An advanced pluripotent stem cell test for developmental toxicity

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Published in:
Proceedings of 11th World Congress on Alternatives and Animal Use in the Life Sciences: 3Rs in transition from development to application

Publication date:
2021

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
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The US Tox21 Program has utilized a quantitative high throughput screening (qHTS) approach to profile thousands of environmental chemicals using a battery of in vitro cellbased assays. An important limitation of these assays, particularly those that measure events associated with DNA damage and repair (i.e., genotoxicity), is the absence of a xenobiotic metabolism capability. The absence of a method to provide for metabolic transformation of parent compounds may potentially lead to mischaracterization of exposure hazard if the parent compound is detoxified in vivo, rendering it less hazardous, or alternatively, bioactivated in vivo to a hazardous metabolite. To overcome this limitation, we investigated methods to incorporate a metabolic component (e.g., human liver microsomes) into existing Tox21 assays. This presentation will provide an overview of the Tox21 efforts to incorporate metabolism into in vitro HTS assays, and will be followed by presentation of a case study of a screening approach to identify acetylcholinesterase inhibitors whose activity is dependent on metabolic activation.

References

Keywords
Tox21 10K compound library; High throughput screening; In vitro assays; Metabolism; Acetylcholinesterase inhibitors
3 THE NEED TO PRIORITIZE ‘REPLACEMENT’ IN ALZHEIMER’S DISEASE RESEARCH

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Animal models of Alzheimer’s disease (AD) have been extensively utilized in the last decades to elucidate the pathophysiological mechanisms of the disease and to test novel therapeutic drugs. However, basic/fundamental and pre-clinical research successes have not translated into effective therapeutic treatments for AD patients. One of the possible reasons behind this translational failure may be the over-reliance on animal models for AD, which have been shown useful to recapitulate some AD-associated features, such as amyloidosis and tauopathy, but have failed to deliver effective treatments for AD patients. On the other hand, the use and the implementation of human-based methods, non-invasive neuroimaging technologies, and large scale epidemiological data set repositories, may contribute to the development of new preventive and treatment strategies.

Here we discuss the need to prioritize replacement, over refinement and reduction, in AD research, and show how we can mitigate this translational gap by employing human-based methods to elucidate disease processes occurring at multiple levels of biological complexity. A paradigm shift towards human-based research, accounting for a multidimensional and multi-disciplinary approach is highly needed to tackle the everincreasing prevalence of AD.

References


Keywords

Alzheimer’s disease; Replacement; Human-based methods; Animal models; Translational failure
5 THE NEED TO ADDRESS HUMAN RELEVANCE AND MEASURE RETURN ON INVESTMENT IN BIOMEDICAL RESEARCH

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Animal models have been traditionally used in biomedical research to recapitulate human disease features and develop new drugs, as they are generally purported to resemble some of the major hallmarks of human diseases. However, these animals do not develop the disease as it occurs in humans, and their use has not paved the way to the development of drugs effective in human patients. Indeed, despite conspicuous research and economical endeavours, the clinical failure rate in drug development still remains very high, with an overall likelihood of approval from Phase I of about 9.6%. On the other hand, the expanding toolbox of non-animal methods, accounting for e.g., induced pluripotent stem cells derived from patients, next-generation sequencing, omics and integrated computer modelling can be used to study human diseases in human-based settings, identify new potential druggable targets, and evaluate treatment effects. The rise of new technological tools and models in life science, and the increasing need for multidisciplinary approaches, have encouraged many research initiatives and the launch of new EU calls for proposals. Research proposals based on the use of both animal and/or non-animal approaches have been extensively funded at European level. Nowadays, it is becoming pivotal to define and apply indicators suitable to measure return on investment of research funding strategies, monitor contribution to innovation, retrospectively assess public health trends, and readdress funding strategies when needed. Here we discuss such issues, describing a list of indicators to measure return on investment in biomedical research.

References

Keywords
Biomedical research; Funding strategies; Indicators; Return on investment
Göttingen Minipigs has for decades been used in biomedical research as sharing many anatomical, physiological and pathophysiological similarities to humans, and as such playing an important role as large animal models in translational studies. In recent years, the number of genetically altered Göttingen Minipigs has increased as advanced genetic techniques simplify the generation of animals with precisely tailored modifications designed to replicate lesions responsible for human disease. As such, genetically altered Göttingen Minipigs are valuable large animal disease models and in addition considered promising donors for xenotransplantation.

To ensure compliance to the 3Rs and ensuring high animal welfare standards, it is crucial to perform a baseline assessment of the welfare of all genetically altered Göttingen Minipigs models; both during the time of creation, but also followed by the time of maintenance of the specified genetically altered model. Such a welfare assessment should be performed by experienced and knowledgeable staff, and should include animals of representative age groups soon after birth, around weaning and again around sexual maturity and include both males and females and data from a minimum of two breeding cycles. Very importantly, all comparisons should be made with similar nongenetically altered animals representing the same background strain. The observations should be performed in a structured way and include appearance, like body condition, coat and skin condition; body functions, like food and water intake; environment, like defecation and urination; behavior, like social interaction, posture and mobility; procedure-specific indicators, based on the individual project and potential adverse effects; plus finally free observations including all unexpected negative welfare impacts. In addition, an individual welfare assessment should be performed in neonatal animals based on skin color and appearance, activity level, interaction with the sow, suckling behavior and litter related data, like gestation length, litter size and development and growth of piglets.

References

Keywords
Animal welfare assessment; Genetically altered animals; Large animal disease models; Göttingen Minipigs
Although re-homing former laboratory animals, such as cats and dogs, has been practiced in some laboratories on a voluntary basis for decades and some national recommendations for the placement of laboratory animals exist, efforts to re-home laboratory animals have been further stimulated by the publication of Directive 2010/63/EU in the EU Member States. For Switzerland, there is currently no national recommendation on the re-homing of laboratory animals.

While there are some well-known re-homing programs for cats and dogs, the re-homing of smaller laboratory animals such as rodents, on the other hand, is less well known. In autumn 2018, a collaboration between the Swiss Animal Protection (SAP / STS) and the University of Zurich resulted in a re-homing project with the aim of giving rodents and rabbits from animal experiments a new life in private homes. For experimental and legal reasons not all laboratory animals can be re-homed after the experiments. However, until now more than 100 rats and mice have already been successfully re-homed. The rehoming project receives great support from the experimental animal husbandries and the research groups involved.

In this talk, I will present the prerequisites, challenges and potential of the UZH rodent rehoming program.

References

Keywords
rehoming; rat; mouse; animal welfare
Evidence-based severity assessment is essential as a basis for ethical evaluation in animal experimentation to ensure animal welfare, legal compliance and scientific quality. To fulfil these tasks scientists, animal care and veterinary personnel need assessment tools that provide species-relevant measurements of the animals’ physical and affective state.

In a three-centre study inter-laboratory robustness of body weight monitoring, mouse grimace scale (MGS) and burrowing test were evaluated. The parameters were assessed in naïve and tramadol treated female C57BL/6J mice.

During tramadol treatment a body weight loss followed by an increase, when treatment was terminated, was observed in all laboratories. Tramadol treatment did not affect the MGS or burrowing performance. Results were qualitatively comparable between the laboratories, but quantitatively significantly different (inter-laboratory analysis). Burrowing behaviour seems to be highly sensitive to inter-laboratory differences in testing protocol. All locations obtained comparable information regarding the qualitative effect of tramadol treatment in C57BL/6J mice, however, datasets differed as a result of differences in test and housing conditions.

In conclusion, our study confirms that results of behavioural testing can be affected by many factors and may differ between laboratories. Nevertheless, the evaluated parameters appeared relatively robust even when conditions were not harmonized extensively and present useful tools for severity assessment. However, analgesia-related side effects on parameters have to be considered carefully.

References

Keywords
Severity assessment; Mouse; Behaviour; Burrowing; Mouse Grimace Scale
Animal models of colitis are used to explore the underlying pathogenesis of inflammatory bowel diseases (IBD) and to develop therapies for the treatment of IBD in humans. Human IBD patients report reduced well-being and pain, it therefore can be assumed that this condition is also burdening and painful in laboratory animals. When performing animal experiments for IBD research, particular emphasis should therefore be given to the monitoring of pain and distress as well as to the implementation of adequate refinement measures.

For a systematic analysis of the implementation and reporting of these refinement measures we performed a systematic literature review on the reporting of details of the DSS induced colitis model, measures to reduce bias and information on clinical monitoring, refinements and humane endpoints.

In general, reporting of many of these important aspects was poor, indicating that this research field has yet to adopt reporting guidelines such as ARRIVE, the Gold Standard Publication Checklist or the colitis methods checklist (Bramhall et al. 2015). While many studies used the common disease activity index (DAI) for monitoring of colitis progression, other available severity assessment tools were rarely used and refinement measures like analgesia were omitted in virtually all studies due to concerns of pharmacological side effects. We conclude that there is a need for evidence based severity assessment and refinement of DSS induced colitis mouse models.

References

Keywords
IBD; colitis; mouse; refinement; reporting
Sharing legacy data from in vivo toxicity studies offers the opportunity to analyze the variability of control groups stratified for strain, age, duration of study, vehicle and other experimental conditions. Historical animal control group data may lead to a repository, which could be used to construct virtual control groups (VCGs) for toxicity studies. VCGs are a established concept in clinical trials (Eichler, H.-G., Sweeney, F., 2018), but the idea of replacing living beings with virtual data sets has so far not been introduced into the design of animal studies.

Given the fact that toxicity studies usually consist of three dose groups plus one control group, the use of VCGs has the potential for a 25% reduction in animal use. Provided regulatory acceptance can be achieved, this would represent the biggest reduction initiative in pharmaceutical toxicity testing.

Prerequisites for such an approach are the availability of large and well-structured control data sets as well as thorough statistical analyses. The IMI projects eTOX and eTRANSAFE have laid the foundation for data sharing among the pharmaceutical industry. Efforts are now being undertaken to share control animal data also from confidential data sets. Since control animal data are not related to the drug candidate and thus pose no IP issues, control group data can be shared without restrictions. Participating companies have started to collect control group data for subacute (4-week) GLP studies with Wistar rats (the strain preferentially used in Europe) and are analyzing these data for its variability. In a second step, the control group data will be shared among the companies and cross-company variability will be investigated. In a third step a set of studies will be analyzed to assess whether the use of VCG data would have influenced the outcome of the study compared to the real control group.

