of drugs/chemicals on respiratory tract [3]. Several applications of MucilAir™ relevant to inhalation toxicity assessment will be presented:

- Acute, long-term and repeated dose inhalation toxicity testing (Transposition of OECD TG412 – 28 days study)
- Recovery/regeneration study
- Inflammatory effect assessment
- Assessment of reversible vs irreversible effects of chemical

Bibliography:

- [1] Huang S, Caulfuty M. A novel *in vitro* cell model of the human airway epithelium. 3R-Info-Bulletin (2009). N°41.
- [2] Huang S, Wiszniewski L, Derouette JP, Constant S. *In vitro* organ culture models of asthma. Drug discovery today. (2009) Vol. 6, No. 4, 137
- [3] Maier MSV, Setting occupational exposure limits for unstudied pharmaceutical intermediates using an *in vitro* parallelogram approach Toxicology Mechanisms and Methods, (2010) 1-10.

Improve neurotoxicity testing: Ready-to use *in vitro* Blood-Brain Barrier model suitable for High-Throughput Screening

Dorothee Hallier-Vanuxeem¹, Lucie Dehouck², Emmanuel Sevin², Yannick Delplace¹,
Maxime Culot², and Roméo Cecchelli^{1,2}

¹Cellial Technologies, Faculté des Sciences Jean Perrin, Rue Jean Souvraz, 62303 Lens Cedex, France.

²Université Lille Nord de France, Laboratoire de Physiopathologie de la Barrière Hémato-Encéphalique, EA 2465 – IMPRT 114, Faculté Jean Perrin, Rue Jean Souvraz, S.P. 18 F-62300 Lens, France.

Prediction of neurotoxicity is a key feature in the toxicological profile of compounds and is therefore required in many regulatory testing schemes. Current *in vivo* test methods for neurotoxicity are inappropriate for screening large number of agents. Neural cell lines have shown promise as alternatives for assessing toxicity to the CNS. However, these *in vitro* cell assays would benefit from biokinetic data that considered the extent and rate of distribution into the CNS compartment to correlate with *in vivo* neurotoxicity studies. CNS penetration is under control of the Blood-Brain Barrier (BBB), a physical and metabolic barrier located at the level of brain capillaries which protects the microenvironment of the brain. Therefore, BBB permeability and functionality are both important parameters in the neurotoxicity profile of compounds. In the early 90's, Cecchelli et al. did pioneering work in establishing a highly predictive BBB model, consisting in the co-culture of bovine brain capillary endothelial cells and rat glial cells. By modifying this well-validated co-culture model, it has been possible to develop a BBB system more easily to use, quick and suitable for High-Throughput Screening. Available in frozen ready-to-use format, this model, consisting in bovine brain capillary endothelial cells frozen onto collagen-coated 24-well cell culture insert plates, considerably eases the technical needs to obtain a functional *in vitro* BBB model and substantially reduces the use of experimental animals. Upon thawing, the cells form a confluent endothelial cells monolayer after only 4 days of culture and then shows the typical expression and localization of tight junction proteins (ZO-1, Claudin 1,5 and Occludin). The tightness of the endothelial cells junctions was confirmed by a reproducible low permeability to Lucifer yellow, a non permeant marker ($Pe < 0,3 \times 10^{-3}$ cm/min). In addition, the presence and functionality of efflux transporters such as P-glycoprotein have been demonstrated. To further characterize the model, drug permeability assays were performed using a set of more than 35 CNS marketed drugs covering a wide range of physicochemical characteristics. These investigations demonstrated that data produced by the "Ready to use" BBB model which considerably facilitate the process of obtaining a functional *in vitro* BBB were similar to the standard co-culture model.

THE BARRIERS FACING PHARMACEUTICAL DRUG DEVELOPMENT

Ian Holyer, GSK (UK)

The pharmaceutical industry invests billions of pounds each year on drug discovery and development (D&D), with only a very small percentage of entities investigated becoming FDA approved. A major reason for this is the lack of robust *in vitro* assay data to enable significant predictions of *in vivo* clinical outcomes. Drug candidates reaching clinical trial phase frequently fail parameters which have been robustly investigated within *in vitro* and *in vivo* animal models. It is clear that optimisation of *in vitro* assays is required for pharmaceutical companies to 'fail fast or proceed with confidence' in regards to candidates progressing into first time in human (FTIH). Our present collaboration with Kirkstall and the encouraging ability of the quasi-*vivo* system to cover the required parameters of a clinically relevant *in vitro* assay, could give great insight into how future drug D&D should progress.

Alternatives for Lung Research: Stuck between a Rat and a Hard Place

Zoe Prytherch, Keith Sexton and Kelly A. Bérubé

Lung and Particles Research Group

School of Biosciences, Cardiff University, Museum Avenue, Cardiff, CF10 3AX, Wales, UK

The respiratory system acts as a portal into the human body for airborne materials which may gain access by administration of medicines or inadvertently during inhalation of ambient air (e.g. air pollution). The burden of lung disease has been continuously increasing to the point where it now represents a major cause of human morbidity and mortality worldwide. In the UK, more people die from respiratory disease than coronary heart disease or non-respiratory cancer. For this reason alone, gaining an understanding of mechanisms of human lung biology, e.g. development, injury and repair events, is now a principal focus within the field of respiratory medicine. Animal models are routinely used to investigate such events in the lung; however, the models do not truly reproduce the responses that occur in humans. Scientists working towards more robust three R's principles (Reduction, Refinement and Replacement) of animal experiments have been developing viable alternatives, derived from human medical waste tissues from patient donors, to generate *in vitro* models that resemble the *in vivo* lung environment. In the specific case of inhalation toxicology, models are especially warranted given the new REACH regulations for the handling of chemicals, the rising air pollution problems and availability of pharmaceutically valuable drugs. Advances in tissue-engineering have made it feasible and more cost-effective to construct human tissue equivalents of the respiratory epithelia than to use whole animal methods. The conducting airways of the lower respiratory system (i.e. bronchial epithelia) are a critical zone to recapitulate for use in inhalation toxicology. Three-dimensional tissue designs using primary cells provide more *in vivo*-like responses, based on the targeted interactions of multiple cell types supported on artificial scaffolds which emulate the native extracellular matrix in which cells differentiate into a functional pulmonary tissue. The 3D models are also designed to be modular and complimentary physiological functions may be built in, such as metabolic activity. When such 3D cell cultures are employed for testing aerosolised chemicals, drugs and xenobiotics more *in-vivo* like responses are captured that mirror the events in the *in situ* human lung, providing robust human end-point data.