References

Keywords
Reduction; Data Sharing; Big Data; Virtual Control Group; Animal Study
The pharmaceutical industry is one of the sectors generating comprehensive high-quality safety assessment data. Recently, the value of these data has been recognized and different initiatives have started to mine this valuable resource (Sanz et al. 2017). The process for exploiting legacy safety assessment data starts with their collection from multiple sources but requires the application of knowledge management and integration tools before it can be used for practical purposes. Here we will introduce eTRANSAFE, an ongoing IMI2/JU funded project bringing together 12 pharmaceutical industries, 6 SMEs and 8 academic institution for collecting and exploiting this kind of data.

eTRANSAFE is developing an integrative data infrastructure supporting the application of computational methods and tools. The data collected can be retrieved, visualized and analysed in multiple ways to answer multiple relevant questions. One of the most ambitious goals is to use this information to improve the reliability of translational drug safety assessment. With this aim, the project is collecting both preclinical and clinical data and mapping the information collected in either domains. The knowledge platform being developed by the eTRANSAFE project contains all the data in an integrated form as well as advanced tools for data extraction, visualization and analysis. Beyond the simple collection of findings, the project is incorporating mechanistic information as a key strategy to improve the translatability between species and better understand the ability of preclinical studies to predict clinical outcomes. Eventually, such a systematic approach might contribute to the avoidance of animal studies lacking predictivity. In addition, the data will be further exploited with the help of machine learning and deep learning methods for translating knowledge into mathematical models and produce reliable predictions for novel compounds.

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (IMI2/JU) under grant agreement No 777365 (eTRANSAFE).

References

Keywords
Data Sharing; Knowledge management; Translational toxicology; In silico toxicology; Toxicity prediction
IN VITRO APPROACH FOR ASSESSING RESPIRATORY TOXICITY IN HUMAN LUNG CELLS

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Approaches to efficiently and effectively assess the toxicity of chemicals on the human respiratory tract using in vitro systems would provide useful information to inform product development and risk management decisions. Presented here is an approach to help better understand the appropriate in vitro system to use and the biological markers to monitor based on the test chemical under evaluation. In this study, BEAS-2B cells (a human bronchial epithelial cell line) were exposed to various concentrations (0.72ppm, 25ppm, and 85ppm) of triethoxysilane vapor at the air-liquid interface using a capillary dosage unit coupled to a VITROCELL 6/4 exposure module. Triethoxysilane is an industrial chemical classified as a GHS category 2 inhalation toxicant based on rat acute inhalation toxicity testing. A significant concentration-dependent decrease in cell viability (resazurin-based assay) and increase in cytotoxicity (lactate dehydrogenase assay) was observed after exposure to the triethoxysilane (test chemical) and nitrogen dioxide (positive control) as compared to clean air (negative control). A significant increase in expression of inflammatory markers [interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL12p70, IL-13, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α)], determined by Meso Scale Discovery technology, was observed at 25ppm. Additional work is underway to test other silanes that vary only in their carbon length to determine if this in vitro system can detect the decrease in toxicity that correlates with increasing carbon-chain length and to determine the advantages of using a 2D cell line (BEAS-2B cell) versus a 3D human reconstructed tissue model. Overall, these results will evaluate the utility of an in vitro system to predict the likelihood of a chemical to cause portal-of-entry effects on the human respiratory tract and could be a useful approach to rank chemical toxicity.

References

Keywords

in vitro; acute inhalation toxicity; air-liquid interface; respiratory toxicity
Safety assessment of cosmetics can no longer be performed by generating new animal studies in Europe. To address this, initiatives based on the use of new approach methodologies – NAMs in exposure-centric and hypothesis driven approaches are led by the cosmetic sector. The purpose of the assessment is to be protective of human health as stated in the principles developed by the International Cooperation on Cosmetics Regulation (ICCR, Dent et al., 2018). In line with these principles, an Integrated Approach to Testing and Assessment (IATA) from the European framework project – SEURAT1 (Berggren et al., 2017; OECD Series on Testing & Assessment No. 275, 2017) was utilized in a next generation risk assessment case study. The case study focused on the use of propylparaben as a preservative in cosmetics. The approach in this case study entailed a ‘learning by doing’ exercise which was designed to assess the value added by NAMs in safety assessments based on read-across. The objectives were to test the methodology and evaluate how data can be applied in decision making. The problem formulation in this assessment was an assumed data gap for reproductive toxicity for propylparaben (available experimental animal reproductive toxicity data were purposefully not taken into consideration). A tiered approach was employed to assess the reproductive toxicity hazard associated with dermal exposure to propyl paraben in cosmetics. NAMs were utilized to support the read-across hypothesis and to inform human-relevant risk assessment and support decision making. This NAM-based IATA approach facilitated testing or modification of read across hypotheses and supported the assessment of analogue chemical(s). Efforts are currently underway to continue to evolve read across in this direction. The case study was led in collaboration by a working group of members of the Cosmetics’ Europe Long Range Science Strategy – LRSS- and the horizon 2020 EUTOxRisk consortium.

References

Keywords
Next generation read-across; Next generation risk assessment; IATA; Propylparaben; New approach methodologies
The potential for pesticides to induce tumors greatly concerns regulatory authorities in Taiwan. For example, triazole pesticides pose a risk of inducing liver tumor. The rapid partial hepatectomy bioassay model (Shirai T., 1997) was established to evaluate the potential of pesticides to cause epigenetic effects (Kobets T et al., 2018) related to liver carcinogenicity. This model requires fewer animals compared to mouse long-term study (Billington R et al., 2010) and also reduces the test period from 18-months to 8-weeks. The objective of this study was to use the rapid partial hepatectomy bioassay model to evaluate the liver carcinogenicity effects of two triazole pesticides: hexaconazole and difenoconazole. Results revealed that the diethylnitrosamine and 2-acetylaminofluorene positive control groups had obvious hepatoma on the liver surface, while the aflatoxin group had less severe hepatoma formation. Conversely, no hepatoma was found in the hexaconazole and difenoconazole groups. Moreover, the hexaconazole and difenoconazole groups did not show significant increases in gamma-GT activity compared to the blank control group (p>0.05). On the other hand, acute pesticide toxicity always leads to cell damage and death in animal cells. It has become increasingly important to assess how pesticides regulate epithelial cell differentiation and cell cycle by repeatedly exposing cells to low dose levels of pesticides. Therefore, an additional objective of this study was to evaluate the extent to which hexaconazole and difenoconazole regulate primary human epithelial cell line differentiation and the cell cycle. Results showed that difenoconazole had no effect, but hexaconazole was found to enhance the effect on cell cycle regulation between the G2 phase and the mitosis phase compared to the blank control. This study, which was conducted in accordance with the 3R principles of animal welfare, provides important information pertaining to carcinogenicity risks associated with triazole pesticides.

References

Keywords
Rapid Partial Hepatectomy Bioassay Model; Epigenetic Carcinogenicity; Hexaconazole; Differentiation; 3R principles
REPEATING PAST MISTAKES: THE BANALITY AND FUTILITY OF NOWADAYS CIGARETTE SMOKE-RELATED ANIMAL EXPERIMENTATION

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Since the 1950s experiments have been performed with different animals like mice, dogs and monkeys, forced to inhale, ingest or otherwise receive large amounts of cigarette smoke (CS) or its derivatives. Because of a low reproducibility between different species and a poor transferability to humans, these experiments have been widely criticized by the scientific community and partly used as an argument by the cigarette industry attempting to deny the link between CS and lung cancer. Eventually, despite these experiments, the collective results from multiple largescale epidemiological studies have conclusively proven that CS is the leading cause of lung cancer and exacerbates many other severe diseases.

Although animal experiments have long been deemed unreliable in assessing the effects of CS in humans, such experiments are still being performed in multiple laboratories. Amongst them are extremely invasive procedures associated with immense animal suffering, including acute trauma, hemorrhagic shock and massive inflammation (Bucher et al., 2017, Jia et al., 2018, Hartmann et al. 2019). Furthermore, with small modifications many studies are merely repeating the effects already described in humans and are frequently failing to reproduce the results of clinical data.

The banality of the outcomes, i.e. that CS contributes to various lung-related health problems, the repetition of studies already performed in patients and the contradiction of clinical results reveals the extreme inadequacy of using animals for the analysis of CSrelated diseases in humans. Luckily, many human-oriented, innovative and personalizable methods like precision cut lung slices, 3D lung epithelium models and lung-on-a-chip systems are readily available and approved for regulatory purposes. Here, we show recent animal experiments and human-based research on CS effects. Taken together, we regard the fact that severely harmful animal experiments are still being performed as scientifically and ethically unjustifiable and demand their immediate replacement with more suitable in vitro techniques.

References

Keywords
cigarette-smoke; animal suffering; untranslatability; human-based lung models
As a diversified global healthcare leader focused on patients’ needs, Sanofi is morally and legally obligated to ensure the quality, safety and efficacy of its medicines, vaccines, medical devices, and consumer healthcare products. Besides the regulatory requirements, the responsible use of animals is essential in the research and production process. Animals remain a small but an integral part of a comprehensive research and testing strategy that includes non-animal methods (such as computerized models and in vitro testing) and clinical research. The standard approach is designed to use animals only when a non-animal method is not suitable for the required use or not accepted by the authorities (replacement), with the smallest number necessary for quality science (reduction) while implementing state-of-the-art practices to promote animal welfare and prevent pain and distress in housing, procedures and treatment (refinement). To go further, a strategy, relying on regulatory sciences, translational medicine and breakthrough innovation, has been developed to increase the proportion of non-animal methods, including clinical research, to reduce significantly the necessity to use animals in research and production. Several decades ago, the development on new drugs and vaccines mainly relied on animal studies. Nowadays, all the projects require and use non-animal data, in vivo studies and clinical research to assess the safety and the efficacy of new drugs. We strongly believe that, based upon the development of regulatory sciences, translational medicine and innovation, reduction of the ratio animal studies versus non-animal methods is effective and it will accelerate.

References

Keywords
Strategy; Non-animal methods; Integration; 3Rs
There is an unmet need for an accurate, non-invasive biomarker test for the diagnosis of early Alzheimer’s disease (AD). To identify new biomarkers, we focused on long noncoding RNAs (lncRNAs) as they are tissue-specific to identify lncRNA panel candidates for diagnostic of early Alzheimer’s disease (AD) and other dementia types. Methods: We performed a screening using NGS RNA-Sequencing to quantify over 127000 lncRNAs in human postmortem brain tissue and blood samples including whole blood, PBMCs and, plasma samples collected in prospective clinical studies that recruited patients with early AD, patients with late AD, patients with one of the other 5 dementia types and healthy controls. Results: We identified for the first time several panels (i) a panel of brain-enriched lncRNAs never described before, (ii) panels of brain-enriched lncRNAs that are either expressed in whole blood or circulating in plasma. (iii) Interestingly, out of these, we also identified panels of lncRNAs that are differentially expressed in the blood of patients with AD or with other dementia types as compared to healthy control subjects. The most accurate lncRNA panel to detect early AD is selected for use as Research-UseOnly test and is being further validated to compile the data dossier for submission to regulatory agencies for approval as an in-vitro diagnostic (IVD) tool diagnostic of AD. Additional clinical applications are for prognostic or theragnostic purposes. Conclusion: Our results from studies combining the use of high-quality samples from welldesigned prospective clinical studies, cutting edge technologies and scientific knowhow enabled to translate novel brain specific lncRNA panels measurable in blood as new noninvasive and accurate diagnostic approaches using highly specific and low represented sequences specific to neurodegenerative diseases.

References

Keywords
Alzheimer’s disease; Diagnostic tool; long non-coding RNA; Biomarker; IVD
66 AIR-LIQUID INTERFACE EXPOSURE FOR INHALATION TESTING: CASE STUDIES

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There is a demand to implement in vitro alternatives for inhalation testing using human relevant lung cell models, realistic air-liquid interface (ALI) exposure systems, and proper dosimetry techniques to increase the predictability and accelerate the shift from in vivo towards in vitro testing. Presented here are case studies. In a first case study, BEAS-2B cells were exposed to various concentrations (0.72, 25, and 85 ppm) of triethoxysilane vapor using a capillary dosage unit coupled to a VITROCELL® 6/4 module. A significant concentration-dependent decrease in cell viability and increase in cytotoxicity was observed after exposure to triethoxysilane and nitrogen dioxide (NO\textsubscript{2}, positive control) as compared to clean air (CA, negative control). A significant increase in expression of inflammatory markers was observed. Additional work is underway to test other silanes to determine if this in vitro system can detect the decrease in toxicity that correlates with increasing carbon-chain length and to determine the advantages of using a 2D cell line versus a 3D human reconstructed tissue model. Another case study is focused on ALI inhalation testing of petroleum-derived substances. A generation facility was successfully developed at VITO to volatilize ethylbenzene (EB) and VITROCELL® 24/48 exposure system was optimized and validated for CA, NO\textsubscript{2}, and EB exposure. A significantly decreased mean cell viability of 86\%, 77\%, and 47\% was observed for exposure of A549 cells to EB of about 30000, 40000, and 50000 mg/m\textsuperscript{3}, respectively. Inflammatory and oxidative stress markers were evaluated as well. The difference between in vivo absorption and in vitro deposition (chemically determined) is a crucial element when setting an ALI dose-range. Quantitative in vitro to in vivo extrapolations from in vitro air concentrations applied for testing cell viability to in vivo air concentrations may be a promising method for screening acute adverse inhalation effects.

\textbf{References}

\textbf{Keywords}
Air-liquid interface exposure; In vitro inhalation testing; Petroleum-derived substances; Silanes
Buddhism as a religion promotes love, compassion and kindness towards all living beings thus promoting the concept ‘do not harm/make them suffer (“avihinsa”)’. Buddhism facilitates mental and physical wellbeing of animals. The first precept makes people aware of killing of animals is a sin and all five precepts are to promote ethical conduct of humans. The concept of ‘cause and its effect’ is the fundamental principle in Buddhism. In this context, science is an accepted phenomenon in Buddhism; however, as scientists we must take steps not to harm animals for the benefit of humans.

According to Hinduism cultural differences play a major role in animal welfare as we are grown up considering animal as a form of God and the principle of AHIMSA (NonViolence). Series of steps taken from 1996-2002 for animal welfare including formation of Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA) to implement guidelines governing use of animals in research and enforcing penalties for non-compliance.

Researchers in Malaysia prone to identify the usage and toxicity of various herbal material which are abundant in the country using laboratory animals such as rodents, rabbits and zebrafish. Since Islam is the main religion of Malaysia, the Islamic views has influenced in various aspects of daily activities including scientific research. Therefore Islamic point of view has become a basic principle for the establishment of the humane practice in using laboratory animals in science.

Many religions promote good and compassionate animal care, it be domestic or wild. The current world uses lab animals to discover new drugs and test toxicities of existing ones. A healthy research program exists if all these religions continue to promote such compassion towards animals. Culture of care is one such educated care that can promote the care of animals.

References

Keywords
Religion; Ethics; Welfare; Animals in science; Culture of care
AN INTEGRATED APPROACH ALTERNATIVE FOR SCREENING REPRODUCTIVE, DEVELOPMENTAL AND ENDOCRINE DISRUPTING ACTIVITY WITH EX VIVO WHOLE RAT EMBRYO CULTURE

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Standard animal tests to evaluate the safety of pesticides can be prohibitively expensive. However, rat post-implantation whole embryo culture (WEC) is a promising alternative test to assess developmental toxicity. In this study, we suggest proposed an integrated approach to screen for reproductive, developmental, and endocrine disrupting activities using ex vivo whole rat embryo culture. Specifically, our proposed approach evaluates adverse outcome pathways (AOPs), chemical structure activity relationships (SARs), morphological scores, receptor activities, and immunohistochemistry in rat WEC. We also employed WEC to assess endocrine-disrupting activity induced by environmental chemicals, which to the best of our knowledge, had not been done before. All experiment results in this study were comparable with those of OECD test guidelines for reproductive toxicity (443, 415, 416), developmental toxicity (414, 421, 422) and endocrine disrupting activity (426, 440, 441). Results revealed that, during rat embryo development, 17ßestradiol, triiodothyronine, triadimefon, penconazole, propiconazole did not significant affect the yolk sac circulatory system; allantois; flexion; the heart caudal neural tube; the hindbrain, midbrain, or forebrain; the otic system; the optic system; the olfactory system; the maxillary process; the forelimb or hind limb; yolk sac diameter; crown-rump length; head length; or developmental score. Immunohistochemistry revealed that 17ßestradiol (which was used as a positive control) positively affected its receptor expressions. These three triazoles induced ERα and ERß expression in WEC. This result illustrates the triazole mode of action, in which triazole compounds disrupt steroid hormone synthesis. Finally, the research performed herein confirmed that our proposed method is both effective and fast, requiring only 10.5 days to detect (1) hormone receptors, such as androgen, estrogen, and thyroid receptors, (2) aromatase receptors and aromatase activity, and (3) developmental and reproductive toxicity. Therefore, our integrated approach to screening for reproductive, developmental and endocrine disrupting has the potential to benefit pesticide testing and assessment applications.

References

Keywords
whole embryo culture (WEC); reproductive; developmental; endocrine disrupting activity; ex vivo
The Tox21 Program has screened a 10,000 compound library in over 80 cell-based quantitative high throughput screening (qHTS) assays. These assays employ 1536-well plates on a robotic platform; all assays are homogeneous (“add, mix, measure”) with no aspiration steps due to the small assay volume per well (~5 uL). In standard in vitro assays used routinely to characterize genotoxicity (e.g., bacterial mutation or mammalian cell micronucleus assays), induced rat liver S9 fraction plus cofactors is employed as a source of Phase I enzymes to mimic in vivo metabolism of the compound under study. However, S9 enzymes are toxic to cells after a few hours of exposure. Therefore, in these standard assays, medium plus S9 mix is removed after ~4 hours and replaced with fresh medium without S9. Due to the inability to remove S9 mix by aspiration, Tox21 cell-based assays have had limited or no metabolic capability. This lack of biotransformation capability is reflected in the inability of the Tox21 DNA damage assays to detect most compounds that require activation to a DNA reactive metabolite (e.g., pyrene). This limitation also risks mischaracterization of compounds that are detoxified by liver metabolic enzymes. Successfully retrofitting Tox21 assays with metabolic capability would achieve a key goal in the new Tox21 strategic plan of providing data with enhanced physiological relevance. We therefore investigated whether low, non-toxic concentrations of human or induced rat liver microsome preparations could provide effective metabolic capability to the cell-based p53 reporter gene assay, which measures activation of p53, a gene that responds to DNA damage. Results showed both microsome preparations were biologically active, but differences were seen in the number and identity of chemicals that were metabolized. Interpretation of these data and applications for use of microsome preparations in high throughput screening assays will be explored.

References

Keywords
HTS assays; metabolic enzymes; p53 assay; genotoxicity; DNA damage
88 REPLACING FOETAL BOVINE SERUM: A PIECE OF CAKE?

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FBS is still being applied as the universal medium supplement to grow and maintain cells and tissues. But, the use of FBS presents five significant issues:

1. the degree of suffering experienced by the calf during blood collection (van der Valk et al, 2004);
2. inappropriate cellular growth profiles and physiological responses of cells cultivated with medium containing FBS;
3. FBS contamination with viruses, prions, etc.;
4. the large variability of FBS such that it is very difficult to even ensure consistent and well-controlled in vitro cell culture between batches;
5. the fraud-problem (Gstraunthaler et al, 2014).

To answer to these issues, the use of FBS in particular, but also the use of other animal-derived products in cell, tissue and organ culture and related experimental techniques should be avoided wherever possible. There are several strategies to avoid the use of FBS. Human platelet lysates (HPLs) can be a valuable alternative to FBS as cell culture supplement. In addition, there is large interest in chemically-defined media (van der Valk et al, 2018). As HPLs are undefined but work for most cell types, chemically-defined media, on the other hand, are cell type-specific. To facilitate the search for serum-free media, the fcs-free.org database was established. This database provides an overview of commercially available serum-free media for cell and tissue culture, as well as medium formulations for specific cell types obtained from scientific literature. Furthermore, the website serves as a platform to exchange information on the quality and applicability of each product. Not for every cell type is yet a serum-free medium available. Strategies will be discussed to develop a serum-free medium for a specific cell type and to adapt cells to the new medium (van der Valk et al., 2010).

References

Keywords
in vitro; foetal bovine serum; chemically-defined medium; database; animal welfare
With more than 80% of national competences being centralised by the European Commission (EC) on behalf of
the EU member states, Brussels is the place to be when it comes to interact with the policy makers (e.g.
Members of the European Parliament (MEP)). EC transparency register has collected in February 2019 almost
12,000 entries claiming lobbying activities in the Belgian capital. Animal Welfare and 3Rs are not always a
central topic being discussed when Brexit, Greek debt, migration crisis, terrorism attacks, glyphosate re-
authorization are also steering the attention. In other words, the 3Rs’ topic competes with all these major
issues that policy makers deal with every day. Therefore, it is essential to map the policy environment in order
to better communicate 3Rs and be efficient with policy makers by 1) identifying the actors, 2) the institution we
are dealing with 3) the pressure participants 4) linking 3Rs with the context and events (i.e. political agenda) 5)
providing appropriate evidence that fits policy concerns at hands and last but not least 6) building trust on the
long term. For the latter, an initiative was designed to provide inter-sector information exchange for future
actions is the "MEP - 3Rs scientists pairing scheme" initiated in 2015 by CAAT-Europe at the European
Parliament. it was the opportunity to identify some of the obstacles when it comes to integrate scientific
advice/expertise with values represented by elected policy makers.