New approaches to modifying epithelial barrier function for targeted drug delivery

S.C. Baker, S. Shabir and J. Southgate

Drug delivery systems require efficient delivery to the target tissue, without side-effects in other tissues. Intravesical delivery to the bladder negates the need for systemic exposure, but maybe inefficient, as the bladder lining (urothelium) develops the tightest epithelial barrier in the body. Approaches to improve uptake of bladder-instilled drugs include direct physical/chemical damage to the urothelium, electromotive drug administration and a variety of indwelling devices. Using in vitro tissue models, we have investigated functional modulation of the urinary barrier and shown that most xenobiotics result in barrier tightening. We have recently identified an agent which rapidly and reversibly modifies barrier permeability. Coadministration of this factor could enhance intravesical drug delivery.

Cardiac Muscle Engineering: Strategies to deliver stem cells to the damaged site

Giancarlo Forte. Lead Researcher, Smart Biomaterial Group, Biomaterials Center
International Center for Materials Nanoarchitectonics (MANA), National Institute for
Materials Science (NIMS)1-1, Namiki, Tsukuba, Ibaraki 305-0044, Japan

Cardiovascular diseases represent the main cause of morbidity and mortality worldwide. Among cardiac diseases, heart failure is the most common end-stage pathology, leading to impaired cardiac output and cardiac performance as a result of the irreversible loss of contractile cardiomyocytes. Tissue engineering holds the promise to provide innovative solutions to the problem of cardiac muscle repair. In the last years, the use of stem cells and biocompatible, biodegradable scaffolds of natural or synthetic origin as well as the possibility to employ dynamic culture conditions has speeded up the search for new protocols to repair cardiac muscle. Indeed, the identification of little reservoirs of stem and progenitor cells within every body district opened new perspectives to the setup of minimally invasive intervention protocols for cardiac diseases. Nonetheless, a number of pre-clinical and clinical trials using stem/ progenitor cells were recently performed; in such trials, different stem cell subsets were directly injected into the myocardium or delivered through bloodstream to the heart, but the results were sometimes astonishing: no or few cells could be found engrafted within host tissue few weeks after the administration. Such results suggest that additional efforts to setup efficient systems to deliver stem cells to the injured site are required. Recently, our group developed original approaches to the issues of stem cell growth, differentiation and delivery. In a first attempt, directional thermally-induced phase separation has been employed to obtain thick scaffolds with oriented porosity to prepare multilayered cardiac constructs. Moreover, thermo-responsive polymers have been used either to obtain scaffoldless, multilayered cardiac patches or to prepare smart surfaces changing their mechano-physical properties with temperature. Thus, the effect of stiffness on stem cell differentiation as well as the role of scaffold micro- and nano-structure in favoring stem cell engraftment and differentiation was explored. Thus, a critical review on the results obtained using different delivery routes (i.e., direct intramural injection, bloodstream delivery, scaffold-based and cell sheet technologies) is given.

A Multilevel Strategy to Differentiate Progenitor Cells to Cardiomyocytes

Paolo Di Nardo

*Laboratory of Cellular & Molecular Cardiology, Dept. of Internal Medicine
University of Rome "Tor Vergata", Rome, Italy*

*Japanese-Italian Tissue Engineering Laboratory (JITEL), Tokyo Women's Medical
University-Waseda University Joint Institution for Advanced Biomedical Sciences
(TWIns), Tokyo, Japan*

The poor knowledge of the progenitor cell biology has determined low viability of the injected cells (<1% of the cells are detectable in the target area after 1 week) hampering the efficacy of cell therapies applied to the injured myocardium. Recent evidences have suggested that adult progenitor cells can be used to fabricate *ex vivo* engineered cardiac tissue to be implanted into the injured myocardium. Engineered tissues can be fabricated in the presence of biocompatible polymeric scaffolds. It is also possible to fabricate scaffoldless sheets made of human progenitor cells, possibly isolated from the heart of the same patient candidate to receive the cell treatment. In order to maximize these technologies by mimicking the natural stem cell environment, bioreactors with novel design matching the specific characteristics of the heart muscle must be validated. In this respect, preliminary results will be discussed.

Utilising Nanosensor Incorporated Scaffolds in the Development of a 3D Lung Model

Helen Harrington (School of Molecular Medical Sciences, Nottingham University)

The development of an immune-competent 3D human lung model within a perfusable bioreactor could provide an effective *in vitro* tool for upper airway research as it is more physiologically relevant than current 2D cell-based assays and could minimise the use of animal models.