References

Keywords
policy makers; evidence
93 MIND THE GAP: IMPROVING LITERATURE SEARCH SKILLS TO ACCESS THE MOST RELEVANT SCIENTIFIC AND TECH KNOWLEDGE.

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About 20 million scientists in the world publish roughly five papers per minute, with an increasing trend of 8-9% per year. Such an overload of information is likely to cause stress and frustration to most researchers, giving the erroneous impression that there might be no much left to research on. However, not only the 2.5 millions scientific publications per year are a proof of the contrary, but they may also turn to be a great source of inspiration. Indeed, underneath this huge amount of information, knowledge and technology gaps are hiding and literature search becomes a treasure hunt. Most of the researchers likely use their favourite web search engine and begin an iterative, often endless, typing. Are there better keywords that could be used? How to search for something that perhaps does not exist? And how to assess the relevance of the retrieved information? Here, we will provide you with clues and a series of critical steps to identify gaps in specific research fields of interest. The systematic search approach allows acknowledgement of relevant literature and provides keys to answer open questions, while saving time and money by avoiding to reinvent the wheel. Finally, and most important, our proposed suggestions will allow to gain insights and seed ideas for future grant proposals and/or patent submissions, thus opening new avenues to research innovation.

References

Keywords
systematic search; knowledge gap; research innovation; data analysis; technology
Both the reported shortcomings of animal studies regarding their predictivity for humans as well as the desire to reduce the dependence on animal testing in drug development inspired the hope for microphysiological systems or organ-on-a-chip. As with any new technology, it became evident, that it was necessary to channel the initial hype into realistic expectations to avoid subsequent delusions. CAAT was instrumental in this process by organizing a workshop in the framework of their transatlantic think tank for toxicology. This workshop, held in 2015, brought together stakeholders from the pharmaceutical industry, academia, technology providers and regulators. The group exchanged the state of the development of the technology and developed a road-map for the implementation of microphysiological systems within the drug development pipeline (Marx et al. 2016). The workshop triggered a series of interactions and collaborations between pharmaceutical companies and technology providers. The success of the workshop is evidenced by a follow-up event held in 2019. The presentation will summarize the results of the initial workshop and discuss the level of implementation in the light of the recent developments.

References
Marx, U., Andersson, T.B., Bahinski, A. et al. (2016). Biology-inspired microphysiological system approaches to solve the prediction dilemma of substance testing. ALTEX 33(3), 272-321. doi.org/10.14573/altex.1603161

Keywords
Drug-induced liver injury (DILI) is one of the major reasons for termination of drug development. Due to the importance of predicting DILI in early phases of drug development, diverse in silico models have been developed to filter out DILI-causing candidates before clinical study. However, no computational models have achieved sufficient prediction power for screening DILI in early phases because 1) drugs often cause liver injury through reactive metabolites, 2) different clinical outcomes of DILI have different mechanisms, and 3) the DILI label on drugs is not clearly defined. In this study, we developed binary classification models to predict drug-induced cholestasis, cirrhosis, hepatitis, and steatosis based on molecular structure of drugs and their metabolites. DILI-positive data was obtained from post-market reports of drugs, and DILI-negative data from DILIRank, a database curated by FDA. Support vector machine (SVM) and random forest (RF) were used in developing models with nine fingerprints and one 2D molecular descriptor calculated from the drug (152 DILI-positives and 102 DILI-negatives) and drug metabolite (192 DILI-positives and 126 DILI-negatives) structures. Models were developed according to OECD guidelines for QSAR validation. Internal and external validation was performed with a randomization test in order to thoroughly examine model's predictability and avoid random correlation between structural features and adverse outcomes. The applicability domain was defined with a leverage method for reliable prediction of new chemicals. The best models for each liver disease were selected based on external validation and applicability domain analysis results on drugs (cholestasis: 70%, cirrhosis: 90%, hepatitis: 83%, and steatosis: 85%) and drug metabolites (cholestasis: 86%, cirrhosis: 88%, hepatitis: 86%, and steatosis: 83%). Compiled data sets were further exploited to derive privileged substructures that were more frequent in DILI-positive sets compared to DILI-negative sets and in drug metabolite structures compared to drug structures with a Morgan fingerprint level 2.

References

Keywords
Drug-Induced Liver Injury (DILI); Structure-Activity Relationship (SAR); Structural Alerts (SAs); Computational Toxicology; Drug metabolism
A new protocol based on a microphysiometric system (McConnell et al., 1992, Hartung et al., 2010, Wiest et al., 2016, Alexander et al., 2018, Brischwein and Wiest, 2019) to analyze cell culture medium (CCM) is described. With the presented cellasys #8 protocol, significant data can be gained in 24 h compared to conventional weaning experiments which need several weeks to perform. First, L929 cells are supplied for 6 hours with DMEM + FBS reference medium to gain initial data. In a second step, 6 hours of the investigated test medium is supplied to see if there are any changes in cellular vitality or morphology. Then again 4 hours DMEM + FBS and 4 hours of test medium to monitor if the effect of the CCM to the cells is reversible. The experiment ends with 4 hours of test medium + 0.2% SDS to induce cell death. In the presented work, two chemically defined CCM and a common serum-containing medium DMEM + FBS were tested on the L929 cell line. Compared to the reference medium, cells in the DMEM/Ham’s F12 + ITS medium pursue a loss in adherence, but no decrease in extracellular acidification rate. This was substantiated by the observation that the acidification rate remained constant and the impedance recovered after changing back to the reference medium. Cells in NCTC 135 retained their impedance values but lost vitality. It seems reasonable to suppose that cells in NCTC 135 medium are slowly suffering as indicated by a slow decrease in impedance and highly fluctuating acidification rates. Further experiments for the presented method could be the improvement of the DMEM/Ham’s F12 + ITS medium. With the new method electrical cell-substrate impedance and extracellular acidification responds of the cells can be measured immediately and consequently the quality of new CCM can be quantified.

References

Keywords
chemically defined cell culture media; FBS free; extracellular acidification; electrical cell-substrate impedance; microphysiometry
As a follow up on the European framework for Endocrine Disruptors (EC 2018), EURL ECVAM is coordinating the validation of 17 thyroid-targeted test methods (OECD 2014). The aim is to assess the potential of chemicals to disrupt the thyroid hormone (TH) axis, including various modes of action. Fourteen laboratories from the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NetVAL), are participating in this two-part validation study: - Part 1, currently ongoing, aims to define the methods by standard operating procedures (SOPs) with assessment of reproducibility and reliability. Known positive/negative control and reference chemicals, respective for each method (OECD 2018) are being used, identified previously during test method research and development. - Part 2 will aim at assessing the overall relevance of the test methods, using 30 validation set chemicals, selected on the basis of known modes of action and available in vivo study data screened using all 17 methods. A challenge for the success of this validation study is the appropriate selection of relevant validation set chemicals to be tested, which should cover the range of expected response from the various modes of action. In support of the chemical selection for Part 2, an expert meeting was organised in November 2019. Prior to this meeting, experts were surveyed to propose potential validation set chemicals (OECD 2018) with known activity in at least one of the methods/mechanisms of action: 87 chemicals were initially suggested. During the meeting, these initial proposals were reduced to a short list of 51 chemicals, including negative control chemicals, which in the collective opinion best cover the methods and their modes of action. The shortlist will undergo further refinement over the coming months to yield 30 validation set chemicals.

References

Keywords
Thyroid disruptors; Validation; Alternative Methods; Chemical selection
We become scientists because we all want the world to be a better and healthy place. In addition, science and research is the most energizing and stimulating experience your brain can live. As researchers, we live everyday asking questions with no answers. The adrenaline pumps up when you start generating bits and pieces of results and having some hints about a plausible, but a still very preliminary, answer. Then we shift to depression when we realize months of experiments were just on the wrong direction. However, we are quickly back to our desk with tons of articles and a new experimental plan to crash the bench. Finally you publish your scientific results, your endorphins and serotonin levels are at the top. You party all night and the next morning you start again. Will this article really make the world a better place? You spread your idea into the world, but you still need someone able to understand that idea and transform it into something real that people can benefit of. And business is a very key piece in this process, named innovation. This is why some of us go from research to business to keep pushing science to make our world a better place.

References

Keywords
Innovation; Life Science and Business; Exploitation; Dissemination; Researcher life
WHAT IS THE ANALYSIS OF BIOMEDICAL RESEARCH LITERATURE TEACHING US ABOUT THE USE OF NON-ANIMAL MODELS?

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Originality is the seed of innovation. Original ideas, questions and approaches are rewarded in life science with high-impact factor publications. High-impact factor publications give us visibility and the possibility to apply for new fundings and to ask more original questions to start again. This is an established reward system in life science. Every researcher is trying to be as much as original as possible to keep up in this system. One of the key factors to be truly original is studying thoroughly the state of art, for not reinventing the wheel and to add real value. However, after screening more than 250,000 publications, my team and I think that a large number of researchers are not good as they should in studying the state of the art. And most importantly, we start believing that originality is hampering non-animal models research. Our literature review analysis in four fields of biomedical research has revealed most of the time poor originality and many limitations in our way of planning, developing, characterizing and using non-animal models. However, thanks to this enormous project funded by the European Commission, we are now able to propose some working solutions to improve the originality of our research and to offer more relevant non-animal models for their use. And one of them is collaboration.

References

Keywords
Innovation; Literature; research system; non-animal models; originality
Chronic diseases, such as Alzheimer’s disease, cancer, and cardiovascular disease are becoming increasingly prevalent in Western countries. Such noncommunicable diseases account for 71% of all deaths globally and are generally the result of a combination of genetic, physiological, environmental, and lifestyle factors (WHO, 2018). Over the last two decades, the European Commission (EC) has invested extensively in biomedical research to increase understanding of the mechanisms underlying disease pathogenesis and consolidation. However, despite this research and economical endeavor, the clinical failure rate in drug development remains very high, with an overall likelihood of approval from Phase I around 9.6% and almost a 95% failure rate in drugs entering human trials (Seyhan, 2019). It is increasingly recognized that advanced human biology-based models contribute to a deeper understanding of human health, how diseases emerge, develop, and spread, and drive the development of safe and effective therapeutics. There has been a noticeable paradigm shift away from the reliance on whole animal models to study human physiology, pathology, and pharmacology toward the utilization of human-relevant model systems. To increase the understanding of the scientific and societal impacts of animal and non-animal approaches in biomedical research we have developed suitable indicators to retrospectively assess the impact of EC-funded research activities in the fields of Alzheimer’s disease, breast cancer, and prostate cancer. Through the collection and analysis of quantitative and qualitative research, we aim to understand how EC-funded research has contributed to innovation and scientific breakthroughs, how scientific results have translated into socioeconomic impacts of benefit to society, the scientific methods and research approaches contributing to the advances made, and measure return on investment in biomedical research.

References

Keywords
Biomedical research; Funding strategies; Indicators; Return on investment
Next Generation Risk Assessment (NGRA) is an exposure-led, hypothesis-driven approach integrating new approach methodologies (NAMs) to ensure the safety of consumer products without the use of animal data. We recently applied an ab initio tiered framework (Berggren et al., 2017), based upon the ICCR principles (Dent et al., 2018), to a hypothetical safety assessment of coumarin in a shampoo, face cream and body lotion. This provided practical experience in applying NAMs as part of an NGRA framework. A workshop was held in October 2019, which focused on the approach to systemic toxicity risk assessment demonstrated in this case study and the underpinning mechanistic science.

Key areas discussed include:
1. Communication and framework development – There is a need for a more general and universal framework for approaching an ab initio NGRA. Also, communication of the inherent uncertainties and the distributions generated for points of departure (PoD) and margins of safety are essential.
2. Decision making – Utility to make informed, meaningful decisions on the available data is a key part of safety assessment, but knowing when we have enough data to be confident can be challenging.
3. Optimal assay design – Characterising and standardising the design of the assays used can reduce the uncertainty of assay outputs and thus of the PoD for internal and external exposures. This topic was discussed in the context of incorporating metabolism and clearance.
4. Making the most of benchmarking – The use of suitable benchmarking reference chemicals is important for both assay evaluation and for interpreting the risk associated with margins of safety derived from NAM-based PoDs.

This example illustrates how case studies are an impactful method for communicating the current capabilities of NGRA, ultimately driving conversations that will lead to change in the understanding and acceptance of non-animal approaches to safety assessment globally.

References

Keywords
NGRA; NAM; Systemic toxicity
While the use of omics techniques is increasing in research, their application in regulatory toxicology is still extremely limited. Omics are commonly criticized for not being sufficiently reliable for regulatory application because the output depend on the applied analysis pipeline. This reticence to trust omics data is further magnified by the lack of internationally agreed upon guidelines and protocols for both the generation and processing of omics data. One way forward would be to reach a consensus on an omics data analysis framework for regulatory application (R-ODAF) based on rigorous data analysis. The authors of this study are proposing an R-ODAF for transcriptomics data for three platforms: microarrays, RNA-Sequencing and TempO-Seq. The R-ODAF will then be reviewed and evaluated by the main regulatory agencies and consensus forums such as the Organization for Economic Co-operation and Development (OECD). This work is running in parallel alongside an OECD initiative to develop a guidance document called a transcriptomics reporting framework (TRF) that will enhance the quality of reporting of omics data when generated for regulatory purposes. The presentation of the project is now published (Verheijen et al., 2020), and the final proposed pipeline will be presented in the event.

References

Keywords
Transcriptomics; Toxicogenomics; Toxicology; Regulatory agencies; data-analysis framework
The principles identified by the International Cooperation on Cosmetics Regulation for the use of new approach methodologies (NAMs) in Next Generation Risk Assessment (NGRA) include that risk assessments should be designed to prevent harm in consumers, be exposure-led and that any data generation should be tiered and iterative. Assessments should be based on robust and relevant methods and strategies (Dent et al, 2018).

An approach to the evaluation of NAMs for use in NGRA for both skin allergy and systemic effects will be discussed. In both cases, detailed information on levels of exposure to benchmark substances that can cause adverse effects in humans drive the evaluation process: either through (1) known levels of skin exposure to materials with documented evidence regarding the ability of that exposure to induce skin sensitisation; or (2) systemic exposure estimates (using physiologically-based kinetic modelling, Moxon et al, 2020) for materials with documented evidence regarding the ability of that exposure to induce systemic adverse effects.

In this context, two approaches to the evaluation of NAMs for use after an initial in silico NGRA tier will be described: (1) an approach for skin allergy using experimental data from DPRA, hCLAT, KeratinosensTM and U SensTM assays and a Bayesian probabilistic model to estimate human sensitiser potency (Reynolds et al, 2019) and (2) an approach for systemic effects using SafetyScreen44TM, Cell Stress Panel (Hatherell et al, 2020) and BioSpyder Tempo-Seq, high throughput transcriptomic data. This approach to evaluation of NAMs for use in NGRA seeks to establish a fit-for-purpose, robust and reproducible set of bioactivity assays that can be used to generate margins of safety for chemicals when combined with information on levels of consumer exposure.

References

Keywords
risk assessment; skin sensitisation; systemic effects; NGRA
Introduction:
Degenerative corneal disease often leads to corneal edema and visual loss in humans. One therapeutical approach is the application of deswelling eye drops. Up to now there is 5% saline solution as a typical deswelling agent. These drops initially tend to have a good effect but, over the course of further treatment are prone to rebound and worsen the problem. We wanted to improve those drops and developed the corneal edema test in the EVEIT to avoid animal experimentation.

Methods:
We modified our standard approach to cultivate rabbit corneas from abattoir in our EVEIT system. For this, we used a culturing medium (MEM) diluted with hypo-osmolal saline solution. Through this, the EVEIT cornea shows considerable edema with thickness of original 500µm up to 800-1000µm. By applying various hyper-osmolal solutions we simulate the therapeutical effect of edema reduction. We used different commercially available preserved and unpreserved hyper-osmolal saline solutions 5% with/without different additives. The goal was to develop a novel formulation more effective than the commercial references, which was expected to improve the long-term stability of corneal deswelling without rebound and without disturbing the corneal epithelial surface.

Results:
The comparison of 5% saline solution with preserved 5% saline solution and another, unpreserved 5% saline containing hyaluronic acid showed deswelling effects but dependent on preservatives surface damage and rebound swelling. We developed formulations with comparable effectiveness, lower saline content preventing the rebound effect.

Conclusion:
There is evidence, that the biological system of the EVEIT can not only be employed to identify corrosive substances, but is also able to guide clinically the development of new therapeutical substances. Here, we simulate corneal edema, its therapy and reversibility. The replacement of live animal experimentation is realised here with the simulation of high exposure rates and existing therapy regiments close to clinical reality.

References

Keywords
corneal edema model; EVEIT; toxicity
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Introduction:
In 2012 we published data on the EVEIT using 38 corrosives (Spöler et al. 2015). The substance set used contained some of the “should not be used” substances according to the analysis of Barroso et al (Barroso et al. 2017) in the DRD Database. The authors recommended against involving these substances in test development and/or validation. We re-evaluated the data and focused on the ability of the EVEIT test to distinguish between Cat 1 and Cat2 or NC corrosives.

Methods:
All substances used in our tests were analysed on “should not be used” and whether or not the cornea was the driving factor. We excluded substances that should not be used and those where the cornea was not the driving factor for the resulting categorization.

Results:
In this analysis we found a 97% specify and a 98% sensitivity of the EVEIT test to identify Cat 1 substances. One substance with a false positive Cat 2 result in our system was Trichloroacetic acid (TCA) which is GHS-classified as NC.

Conclusion:
The false positive result is a consequence of superficial burns being the main effect of TCA. In living animals this is subject to shedding of epithelial cells motivated by lid action which is not realised in the EVEIT model system. We introduced a rinsing procedure to simulate epithelial renewal. We are certain that this type of superficial burns, which are healing over the course of an animal experiment, can be simulated in the EVEIT system.
Overall the CAT1 - detection rate of our model is the highest amongst all live-animal-free experimental approaches used in toxicology.

References

Keywords
EVEIT; toxicity; Classification
125 A PRECISELY ADJUSTABLE, LIVE ANIMAL FREE OCULAR CORROSION MODEL

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Introduction
The need for live animal free toxicological tests has increased in numerous branches of science, medicine and industry. In this project we improved the EVEIT ex-vivo ocular chemical injury model. Now we are able to induce and measure ocular corrosive effects on a precisely adjustable and consistent scale.

Methods
The model is utilizing the ex-vivo organ culture technique employed by the Ex Vivo Eye Irritation Test (EVEIT). Briefly, rabbit corneas from food industry are installed into specialised culturing chambers. Corneas are supplied with nutrients from the endothelial side in a continuous flow. Physiological pressure and flow conditions are realised by an artificial anterior chamber. A three axis corrosive applicator workstation (BioFluidix GmbH, Germany) with a specialised impulse based application mechanism is able to precisely place substance droplets in the nanolitre range contact free onto the corneal surface. Timing and frequency of droplet application can be exactly adjusted, so that multiple positions on one cornea can be treated sequentially. The depth of corneal injury induced by the substance application is detected by means of a high resolution OCT system. The quantification of the damage zone in OCT images is performed by analysis software.

Results
An application of 10 – 80nL of NaOH in increasing concentrations (250mM; 500mM, 1000mM, 2000mM) to the corneal surface yielded significantly different OCT signals for each concentration used. The detected penetrative effect ranged from superficial to extensive. Further, a direct strong correlation was established between the volume and the extent of the measured OCT signal.

Conclusion
With this novel live-animal-free method, we were able to induce ocular corrosive effects in a precise and consistent manner. The effects, which ranged from superficial to extensive, were controllably and consistently triggered. Thus, this method could provide a basis for future ex-vivo investigations into the treatment of ocular corrosive injuries.

References

Keywords
Ocular corrosion model; EVEIT
126 THE VALIDATION OF A SEMI-HIGH THROUGHPUT AUTOMATED OCULAR TOXICITY TEST

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Introduction
The necessity of a live-animal-free ocular toxicity test is strongly represented in multiple branches of science, medicine and industry. The Ex Vivo Eye Irritation Test (EVEIT) is providing the platform for various ex-vivo testing applications. To improve the efficiency of the test, we adapted an automated substance application apparatus that is able to precisely place quantified test substance droplets in the nanolitre range onto the corneal surface. With this technology we are able to test multiple test substances on a single cornea.

Methods
The model is utilizing the ex-vivo organ culture technique employed by the Ex Vivo Eye Irritation Test (EVEIT). Briefly, rabbit corneas from food industry are installed into specialised culturing chambers. Corneas are supplied with nutrients from the endothelial side in a continuous flow. Physiological pressure and flow conditions are being realised by the artificial anterior chamber.

A substance applicator workstation with a specialised impulse based application mechanism is able to precisely place substance droplets contact free onto the corneal surface. Timing and frequency of droplet application can be exactly adjusted, so that multiple positions on one cornea can be treated sequentially.

Categorization into NC, CAT2 or CAT1 is based on observations via OCT and live fluorescein staining immediately after substance exposure and two days later to integrate acute and long-term effects respectively.

Results
The application mechanism, in combination with the ex-vivo organ culture model, is very precise with a high degree of repeatability. Further, the setup allows for the instillation of minute adjustments. Correlations of application and damage patterns with strong corrosives are highly repeatable.

Conclusion
We aim to be able to recreate the results of the substance categorization created in the pre-validation project. The novel application mechanism exhibits a high degree of flexibility which enables us to fine-tune the testing system according to our needs.