To allow long-term experiments to be performed without disrupting the developed 3D model we have created biocompatible scaffolds that incorporate analyte responsive nanosensors. These scaffolds have morphological similarities to the fibrous lung matrix and are capable of supporting cell attachment and proliferation. Utilising nanosensor incorporated scaffolds as the matrix for our 3D lung model would permit microenvironment analyte concentration to be monitored *in situ* and in real-time. This would allow repeat testing of 3D constructs and offers the potential to monitor factors such as nutrient exchange and metabolic waste removal during different stages of tissue differentiation. The use of the developed self-reporting scaffolds for tissue engineering applications presents the opportunity to fully understand, monitor and optimise the growth of 3D model tissue constructs *in vitro* non-invasively and in real-time.

Preferential hepatocyte differentiation directed by shape constraint in stem-like hepatic HepaRG cells

Pernelle K.¹, Tsai YH²., Hsieh JY²., Le Guevel R., Lambert K., Chesné C.³, Wang HW²., Corlu A.¹ Guguen-Guillouzo C.^{1,3}

1 INSERM U991, Rennes1 University, 35043, RENNES Cedex, France

2 Institute of Microbiology and Immunology, National Yang-Ming University, Taiwan

3 BIOPREDIC International, Parc d'activité Bretèche batA4, 35760 Saint-Gregoire, France

Developments of liver cell applications to pharmaco-toxicology and to clinical use in liver replacement therapy depend on the ability to efficiently produce mature human hepatocytes. Embryonic stem cells (ESC) and more recently, induced pluripotent stem cells (iPSC) have shown great potential as sources for liver development modeling and for cell replacement in regenerative medicine. Selective and temporal action of different biological factors chosen for their involvement in liver organogenesis is the mainly used strategy. However, till now only mixed populations containing a limited number of differentiated hepatocytes have been obtained. Recent findings have highlighted a new differentiation pathway through the application of active stresses to ESCs induced by the cell's surrounding environment.

We bring evidence showing that physical forces such as cell shape constraint, direct stem-like cells from the hepatic human HepaRG cell line, to preferentially differentiate into hepatocytes. HepaRG cells are early bipotent progenitor cells able to undergo hepatocyte and biliary cell differentiation programmes in an equal rate of 50% in basal conditions. The compression stress induced by seeding HepaRG stem-like cells at high density greatly favoured the decision to commit towards the hepatocyte differentiation programme. This includes early down-regulation of genes involved in very early stages of development and characterizing the stem cell fate, such as oct1, oct4 and Sox 17, and up-regulation of genes involved in early liver organogenesis such as HNF4 α , Notch3, β -catenin/GSK3 or oncostatin and then, gradually in hepatocyte differentiation with expression of liver specific genes such as aldolase B and CYP 3A4. These changes occurred at the gene transcription, protein expression and activity levels, in a sequential and coordinated manner, from day 1 after the compression stress initiation up to day 6. They were tightly associated with strong morphological modifications of cell shape and cytoskeleton organization, cell to cell interactions and cell polarity. Noteworthy, nuclear shape also evidenced deep modifications with a 2-fold surface reduction by day 6, and nucleolar condensation. These changes strongly suggest the occurrence of mechanotransduction signals up to nuclei that make possible the deep transcription profile changes performed by compressed cells within 1 to 3 days.

All together these results show that HepaRG cells may reproduce the different steps of early hepatic development. They represent a unique model for setting experimental conditions supporting production of human hepatocytes from embryonic stem cells. Overall, owing to their capacity to both actively grow and to undergo a complete hepatocyte differentiation programme, HepaRG cells could be a source of reproducible hepatocyte production for the next years.

Peroral delivery of paclitaxel aided by biodegradable nanoparticles: in vitro and in vivo evaluation

Vivekanand Bhardwaj

With the aim of preparing an oral, non-toxic stable dosage form, the anticancer drug paclitaxel was incorporated in biodegradable PLGA nanoparticles by a simple emulsification process using a cationic surfactant. The developed formulation was characterized, freeze-dried and subjected to *in vitro* cell culture studies to establish the safety of the delivery system and efficacy of paclitaxel. The product was assessed in pre-clinical efficacy study in a chemical induced cancer model in rats whereby it was established that nanoparticles not only enable the oral delivery of paclitaxel, but also increase its efficacy. The formulation also increase potency of paclitaxel in a drug-resistant cancer model in mice. The uptake of the orally administered nanoparticles and the disposition of the drug were shown by a tissue distribution study using radiolabeled drug. The developed formulation presents a strong case of improving drug delivery using polymeric nanoparticles.

Abstracts: Poster presentations

Assessing the free brain/free plasma ratio *in vitro* in early drug discovery

Cecchelli Romeo^{1,2}, Da Costa de Matos Anaëlle¹, Culot Maxime¹, Hallier-Vanuxeem Dorothée², Sevin Emmanuel¹, Vandenhautte Elodie¹, Dehouck Marie-Pierre¹, Lundquist Stefan³

¹Université Lille Nord de France, UArtois, BBB Laboratory EA 2465 62307 Lens Cedex, France

²Cellial Technologies, rue Jean Souvraz, 62303 Lens Cedex, France

³AstraZeneca R&D, Södertälje, S-151 85, Sweden.

The market for neuropharmaceuticals is regarded as one of the potentially largest sectors of the global pharmaceutical market owing to the increase in average life expectancy and that many neurological disorders have been largely refractory to pharmacotherapy.

The value of many promising CNS drug candidates is diminished by the presence of barriers between blood and brain, which possess both structural and enzymatic components at the level of the cerebral capillaries (commonly referred to as the Blood-Brain Barrier or BBB), the epithelia of the choroid plexuses and the other circumventricular organs (often referred to as the blood-cerebrospinal fluid barrier) and the arachnoid membranes. Since the cerebral capillaries comprise an estimated 95% of the total area of the barriers between blood and brain, it follows that this is the principal route for the entry of most molecules into the CNS as well as it is the major hurdle that prevents many neuropharmaceuticals from eliciting a desired pharmacological effect at an attainable dose. Consequently, by modelling the BBB it is possible to make predictions about brain uptake of potential drug candidates and to study the effect of therapeutic interventions at the level of the cerebral capillaries. This provides not only powerful means to assess the risk for taking compounds further in the pharmaceutical development process, but also generates important information to allow for rational drug design.

In the early 90's, our group did pioneering work in establishing an *in vitro* model of the BBB by co-culturing bovine brain capillary endothelial cells together with rat glial cells. This model has been successfully used for screening and mechanistic purposes in a number of major pharmaceutical companies for more than a decade. Historically, the focus has been to use this kind of *in vitro* models to optimize rate of drug delivery to the CNS and *in vivo* total brain/plasma ratios for optimizing extent. However, it can be argued that the rate of transport across the BBB is only important for primarily indications such as pain, epilepsy and stroke while for many other CNS targets at steady-state following chronic administration, peak concentrations are not critical. Total brain/plasma ratios on the other hand often show a poor correlation with receptor occupancy data or pharmacodynamic readouts. Since it is thought that only the free brain concentration (C_{ubr}) is available for interaction with the majority of CNS receptors it may be essential to determine this parameter for CNS compounds. Microdialysis has been used to measure C_{ubr} but the technique is resource-demanding and is not broadly applicable because of probe recovery problems for lipophilic compounds. Cerebrospinal fluid (CSF) concentrations may provide a surrogate measure of C_{ubr} (with various caveats) but are difficult to obtain routinely. Recently, equilibrium dialysis technologies has been used to determine the free fraction of a compound in brain homogenate (f_{ubr}) and then using this free fraction to correct total brain concentration for C_{ubr}. However, although this is so far the simplest way of determining C_{ubr}, both *in vitro* and *in vivo* experiments need to be carried out. The objective of this study was to investigate whether it was possible to modify the use of our current *in vitro* model of the BBB so that in addition to permeability it could also generate a quantitative parameter such as the free brain/free plasma ratio.

ZnO AND TiO₂ NANOPARTICLES PASSAGE THROUGH AN IN VITRO MODEL OF INTESTINAL BARRIER

I. De Angelis¹, L. Bizzarri¹, F. Superti², A. Tinari², B. De Berardis¹

¹ *Environmental and Primary Prevention Dept. Istituto Superiore di Sanità – Rome, Italy*

² *Technology and Health Dept. Istituto Superiore di Sanità – Rome, Italy*

Peculiar characteristics of nanoparticles (NPs), as size, shape and surface, might strongly influence their behavior and reactivity towards biological structures. In particular, interaction with epithelial barriers was considered crucial to NPs entrance into the body. Intestinal barrier is one of the main portal of entry for foreign chemicals and Caco-2 cell line, derived from a human carcinoma, represents a very well known and characterized in vitro model of this barrier. Aim of this work is to investigate the passage through differentiated Caco-2 cells, grown on semi-permeable inserts, of ZnO and TiO₂ NPs. Preliminary TEM analysis, performed after 24 hr of exposure (2.5 μg/cm² ZnO and 5 μg/cm² TiO₂), show that NPs cross the cellular brush border located in both cytoplasm and nucleus. Cellular uptake doesn't seem to be mediated by endocytotic process. Nevertheless, barrier integrity, evaluated by TEER measurement, was not impaired by NPs treatment. Further experiments are ongoing to better clarify the uptake of NPs by Caco-2 cells.

MODEL DEVELOPMENT OF IN VITRO INTESTINAL TOXICOKINETICS OF THE CYANOTOXIN MICROCYSTIN LR

Henri Jérôme, Huguet Antoine, Delmas Jean-Michel, Sanders Pascal, Fessard Valérie
Anses, Toxicology of contaminants, La Haute Marche, BP 90203, 35 302 Fougères
cedex, France
valerie.fessard@anses.fr

Blooms of cyanobacteria, photosynthetic prokaryotes organisms, are frequently occurring in freshwaters for drinking and recreational activities. Some cyanobacteria produce cyanotoxins involved in human and animal intoxications. Among the cyanotoxins, the heptapeptides microcystins including more than 80 variants are potent hepatotoxins. Microcystin LR (MC-LR) is known to be the most toxic variant to rodents. However, its acute toxicity is 100-time lower after oral treatment than after intraperitoneal injection suggesting a weak oral bioavailability. In order to confirm this hypothesis, we conducted in vitro toxicokinetics studies on the human intestinal Caco2 cells. Transfer experiments (apical to basal and the other way round) were conducted on differentiated Caco2 cells. Four concentrations of MC-LR (1, 10, 48 and 75 μM) and 6 times of treatment (0.5, 1, 2, 4, 6 and 24 hours) were tested. MC-LR concentrations were quantified by ESI-IT-MS/MS. Where exposed on the apical side, a decrease of MC-LR amount between 20 and 30% according to the concentration was observed within only 0.5 h due to cellular uptake. A slight increase was further obtained with longer duration (24h) suggesting a mechanism of secretion. After 24h, MC-LR reached a mean of 1.5% of the introduced amount for the highest concentrations in the basal medium. Where exposed on the basal side, the amount of MC-LR remained constant even if a slight amount (less than 5%) can be detected after few hours in the apical medium. All observed data were fitted (Nelder-Mead algorithm) to a mathematical model (derived from a catenary model). This model describes the different clearances (absorption, secretion, influx, efflux as well as binding and metabolism) into the system. Values for parameters (with a physiological significance) are then obtained. Influence of the microcystin concentrations on each parameter describing the model was estimated. Based on our results, hypothesis on the mechanisms regulating intestinal toxicokinetics of microcystin will be suggested.