References

Keywords
Ocular toxicity; EVEIT; automatization
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The recent publication of Principles of Animal Research Ethics, 2020, Oxford University Press by Tom Beauchamp and David DeGrazia outlines the Beauchamp-DeGrazia Framework of Principles (Beauchamp and DeGrazia 2020). This presentation will explore the existing ethical gap during IACUC (or equivalent) review, even with the application of Harm/Benefit analysis and the 3R’s of Replacement, Reduction and Refinement. This gap exists because HBA and 3Rs do not include a robust enough framework for ethical debate on animal studies. Beauchamp and DeGrazia’s analysis of the gap resulted in 3 premises: (1) sentient animals have moral status and therefore are not merely tools of research, (2) the only possible justification for (nontherapeutically) harming animals with moral status, including animal research subjects, is the prospect of substantial and otherwise unattainable social benefits, and (3) any permissible harming of animals in research is limited by considerations of animal welfare. In turn these premise lead to two core values of social benefit and animal welfare. The principle of Social Benefit in turn has 3 principles: (1) The Principle of No Alternative Method, (2) The Principle of Expected Net Benefit and (3) The Principle of Sufficient Value to Justify Harm. The Principles of Animal Welfare include an additional three principles: (1) The Principle of No Unnecessary Harm, (2) The Principle of Basic Needs and (3) The Principle of Upper Limits to Harm. This session will examine these new principles and what they can mean in the future, from the perspective of laboratory animal medicine, governance, ethics and philosophy.

References

Keywords
Ethics; Principles; Animal Research
Compassion stress and fatigue are occupational hazards in human and veterinary medicine. They are a normal consequence of caring people giving to others while not taking the necessary steps to take care of themselves. Those working with research animals can be profoundly affected by the work they do, including feelings of grief and sadness, or a profound sense of loss that they may not even be able to articulate. Left unaddressed, caregivers may become desensitized and numb, which can affect the animals that they are entrusted to care for, making this not only a human issue, but potentially an animal welfare issue. To identify the root causes of compassion stress and fatigue in a research setting, understand coping mechanisms to help individuals deal with feelings of compassion fatigue, and develop an impactful corporate program that would meet the needs of personnel across multiple sites we conducted multiple surveys. Internally, we developed and administered a pre- and post-workshop survey tool to >700 employees attending a multi-day compassion fatigue workshop. Externally, a survey was also administered to the larger laboratory animal community in North America. Results of these surveys were used to develop internal compassion stress training and build an internal network of ‘resiliency building ambassadors’ to extend the program to multiple sites across the organization, focusing on tools for building resiliency, peer counseling, personal wellness, providing tributes to research animals worked with, enhancing communications during research projects, promoting animal adoption and rehoming programs, and sensitizing senior management to compassion stress and their role in helping personnel deal with difficult situations at work. The program includes flexibility for sites to select content to ensure that culturally appropriate tools and materials are available across sites.

References

Keywords
Compassion fatigue; Resiliency; Well-being
141 TRADITION, NOT SCIENCE, IS THE BASIS OF ANIMAL MODEL SELECTION IN DRUG DEVELOPMENT

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National and international laws and regulations exist to protect animals used for scientific purposes, e.g. Directive 2010/63/EU. Therefore, we investigated how animal model selection was reported in project application forms for animal procedures for scientific purposes. We evaluated the choice of a specific animal model using thematic content analysis in project application forms, issued in 2017-2019 by the national Central Authority in the Netherlands. In total, 125 animal models for translational and applied research, from 110 project applications were assessed. Explanations to select a specific model included: the model’s availability (79%); the availability of expertise (62%); and the model showing similar disease pathology/symptoms (59%). Therefore current selection of a specific animal model seems to be based on tradition rather than its potential predictive value for clinical outcome. The applicants’ explanations for the implementation of the 3Rs principles (replacement, reduction and refinement) as to the animal model were often unspecific: replacement was achieved by using data from prior in vitro studies, reduction by optimal experimental design and any statistics, and refinement by reducing of discomfort. Additionally, due to the need for a test model with high complexity (47%) and intactness (30%), the full replacement of animal models with alternative (non-live animal) approaches was thought unachievable. Without a clear, systematic and transparent justification for the choice of a specific animal model, the likelihood of poorly translatable research remains. It is not only up to the researcher to demonstrate this, as ethical committees and funding bodies can provide positive stimuli to drive this change.

References

Keywords
animal model; drug development; efficacy model; 3R; translational research
ARE WE CAR SALESMEN, BOY SCOUTS OR AIRLINE PILOTS? PREPARING FOR ROBUST AND HUMANE RESEARCH

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Many explanations for the so-called “reproducibility crisis” in science focus on the "mathematical" aspects of planning and conducting preclinical experiments, such as the appropriateness of statistical analyses, or the failure to prevent bias. Whilst undeniably important, this approach ignores the many animal-related factors which produce artefacts and reduce both internal and external validity. Unfortunately it is more difficult to produce metrics which reflect these factors, or attempts to avoid their effects.

The last few decades have seen major advances in most aspects of laboratory animal husbandry and care. It is unreasonable to expect scientists (who use animals in their research but whose main focus is naturally elsewhere) to be fully aware of current ‘best’ practice. Scientists need guidance before embarking on animal-based experiments and this should be provided at the earliest possible stage by key personnel from the animal facility in which the research is to be conducted.

The PREPARE guidelines for planning animal research and testing are an attempt to provide an overview of the issues which should be considered, from day one. Unlike reporting guidelines, they are not intended to be a mandatory requirement. They were conceived over a 30-year period to be a voluntary aide memoire for scientists. The PREPARE checklist provides a quick overview of 15 central topics, but importantly, the PREPARE website expands on each of these topics and provides links to the latest advances within each area (https://norecopa.no/PREPARE).

References

Keywords
PREPARE; best practice; planning; reporting; guidelines
While exposure of humans to environmental hazards often occurs with complex chemical mixtures, the majority of existing toxicity data and tools are for single compounds. The Globally Harmonized System of chemical classification (GHS) recommends to use the additivity formula for acute oral toxicity classification of mixtures which is based on the acute toxicity estimate (ATE) of ingredients. We used toxicological data collected in the Integrated Chemical Environment (ICE) developed by the National Toxicology Program for assessment of acute oral toxicity of mixtures. The ICE database contained in vivo acute oral toxicity data for ~10,000 chemicals and for more than 500 mixtures. By using available experimental data for single compounds we were able to calculate GHS category only for 273 mixtures. For 205 mixtures (or 75%) the predicted category was the same as experimentally measured while for incorrect predictions the difference was mostly within one category. To expand a set of chemicals with acute oral toxicity data we used the Collaborative Acute Toxicity Modeling Suite (CATMoS) developed by EPA and implemented in the Open Structure-Activity/Property Relationship App (OPERA). CATMoS is the result of a global collaboration to develop QSAR models to predict acute oral toxicity of chemicals. As the result, we were able to make predictions for 487 mixtures with 69% of accuracy for GHS classification. For 172 mixtures with 2 or more active ingredient the accuracy rate was 78%. Our results demonstrate that CATMoS together with additivity formula can be used to predict GHS category for chemical mixtures.

References

Keywords
mixtures; acute oral toxicity; GHS additivity formula; QSAR modeling; Collaborative Acute Toxicity Modeling Suite (CATMoS)
Increasingly, scientists, regulators, and the public are acknowledging the need to overcome a cultural and historical reliance on using animals in research. Habitually, animals are used in biomedical research to recapitulate characteristics of human disease and to drive the development of therapeutics. However, human biological, pathological, and pharmacological events often cannot be simulated in animals due to inherent genetic, molecular, anatomical, and physiological differences (Sousa et al., 2017). The advancement of in vitro and in silico models has led to fundamental improvements in human disease modeling and drug development (McAleer et al., 2019). However, minimal funding and opportunities exist for using human-based models for biomedical research. Understanding the importance of early-stage scientific mentorship, the Physicians Committee designed the Early-Career Researchers Advancing 21st Century Science (ERA21) program, to strategically intervene with up-and-coming scientists to help build their careers without using animals, from the start. ERA21 aims to provide education and early-career experiences that will instill practices to shape professional lives for decades. Using a multifaceted approach specifically tailored for emerging biomedical researchers, the ERA21 initiative aims to: increase the understanding of the scientific benefits of using human-relevant research practices, educate emerging scientists about the wide range of modern, human-relevant methods available, and connect them with opportunities in this field. ERA21 offers programs to reach students and early-career scientists and launch promising professions. Activities include educational seminar series, hands-on training and in-depth interactive learning, incentivizing student researchers toward human-relevant projects through travel awards, identifying and connecting students with laboratories using nonanimal methods, a monthly newsletter, social media groups to facilitate networking and share funding and job opportunities, presentations and outreach at relevant biomedical conferences, and more.

References

Keywords
Biomedical research; Training; Education; Outreach; Program development
In order to guarantee safety for the end-users, medical devices and other solid products have to be tested for adverse reactions on the skin before market authorization. Animal testing is still state of the art, but ethically questionable. One of the key aspects of our recent research was to establish an in-vitro testing battery to examine the biocompatibility of medical devices. With the help of skin models, cell based testing methods and a chromatographic method, extracts of solid products can be assessed for cytotoxicity, irritation and sensitisation. In general, skin sensitization is defined as an Adverse Outcome Pathway (AOP) reaction caused by immunological responses and is divided into 4 successive key events. To identify a possible sensitization potential, we have developed a screening method based on a weight of evidence approach by concentrating on the two first key events of the AOP. The molecular initiation event, binding of so called haptens (molecules that become allergenic when binding to a protein) to a peptide can be assessed by the chromatographic method DPRA (Direct Peptide Reactivity Assay). The molecular and cellular responses include the activation of an antioxidant pathway in keratinocytes which can be determined via the reporter gene assay ARE-Nrf2. So far, various samples have been tested in the in-vitro assay and additionally samples were examined with animal testing in order to compare the results, demonstrating more sensitive responses in the in-vitro assay. These assays were developed not only with a sufficient sensitivity, but also to be robust, simple to use, ethically correct and inexpensive offering a forwardlooking alternative to animal testing.

References

Keywords
Biocompatibility; Medical devices; In-vitro; Skin sensitization; Adverse outcome pathway
Lung cancer is the major cause of cancer death in both men and women worldwide showing the lowest 5-year survival rate among all cancer types. Small cell lung cancer (SCLC) is a highly metastatic, neuroendocrine subtype representing about 10% of all lung cancers. Therapeutic discovery for SCLC is extremely challenging due to the relapse of the disease with chemoresistance. Most of the drugs fail in the pre-clinical and clinical trial phase because the existing drug testing models are unable to recapitulate the actual tumor pathophysiology. Patient derived xenografts (PDX) are considered the gold standard for drug development in pre-clinical settings, but this has several limitations, including chances of engraftment failure, long development timeline, dissimilarity of tumor microenvironment between human and murine models, substantial cost and a huge sacrifice of animal lives.

To address these issues, here, we present a bioengineered 3-dimensional (3D) lung SCLC organoid model to study tumor growth kinetics and response to chemotherapy. We custom made functionalized alginate microbeads coated with human lung fibroblasts and heterogeneous SCLC cell lines in a specially designed bioreactor to build a 3D model of distal human lung with SCLC tumors. We found that SCLCs in the 3D model proliferated and invaded the microbeads and formed co-culture 3D tumors within a very short duration (72h). We compared this bioengineered model with patient tumors and found it to reproducibly recapitulate the pathology and immunophenotyping of the patient tumors. When treated with dose courses of chemotherapy drugs, Cisplatin and Etoposide, alone or in combination, the model showed significantly higher drug resistance than the 2D cell cultures with a relapsing pattern similar to that of the existing PDXs. Being amenable to high throughput drug screening, this co-culture model can be a faster and advanced alternative to animal PDX models to study SCLC.

**References**

**Keywords**

3D lung organoid; Small cell lung cancer; Patient-derived xenografts; chemoresistance
Recent advances in overcoming both immunological and pathobiological barriers across species (Liu et al., 2017) make xenotransplantation a potential solution to ongoing shortage of human organs. Genetically engineered pigs are currently being raised in the U.S. for clinical xenotransplantation trials in humans (Mullin 2019). The Animal Welfare Act (AWA), enforced by the United States Department of Agriculture (USDA), is the only federal law regulating the treatment of animals used in research in the U.S. (USDA 2017), while the Center for Biologics Evaluation and Research (CBER), under the Food and Drug Administration (FDA), has regulatory oversight of xenogenic products and xenotransplantation in humans (HSS 2016). Animals involved in xenotransplantation are not specifically described under the AWA but are addressed under the FDA’s “Guidance for Industry” documents instead. These guidance documents do not establish legally enforceable responsibilities and focus primarily on public health concerns rather than the welfare of source animals. Current animal protection laws other than the AWA, such as anti-animal cruelty laws, vary from state to state, while animal welfare monitoring for livestock production relies heavily on voluntary third-party audits and certification programs. Given the unique functions and needs of animals raised for tissue and organ harvest for human use, the production and housing of such animals will necessitate specialized regulatory oversight different than that for animals in food production and non-xenotransplantation laboratory research. Collaboration between the USDA and the FDA is critical as well as mandatory auditing or accreditation for all facilities housing animals for xenotransplantation programs. There is a pressing need for U.S. regulatory authorities to review and revise current federal and state laws in order to customize legislation to protect the welfare of this unique group of xenotransplantation-bound laboratory animals until alternatives and replacement for animal use become available.

**References**


**Keywords**

Xenotransplantation; Animal Welfare; Legislation; U.S.
The blood brain barrier (BBB) functions as a barrier to toxins reaching the brain. It also, however, hampers delivery of therapeutics for neurological diseases - including malignant tumours - to the brain. Attempts to model the human BBB in vitro in order to closely investigate the functional complexity of the barrier have largely been based around the use of vascular endothelial cells derived not from the brain, but from other organs, or, indeed even from other species (both in vitro and in vivo). We have engineered 3D dynamic (shear stress/flow) models from human brain-derived cells (endothelial cells, astrocytes and pericytes) and vascular basal lamina proteins which yield hitherto unreported high TEER levels of >1,200 ohms, akin to those reported in situ. (Maherally et al 2018). These values were achieved by the specific use of perlecan and agrin (where previously knock down of these proteins in laboratory mice had been reported to totally disrupt the BBB). Our new models, constructed under human serum supplementation and oxygen levels of 0.1% to 20% have been successfully used to investigate NSCLC metastasis to the brain (Jassam et al 2015; Jassam et al 2017; Jassam et al 2019) as well as in in vitro experiments to ‘verify’ the transient opening of the barrier in human melanoma to brain metastasis in xenografts by way of A16ApoE (Aasen et al 2019). These 3D all-human in vitro BBB models are not only more representative of the human brain in situ than in vitro and in vivo animal models, but they pave the way for accurate pre-clinical assessment of therapeutic drug delivery to the brain for a wide range of neurological disorders and diseases, including malignant brain tumours.

References

Keywords
Brain; Blood-brain; Delivery; in vitro; Model
Finding a chemically-defined cell culture medium as a replacement for FBS is a timeconsuming and costly process (van der Valk et al., 2018). In this work, we present a three-stage approach for evaluating the chemically-defined DMEM/F12+ITS medium (SOP-G200-005_DME_F12+ITS) for the Caco-2 cell line. The three stages are increasingly lengthy and identify adhesion, proliferation and metabolism changes of the cell culture. The first stage utilizes the microphysiometric system intelligent mobile lab for in-vitro diagnostic (IMOLA-IVD) to reveal any short-term effects caused by the chemically-defined medium (Weiss et al., 2013; Brischwein and Wiest, 2019). The IMOLA-IVD device enables an automated cell analysis by label-free measurements of the acidification rate and impedance. Additionally, the device provides fresh nutrients to the Caco-2 cells regularly by a pre-programmable protocol. Herein, the used protocol cellasys #8 is defined as follows: 6 h DMEM + 5% FBS, 6 h DMEM/F12+ITS, 4 h DMEM + 5% FBS, 4 h DMEM/F12+ITS, 4 h positive control with 2% Sodium dodecyl sulphate (SDS). In the experimental results no short-term effects and cellular stress responses are visible. This suggests that all major nutrients are present in the chemically-defined medium. The second stage is a differentiation cultivation in a T25 flask for 40 days while the third stage is a long-term cultivation for 100 days. The qualitative results of these stages obtained by a light microscope show that the proliferation and adhesion is reduced compared to DMEM + 5% FBS cultivated cells but is constant during the long-term cultivation suggesting that there are no missing nutrients. With the IMOLA-IVD system, the screening time for finding minimal, chemically-defined media can be reduced. The real-time measurement of the device shows any cell stress caused by missing nutrients in a chemically-defined medium formulation. This enables a rapid first stage screening with a duration of 24 hours.

References

Keywords
microphysiometry; chemically-defined medium; rapid screening; label-free; Caco-
Background: Abutment surfaces are being designed to promote gingival soft tissue attachment and integration. This forms a seal around prosthetics and consequently ensures long-term implant survival. New scalable and reproducible models are necessary to evaluate and quantify the performance of these surfaces. Purpose: To evaluate a novel implantation model by histomorphometric and immunohistochemical characterization of the interactions between human oral gingival tissue and titanium abutments with either novel anodized or conventional machined surface.

Materials and Methods: Abutments were inserted into an organotypic reconstructed human gingiva (RHG) model consisting of differentiated gingiva epithelium cells on a fibroblast populated lamina propria hydrogel following a tissue punch. Epithelial attachment, down-growth along the abutment surface, and phenotype were assessed via histomorphology, scanning electron microscopy, and immunohistochemistry 10 days after implantation.

Results: The down-growing epithelium transitioned from a gingiva margin to a sulcular and junctional epithelium. The sulcus depth and junctional epithelial length were similar to previously reported pre-clinical and clinical lengths. A collagen IV/laminin 5 basement membrane formed between the epithelium and the underlying connective tissue. The RHG expanded in thickness approximately two-fold at the abutment surface. The model allowed the evaluation of protein expression of adhering soft tissue cells for both tested abutments.

Conclusions: The RHG model is the first in vitro 3D model to enable the assessment of not only human epithelial tissue attachment to dental abutments but also the expression of protein markers involved in soft tissue attachment and integration. The two abutments showed no noticeable difference in epithelial attachment.

References

Keywords
reconstructed human gingiva; dental implant; soft tissue attachment; junctional epithelium; reconstructed oral mucosa
NOVEL HOME CAGE ACTIVITY METRICS FOR POSTOPERATIVE CARE REFINEMENT IN A MOUSE SURGICAL MODEL

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Postoperative monitoring is essential for animal welfare and scientific reliability, as unexpected complication can result in unalleviated pain and impact the quality of data generated. Due to the inter-individual variability between operators when assessing animal condition, objective quantifiable parameters, such as body weight, are often used. These methods can be time consuming and stressful for the animal. In this study we used digital ventilated cages to non-invasively monitor home cage activity in a mouse surgical model. The primary objective was to identify and validate a novel measure of activity reflective of animal condition during the pre and post-operative phases. C57BL/6Crl male mice were housed three per cage and divided in control, sham and surgery groups (n=5 cages per group). The surgery group underwent full tenotomy of the right hind limb while sham group underwent skin incision without tenotomy. All animals were weighed weekly and clinically scored three times per week. Operated animals were treated with buprenorphine for three days. While clinical scoring and body weight did not differentiate between groups, post-surgery activity was reduced in both sham (-25%) and surgery (-50%) groups the first night without analgesic injection. From this time point, activity and response to lights-on increased in both sham (p=0.001) and surgery (p=0.003 – 0.04) groups. Where the sham group returned to pre-surgery activity levels eight days post-operation, the surgery group activity was less than sham (p<0.0001) for six weeks post-operation. Analysis of distribution of activity showed similar post-surgery activity reduction in both groups in the front row. Only sham group activity in the front row returned to pre-surgery levels eight days post-operation. Our results show how home cage activity metrics can be used as valuable complementary tool for postoperative care refinement and for sensitive assessments of degree and time to return of function in surgical mouse models.

References

Keywords
The zebrafish embryo is increasingly used as an alternative animal model, not only for toxicity studies but also for disease models and efficacy studies in different therapeutic areas. The advantages of the zebrafish are mainly its low cost, ease of maintaining and breeding and high genetic homology with humans. Moreover, much of the research and assays conducted in zebrafish have been carried out in embryos to take advantage of zebrafish fecundity, small size, ease of handling and transparency. In compliance with international animal welfare regulations (e.g. Dir 2010/63/UE) and the 3Rs principle, the zebrafish embryos provide an ethically acceptable higher throughput with enhanced biological complexity over in vitro models.

All these advantages make the zebrafish unique for studying human diseases and several neurodegenerative and neuromuscular rare disease zebrafish models have been generated for target validation as well as for screening of potential drugs. We, at Biobide, have set up efficacy studies for the following rare neurodegenerative/neuromuscular diseases: Amyotrophic Lateral Sclerosis (ALS), Duchenne Muscular Dystrophy (DMD) and Dravet Syndrome (DS). First, we have internally characterized the specific zebrafish lines for each of the diseases: transgenic zebrafish overexpressing mutant Sod1 for ALS (Ramesh et al., 2010), dystrophin deficient zebrafish mutant for DMD (Basset et al., 2003) and zebrafish line harbouring a mutation in the scn1lab gene for DS (Schoonheim et al., 2010). After the characterization of the different lines, specific efficacy studies for drug screening purposes have been set up, such as behavioral assays for functional measurements, birefringence or immunofluorescence assays and others.