Nanoparticle toxicity in a human hepatocyte cell line

Birgit Gaiser, Kleanthis Fytianos and Vicki Stone

Heriot-Watt University, Edinburgh, UK

Nanoparticles (NPs) are increasingly used in a large number of consumer products, and risk assessment is essential, as the likelihood of exposure both in the occupational setting and for end users, as well as of environmental exposure, is increasing. Mechanisms by which NPs cause adverse effects include generation of oxidative stress [1] and up-regulation of inflammatory markers [2]. Using *in vitro* methods for risk assessment of NPs is encouraged by the legislators and public opinion, and in addition, validated *in vitro* methods are more economically viable for manufacturers than *in vivo* testing.

To investigate hepatotoxicity of NPs, we used a set of particles of different compositions and sizes, including polystyrene beads (50 and 200 nm), silver (Ag, nominal diameter <25 nm), titanium dioxide (TiO₂ rutile-anatase, nominal diameter 7 nm), and gold (Au, 15 and 80 nm). All studies were performed on the human hepatocyte cell line C3A.

Using the LDH assay for cytotoxicity and the Alamar Blue assay for mitochondrial function, we found that the fluorescent beads of both sizes only impacted on viability at an extremely high concentration of 625 µg/cm², and TiO₂ NPs had no toxic effects at any concentration up to 625 µg/cm². Gold particles were non-toxic up to the highest concentration used (40 µg/cm²). In contrast, Ag NPs were highly toxic with an LC₅₀ between 2.5 µg/cm² (LDH) and 15 µg/cm² (Alamar Blue).

Initial experiments revealed a reduction of albumin secretion into the medium by Ag NPs, indicative of cellular function being compromised, at doses of and below the LC₅₀, whereas TiO₂ did not reduce albumin secretion. Further preliminary experiments, examining the effects of particles on levels of the intracellular antioxidant glutathione, showed a reduction in the levels of reduced glutathione (GSH) after exposure to Ag nanoparticles at concentrations below the LC₅₀.

These experiments will be repeated and extended to the other particles in the test set over the next few months. Our results so far confirm the toxicity of Ag NPs previously reported [3], and indicate that even at non-toxic concentrations, cellular function and antioxidant levels are compromised, potentially providing a means to test for adverse effects of NPs at low concentrations. In contrast, cellular function does not appear to be impaired even at high concentrations of the low-toxicity TiO₂ NPs.

[1] Brown, D et al. 2004. Calcium and ROS-mediated activation of transcription factors and TNF-alpha cytokine gene expression in macrophages exposed to ultrafine particles. *Am. J. Physiol. Lung Cell Mol. Physiol.* 286: L344-L353.

[2] Duffin, R et al. 2007. Pro-inflammatory effects of low toxicity and metal nanoparticles in vivo and in vitro: Highlighting the role of particle surface area and surface reactivity. *Inhal. Toxicol.* 19 : 849-856.

[3] Gaiser, B et al. 2009. Assessing exposure, uptake and toxicity of silver and cerium dioxide nanoparticles from contaminated environments. *Environ Health* 8 Suppl 1:S2.

ZnO toxicity is not a nanoparticle specific effect but largely due to a substantial release of toxic zinc ions

Tina Bürki-Thurnherr¹, Liliane Diener¹, Osman Arslan², Xenia Maeder-Althaus¹, Bruno Wampfler¹, Peter Wick¹, Sanjay Mathur², Harald F. Krug¹

¹ Swiss Federal Laboratories for Materials Testing and Research, Laboratory for Materials-Biology Interactions, St. Gallen, Switzerland

² Inorganic and Materials Chemistry, University of Cologne, Cologne, Germany

Metal oxide nanoparticles exhibit unique properties which are exploited in many fields of technology and medicine. Among these novel materials ZnO is already produced in high tonnage, and their intentional use in commercial applications such as for antibacterial coating or UV absorber in sunscreens and textiles requires immediate knowledge on their toxic potential. The interaction of nanomaterials with immunocompetent cells is of special interest as these cells are present throughout the body and are crucial for the efficient recognition and elimination of pathogens and foreign materials.

Here, we investigated the adverse effects of large-scale produced ZnO nanoparticles on a human T cell leukemia-derived cell line (Jurkat) and the underlying mechanisms. Already low concentrations of ZnO nanoparticles induced major cell death including apoptotic and late apoptotic/necrotic cells. Using mutant cell lines deficient in various components of apoptotic signaling pathways we found that ZnO nanoparticles activate none of the classical apoptotic mechanisms, namely extrinsic or mitochondrial apoptosis pathways. However, reactive oxygen species are involved as the antioxidant agent N-acetylcystein significantly reduced ZnO-induced apoptosis. No ZnO nanoparticles were observed in treated cells but instead rapid dissolution and high concentrations of Zn-ions were found both extra- and intracellularly. Removal of these ions in the medium and/or within the cells completely inhibited ZnO-induced apoptosis. To conclude, our study indicates that ZnO nanoparticle toxicity in Jurkat T cells is predominantly mediated by Zn-ions and involves oxidative stress signaling mechanisms but not the classical apoptotic pathways.

Sponsored by the Seventh Framework Programme of the European Commission (FP7-NANOMMUNE, grant no. 214281)

Comparison of physiological endpoints for developmental neurotoxicity in different cell-based in vitro systems.

Grzegorz Podrygajlo^{1,2}, Samora Okujeni^{1,2,3}, Patrick Dini^{1,2,3}, Matthew Goddard^{1,2}, Ulrich Egert^{1,2}.

¹ Biomicrotechnology, Department of Microsystems Engineering – IMTEK, University of Freiburg, Germany

² Bernstein Center Freiburg, University of Freiburg, Germany

³ Neurobiology and Biophysics, Institute of Biology III, Faculty of Biology, University of Freiburg, Germany

Examination of the chemical substances with respect to their potential toxicity on the brain development is of major concern. A predictive in vitro test for potential developmental neurotoxicity (DNT) needs to be an inexpensive, quick and standardized evaluation to present in vivo methods. Here we are presenting a project combining attachment, proliferation, differentiation as well as morphology and electrophysiological analysis comparing three different cell culture systems (murine embryonic stem cells, human neural progenitor cells and human teratocarcinoma cells) grown on microelectrode arrays (MEAs). Electrophysiological recordings with MEAs showing an electrical activity of the derived neuronal networks are supplemented with Ca^{+2} transient measurements.

This assay allows us to identify a functional endpoint for electrical activity and network properties for several maturation stages that will be used in a robust, medium-throughput system for DNT testing in vitro.

Improving *in vitro* methods by developing and using defined culture media

J. van der Valk^{1*}, M.L. Scarino², L. Knudsen³, G. Gstraunthaler⁴

¹ 3Rs-Centre Utrecht Life Sciences, Fac. of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands, (J.vanderValk@uu.nl); ² Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Rome, Italy; ³ Department of Public Health, University of Copenhagen, Denmark; ⁴ Department of Physiology, Innsbruck Medical University, Innsbruck, Austria

In vitro methods are widely used to study activities at the cellular level. Furthermore, *in vitro* methods are powerful tools to replace or reduce animal experiments, either as stand-alone, or as part of a testing strategy.

Cells are maintained under well-established conditions. An often used basal medium is Dulbecco's Modified Eagles Minimal Essential Medium (DMEM). Dependent on the cell type this medium is supplemented with factors essential for proliferation, migration and differentiation of the cells. Usually, serum is used as supplement, often fetal bovine serum (FBS). The use of serum involves several problems: possible contamination, undefined (binding) factors, batch-to-batch variation, etc. The use of FBS also involves an ethical problem; blood collection may cause severe suffering to the animal (van der Valk et al. 2004). It was therefore concluded that the use of FBS should be strongly discouraged and chemically defined media should be preferred when using *in vitro* methods (Coecke et al. 2005; ESAC 2008; van der Valk et al. 2004). Around 450 serum free media are now available (www.goodcellculture.com). Still, not for every cell type is a defined medium developed. In addition, the formulation of most commercially available media is not released, and these can thus not be regarded as strictly defined.

To discuss the development of culture media for specific cell types a workshop was organised by the Dutch-Belgian Society for In Vitro Methods (INVITROM), the European Society for Toxicology In Vitro (ESTIV) and the Danish In Vitro Toxicology Network, November 2009, in Copenhagen.

It was concluded that the development of serum-free media and cell adaptation processes is an ongoing process in several laboratories, often without knowledge of research processes, experiences or results of other laboratories regarding this topic. This information, particular with regard to precise formulations, should be collected and made publicly available to facilitate the further development and use of defined cell and tissue culture media.

The workshop participants supported the recent statement of the ECVAM Scientific Advisory Committee (ESAC) in which the use of serum and other animal components in cell and tissue culture was strongly discouraged and the development of defined media was required (ESAC 2008). The use of defined media is also suggested when applying Good Cell Culture Practice (GCCP) (Coecke et al. 2005; Hartung et al. 2002). It was recommended to make GCCP part of Good Laboratory Practice and/or Good Manufacturing Practice to give it a legal basis.

During the workshop, the different components of a defined medium were discussed to facilitate the development of defined culture media. Furthermore, several approaches to adapt cells to serum-free media were discussed.

Details of the workshop and its recommendations are published in a workshop report in *Toxicology In Vitro* (van der Valk et al. 2010).

References:

- Coecke, S., Balls, M., Bowe, G. et al. (2005). Guidance on good cell culture practice. A report of the second ECVAM task force on good cell culture practice. *ATLA*, vol. 33: 261-287.
- ESAC (2008). ESAC statement on the use of FCS and other animal-derived supplements.
- Hartung, T., Balls, M., Bardouille, C. et al. (2002). Good Cell Culture Practice. ECVAM Good Cell Culture Practice Task Force Report 1. *ATLA*, vol. 30: 407-414.
- van der Valk, J., Brunner, D., De Smet, D. et al. (2010). Optimization of chemically defined cell culture media - Replacing Fetal Bovine Serum in mammalian *in vitro* methods *Toxicol In Vitro*, vol. 24, 1053-1063
- van der Valk, J., Mellor, D., Brands, R. et al. (2004). The humane collection of fetal bovine serum and possibilities for serum-free cell and tissue culture. *Toxicol In Vitro*, vol. 18: 1-12.

“Detection of cell-physiological changes induced by poly-unsaturated fatty-acids (PUFA) with phenotypic microarrays”

Florian Eiserlo (University of Mainz), Ralph Weyandt (SGS INSTITUT FRESENIUS GmbH)

Beyond the issue of biocompatibility, and especially cyto-toxicology, there are only few experimental approaches available to perform a suitable, customary and non-specific effect screening on defined mammalian cell lines. An innovative phenotypic micro array is able to create a standardized, metabolic fingerprinting by detecting several hundred parameters coupled with energy-producing pathways.

First we characterized and compared various mammalian cell types (eg. L929, 3T3) based on this ready-made metabolic assay. To detect possible metabolic effects by selected substances (e.g. PUFAs = poly unsaturated fatty acids), we cultivated these cell lines for two cell cycles in the presence of the test substances (at the NOEC-concentration out of acute toxicity tests). After harvesting and washing the adherent cells, the suspensions were assayed again for fingerprinting via the multiple energy-producing pathways/metabolic reactions.

In the poster presented we are going to stress the specimen-specific similarities and differences of the metabolic pathways and specific reaction kinetics between different cell lines, assessing the applicability of the method as well as the sensitivity for cell specific responses.

Accumulation and translocation of nanomaterials across the human placenta

P. Wick¹, C. Hirsch^{1#}, A. Malek², P.A. Diener³, A. Zisch², H. F. Krug¹, and U. von Mandach²

¹Swiss Federal Laboratories for Materials Testing and Research, Laboratory for Materials-Biology Interactions, St. Gallen, CH

²Dept of Obstetrics, University Hospital Zurich, CH

³Institute of Pathology, Cantonal Hospital St. Gallen, CH.

#presenting author

Background and Objectives:

Exposure to fine and ultrafine particles occurred throughout human history. However, sources, concentrations and types of nanoparticles dramatically changed since the Industrial Revolution. Due to rapid developments in the field of nanotechnology the production volumes of various engineered nanoparticles possessing novel physical and chemical properties considerably increased. Whether exposure to such nanosized particles is intended or not, the assessment of possible adverse effects on human health is absolutely inevitable. Furthermore, recent studies indicate that exposure of pregnant women to air pollutants might negatively influence the development of the unborn child. These findings led to growing concern about potential risks of *in utero* nanoparticle occurrence. Even though a substantial number of projects investigated consequences of combustion-derived as well as engineered nanoparticles for human health, the question of whether nanoparticles may cross the placenta has so far been largely unaddressed.

Methods:

We made use of the human *ex vivo* placenta perfusion model to assess nanoparticle transfer across this natural barrier. Moreover, we examined the potential size dependency of this process by using fluorescently labeled polystyrene beads with diameters of 50, 80, 240, and 500 nm.

Results and Conclusion:

Polystyrene particles with diameters of up to 240 nm were taken up by placental tissue and were able to cross the placental barrier without affecting the viability of the explant. These findings suggest that transplacental transfer might also be possible for different other types of nanomaterials, necessitating further nanotoxicologic studies in this important organ system.

Assessing the toxicological impact of a panel of engineered nanoparticles for risk assessment purposes

Ali Kermanizadeh¹, Vicki Stone¹, Birgit K Gaiser¹, Gary R Hutchison²
Heriot Watt University¹, Edinburgh Napier University²
Email: ak435@hw.ac.uk

Nanotechnology has become a global industry. Nanoparticles (NPs) arise from a wide variety of natural and man-made sources and have a diverse array of biological, chemical, and physical properties. As with any new technology there are a number of potential risks to consumers and workers exposed to these particles. It is paramount that these risks are assessed and managed promptly as any failings to do so could slow down this rapidly expanding field and more importantly could have hazardous consequences for human and environmental health (1, 2).

It is now known that following exposure via a number of routes (inhalation, instillation, dermal or ingestion) some NPs can translocate to secondary tissues and can be potentially toxic in these target organs. One organ identified as a site for accumulating blood borne particles is the liver.

The initial phase of this study has focused on C3A cells (human liver cell line derived from a hepatoblastoma). The impact of a panel of engineered nanomaterials consisting of five titanium dioxide (TiO₂), two zinc oxides (ZnO), two multi walled carbon nano-tubes (MWCNT) and one silver (Ag) particles were observed on hepatocyte toxicity and function.

It was observed that the silver particles elicit the greatest levels of toxicity followed by the ZnO uncoated and ZnO coated particles. It was also discovered that LC50 was not reached in the presence of any of the other nanomaterials after a 24 hour period of exposure. It was deduced that C3A cells produce significantly increased levels of IL8 following exposure to these engineered NPs, with these levels peaking around the LC50. Meanwhile it was found that there was no significant increase or decrease in the levels of TNF- α , IL6 or CRP secreted from these cells after NPs exposure.

Experiments were conducted to ascertain the potential mechanism driving inflammation in C3A cells post NPs exposure. Intracellular ROS levels were assessed and shown to increase, following exposure of the C3A cells, in a similar pattern to the equivalent levels of IL8 produced.

In order to evaluate the ability of NPs to induce oxidative stress (decrease of antioxidants - total GSH levels), C3A cells were exposed to the NPs at increasing concentrations. Decrease in GSH concentrations was determined using the fluorescent probe – o-phthaldialdehyde. We found that there was a dose dependant decrease in levels of total GSH across the ten particles however these effects were greatest in the presence of the Ag and the two ZnO NPs.

We also found that there was no significant increase or decrease in the levels of urea or albumin produced by the C3A cells in the presence of the investigated NPs compared to the control – with the exception that there was a significant reduction in the levels of albumin produced at LC50 concentrations of both ZnO NPs.

In conclusion, the *in vitro* hepatocyte model demonstrated that Ag and ZnO NPs were constantly more potent than MWCNT and TiO₂ nanomaterials with respect to cytotoxicity, cytokine production and oxidative stress.

1. Hood, E., 2004. Nanotechnology: look as we leap. Environmental Health Perspectives. 112, 740-749.

2. Maynard, AD., Aitkin, RJ., Butz, T., Colvin, V., Donaldson, K., Oberdorster, G., Philbert, MA., Ryan, J., Seaton, A., Stone, V., Tinkle, S., Tran, L., Walker, NJ., Warheit, DB., 2006. Safe handling of nanotechnology. Nature. 444, 267-269.

The InLiveTox system – A novel microfluidic in vitro test system

Julia Susewind¹, Tommaso Sbrana², Mélanie Favre³, Arti Ahluwalia², Claus-Michael Lehr^{1,4}, Eva-Maria Collnot^{1,4}

¹ Department of Biopharmaceutics and Pharmaceutical Technology, Saarland University, 66123 Germany

² Università di Pisa, Pisa, 56126 Italy

³ Centre Suisse d'Electronique et de Microtechnique SA (CSEM), Neuchatel, 2002 Switzerland

⁴ Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarland University, Saarland, 66123 Germany

The aim of InLiveTox project is the development of a microfluidic cell culture system to test the response of different cells and tissues to ingested nanoparticles. The target tissues are gastrointestinal tract, liver and endothelium. The tissue models are connected to each other via a microfluidic system.

Caco-2 cells as a model of intestinal epithelium were evaluated in the system. Cells were grown for 21 days to confluency on porous silicon nitride membrane wafers. Silicon wafer chips were then transferred into the microfluidic system and cells were maintained there under varying flow rates for up to 48 h. Electrodes for measurement of transepithelial electrical resistance (TEER) were integrated into the setup allowing for automated resistance measurements via contacts on the chip.

Caco-2 cells grown in the InLiveTox system formed confluent monolayers with TEER values up to $450 \Omega \cdot \text{cm}^2$ comparable to standard cultures on permeable polyester cell culture inserts. TEER values were stable in dynamic flow conditions up to flow rates of $200 \mu\text{l}/\text{min}$. TEER could be measured continuously over 24h.

Thus it is possible to maintain a functional monolayer of intestinal epithelial cells in the microfluidic system under dynamic conditions. Different cell types can be connected with each other so that cross-talk can be monitored. TEER will be used as an indicator of toxicity of ingested nanoparticles in addition to soluble biomarkers.

Complex Characterization of Drug Formulation by means of combined Dissolution and Permeation Measurement

Sandra Gantzsch¹, Ulrich F. Schäfer¹, Petra Loos², Stefan Balbach², Harald Berchtold², Thomas Eichinger², Claus-Michael Lehr^{1,3}

¹ Department of Biopharmaceutics and Pharmaceutical Technology, Saarland University, Campus A4.1, D-66123 Saarbruecken, Germany

² Lead Generation to Candidate Realization, Sanofi-Aventis Deutschland GmbH, D-65926 Frankfurt/Main, Germany

³ Department of Drug Delivery, Helmholtz-Institute for Pharmaceutical Research Saarland, Saarland University, Campus A4.1, D-66123 Saarbruecken, Germany

Abstract:

The most prominent way of drug application is the oral administration. In the early development of a new drug the physicochemical characterization plays an important role. Based on the solubility and permeability data the substance can be assigned in a BCS class system according to the FDA. Thereby the drug behavior in a formulation may be predicted. Nevertheless, the results are detached from each other and are not so close to physiological conditions. Therefore, a system combining dissolution and permeation tests (d/p system) was developed in our group. The possibility to mount an insert with a CaCo-2 cell monolayer into this apparatus enables to study the effect of excipients and food on the permeation simultaneously under dynamic conditions. The tool for online TEER measurement is an advantageous option to control the integrity of the monolayer during the whole experiment. The apparatus was already validated with a BCS class I substance (propranolol). (1-3)

The aim of our study was to demonstrate the feasibility of the system for BCS class IV substances as well. The dissolution and permeation of two dose strengths of Lasix® tablets (furosemide) was analyzed. First results led to the expected results of a ratio of almost two comparing the AUCs. This shows clearly that the d/p system makes the analysis of low soluble and low permeable substances possible which qualifies it for future studies of various formulation factors also with BCS class III and IV substances.

1. Motz SA, Klimundová J, Schaefer UF, Balbach S, Eichinger T, Solich P, et al. Automated measurement of permeation and dissolution of propranolol HCl tablets using sequential injection analysis. *Analytica Chimica Acta*. 2007;581(1):174-80.
2. Motz SA, Schaefer UF, Balbach S, Eichinger T, Lehr CM. Permeability assessment for solid oral drug formulations based on Caco-2 monolayer in combination with a flow through dissolution cell. *European Journal of Pharmaceutics and Biopharmaceutics*. 2007;66(2):286-95.
3. Muendoerfer M, Schaefer UF, Koenig P, Walk JS, Loos P, Balbach S, et al. Online monitoring of transepithelial electrical resistance (TEER) in an apparatus for combined dissolution and permeation testing. *International Journal of Pharmaceutics*. 2010;392(1-2):134-40.

Development of a Tissue Engineered 3D Immunocompetent Model of the Human Upper Respiratory Tract

P.A. Cato^{1,3}, H.C. Harrington^{1,2}, F.R.A.J. Rose³, A. Ghaemmaghmi¹

¹Allergy Research Group, School of Molecular Medical Sciences, University of Nottingham, UK. ²Laboratory of Biophysics and Surface Analysis, School of Pharmacy, University of Nottingham, UK. ³Division of Drug Delivery and Tissue Engineering, Centre for Biomolecular Sciences, University of Nottingham, UK.

Current research tools for modelling human disease are dependent upon animal models, with limited physiological relevance, or 2D *in vitro* cell-based assays lacking complexity. Advances in the field of tissue engineering are progressively leading toward more complex *in vitro* models that better mimic native functional tissue. Using these techniques, we aim to develop a perfusable, immunocompetent 3D human upper respiratory model. Specifically, we aim to construct a tri-culture of human epithelial, fibroblast and dendritic cells each supported on multi-layered biocompatible scaffold sheets that mimic the extracellular matrix. Polymer scaffold sheets that mimic the native fibrous network of the extracellular matrix have been constructed and optimised to better support epithelial, fibroblast and dendritic cells for *in vitro* culture.

It is anticipated that such a model would simulate the natural microenvironment in which cells co-exist *in vivo* and therefore provide an effective *in vitro* tool for upper airway research.

Exhibitors Include



RANDOX



National Centre for the Replacement, Refinement
and Reduction of Animals in Research

IVTIP In Vitro Testing Industrial Platform