The results obtained during the characterization and set up of the studies demonstrate that the zebrafish is a powerful model for rare disease research and their application in central nervous system related drug screening, target validation and other early research and development activities.

References

Keywords
Zebrafish; Alternative model; Rare Disease; Neurodegenerative disease; Neuromuscular disease
In vitro tools are inexpensive and scalable for high-throughput platforms, however, they pose low relevance to vertebrate species. On the other hand, work with adult animals is expensive and represents ethical issues. Small fish like zebrafish (Danio rerio) are an excellent alternative to in vitro and in vivo model. They offer a unique experimental system where screening assays can be performed at the whole animal level, being at the same time compatible with the 3R principles (replacement, reduction and refinement in animal testing). Endocrine disruptors (EDCs) are chemicals that by interfering with the endocrine system can have an adverse effect at developmental, neurological, immune and reproductive levels. Thyroid Disrupting (TD) compounds specifically alter the function of the thyroid gland through the interference with the synthesis, transport and/or binding of the thyroid hormones. The negative impact of EDCs is becoming a real public health issue, therefore the necessity of tests to assess the potential risk of new chemicals before they are marketed is increasing. The zebrafish is currently used as a model for the evaluation of acute and developmental toxicity and for the screening and testing of potential endocrine disrupters (EDCs), as described in the OECD Guidelines. The two major endpoints used to evaluate EDCs, vitellogenin concentration and change in sex ratio, have several limitations. With the purpose of expanding the number of tests available to identify estrogenic, androgenic, and thyroid disrupting substance, we evaluate gene expression of 4 biomarkers in 5 dpf zebrafish larvae after exposure, from 48 hpf to 10 compounds reported as EDCs. A transgenic strain, expressing fluorescence coupled to the induction of thyroglobulin was also used to evaluate 9 environmentally relevant TD substances. This screening methodology showed to be a sensitive and cost-effective assay to screen and evaluate potential EDCs chemicals.

References

Keywords
Zebrafish; Endocrine Disruption; Thyroid Disruption; 3R
Limitations of the current standard, regulatory in vivo developmental neurotoxicity (DNT) testing and assessment (OECD DNT TG 426) are well known, such as the 3R conflict, the low throughput, the high costs, the high specific expertise needed and the lack of deeper mechanistic information. Moreover, there is uncertainty for the possible standard in vivo DNT data variability and for the experimental animal to human real life extrapolation. Some quantitative information and qualitative considerations indicate the potential concern. Within this poster, major limitations and aspects of uncertainty for in vivo DNT testing and assessment are systematically illustrated. The underlying comprehensive knowledge base shall inform decision makers, what performance of alternative approaches may be acceptable and permitting faster and more reliable overall improvement of children health protection. We also outline a hypothesis, which limitations and uncertainties could be reduced with ongoing OECD and EFSA projects (Sachana et al. 2019) (Bal-Price et al. 2018): The development of alternative, fit-for-purpose Integrated Approaches to Testing and Assessment for DNT shall permit: relative gains in 3R compliance, reduced costs, higher throughput, improved basic study design, higher standardization of testing and assessment and validation without 3R conflict, increasing the availability and reliability of DNT data. This could allow a more reliable comparative toxicity assessment over a larger proportion of chemicals within our global environment. The use of early, sensitive, mechanistic indicators for potential DNT could better support human safety assessment and mixture extrapolation. Combined with QVIVE modelling ideally this could provide - eventually context dependent - at least the same level of human health protection. Such new approaches could also lead to a new mechanistic understanding for chemical safety, permitting determination of a dose that is likely not to trigger defined toxicity traits or pathways, rather than a dose not causing the current apical organism level endpoints.

References

Keywords
Uncertainty; Chemical Safety; Developmental Neurotoxicity; Animal Testing; Alternative Methods
166 LIMITATIONS AND UNCERTAINTIES FOR THE USE OF THE ACUTE FISH TOXICITY TEST—CAN THEY BE REDUCED BY ALTERNATIVES?

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Information about acute fish toxicity is routinely required for environmental risk assessment of chemicals in many jurisdictions. This information is assumed to mitigate the most obvious chemical hazards for the environment and is typically obtained by a 96 hours juvenile/adult fish test for lethality conducted according to OECD test guideline (TG) 203 or regional guideline equivalents. However, TG 203 has not been validated using criteria currently required for new test methods including alternative test methods. A characterization of all aspects of practicality and validity of TG 203 is important to provide a benchmark for alternatives to acute fish toxicity testing. This poster summarizes available knowledge about limitations and uncertainties of TG 203, based on methodological, statistical, and biological considerations: Uncertainties for the variability estimate stem from the historically flexible test design (e.g. eleven fish species, specific water conditions and the related variable metabolism) and the constraints of the basic test design (e.g. test duration and 7 fish without replication, which can lead to low confidence). Other uncertainties relate to the use of TG 203 data, e.g. for environmental safety extrapolation engaging pragmatic assessment factors. Examples are provided as to why alternative approaches may reduce several of the uncertainties. Environmental extrapolation models combined with data from alternative methods including early sensitive mechanistic indicators of toxicity can provide at least the same level of environmental safety. Yet, most importantly, better standardization, characterization and improved basic study design without conflicting with the 3Rs can support higher data reliability and thus comparison of chemical toxicity in terms of GHS classification, PNEC (Predicted No Effect Concentration for ecosystems) derivation and PBT (Persistent, Bioaccumulative, Toxic) assessment. Combined with the potential for higher throughput, a reliable assessment of many more chemicals can be achieved, expectedly allowing an overall improved environmental protection.

References

Keywords
Uncertainty; Chemical Safety; Acute Fish Toxicity; Alternative Methods; Environmental Toxicity
To increase physiological relevance and to develop new therapies 3D spheroids are of increasing interest. The addition of superparamagnetic iron oxide nanoparticles (SPIONs) into the spheroid allows for investigation of new therapeutic strategies (Zhang et al., 2014) without the use of animals (Lei and Schaffer, 2013; Whatley et al., 2014; Marx et al., 2016). L929 fibroblasts were maintained in 25 mL Greiner Bio-One AdvancedTC® cell culture flasks in chemically defined DME/F12+ITS cell culture medium. Cells received fresh medium twice a week and were passaged at about 80% confluency once a week. Spheroids were created similar to a previously developed method (Alexander, Eggert and Wiest, 2018). The 20 nm SPIONs beads were obtained from micromod Partikeltechnologie GmbH (Rostock / Germany) and a stock solution with 100 µg/mL was prepared. Spheroids were prepared in a 96 well-plate with cell-repellent surface (Greiner Bio-One GmbH, #650970). Each well was filled with 190 µL DME/F12+ITS containing 10.000 L929. For the SPION loaded spheroids (SPION-LS), 10 µL of magnetic beads stock solution was added, whereas for the non-magnetic control spheroids (NM-CS) 10 µL of cell culture medium was added. Then the 96well plate was centrifuged at 1000 g for 5 min and finally incubated at 37°C with 5% CO2. 100 µL of medium in each well was replace by fresh DME/F12+ITS (preheated to 37°C) daily. Spheroids were used for the experiments on day 5. For manipulation of the spheroids a neodym magnet (length 25 mm, diameter 25 mm) was used in a distance of approximately 30mm. The calculated field force acting on the spheroids was approximately 30mT. To investigate if the SPIONs are incorporated into the spheroids one SPION-LC was transferred to a well with one NMCS. The movement of the SPION-LC due to the applied magnetic field can be seen at https://youtu.be/ev9bZnKyvGA.

References

Keywords
Dynamic magnetic field; Superparamagnetic iron oxide nanoparticles; Spheroids; Chemically defined
In recent years, rapid advances in laboratory-based in vitro methods and improved imaging analysis have increased the relevance of these methods as potential alternatives to animal-based tests. Therefore, Chinese Hamster Ovary (CHO) cell assay, based on the clustering effect of pertussis toxin (PT) on the cells has been developed to test for residual PT and reversion to toxicity of acellular pertussis vaccines as an alternative to animal-based test. The use of a standardized protocol including scoring of the cellular morphologic change by microscopic observation is important to ensure the assay reliability, but there are difficulties with the microscopy and identifying clusters. With this background, we examined the method where we can conduct automatic image capture of whole well of a microtiter plate. This allows us to detect objects and define the PT positive concentration on CHO cells using image analysis.

Methods: Briefly, we prepared a PT two-fold dilution series and added them to cultured CHO cells in 24 and 96 well plates. Cell images of the cells growing in the wells were captured 48 hours later and analyzed using the cell imager Cell3iMager CC-8000 duos (SCREEN Holdings Co. Ltd, Japan).

Results: We observed that shape of the cell colonies was round, when higher concentrations of PT were added. Therefore, we adopted ‘circularity’ as a colonies’ image characteristic and analyzed the colony shapes. We observed that the average circularity of the colonies correlated with the PT concentration. These results were reproducible and obtained by the assays using both 24 and 96 well plates.

In conclusion, we propose this improved method of the CHO assay which have advantages, such as, a) short time for the analysis required because of automatic imaging and analysis, b) allowing quantitative measurement instead of binary (positive/negative) or semi-quantitative detection.

References

Keywords
A VALIDATION STUDY OF THE IATA-BASED READ-ACROSS IN NEPHROTOXICITY OF AMINOPHENOLS

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Integrated Approaches to Testing and Assessment (IATA) is one of the useful concepts to enhance accuracy of read-across conclusion for systemic toxicity (Sakuratani et al., 2018). However, there are few case studies that have verified how much IATA can contribute to improving accuracy of read-across. Under this circumstance, we developed case studies for hepatotoxicity previously (Nakagawa et al., 2020). Considering the variety of target organs, we focused on nephrotoxicity because the kidney is likely to become the target organ of toxicity following the liver (Vinken et al., 2012) and there are few case studies of read-across for nephrotoxicity. Aminophenols, which have the potency of nephrotoxicity, were chosen as the target categories in this study. Category compounds including 6 compounds which have in vivo data are structurally similar, but have different toxic intensities. For example, paminophenol and p-methylaminophenol induced clear toxicological effects like renal tubular necrosis in vivo, while m-aminophenol only cause brownish pigment. In order to validate the IATA-based read-across predicts these toxicological information properly, we estimated the adverse outcome pathway, and then performed in silico/vitro evaluation to compare them in the target category. First, we analyzed the cell death to renal tubular epithelial cell lines; NRK-52E by measuring cell viability 24 hours after exposure. Next, we analyzed the oxidative stress that is assumed as a putative cellular key event in adverse outcome pathway of p-aminophenol by measuring the expression level of HO-1 in western blotting. These analyses showed that the cytotoxicity and oxidative stress intensity in vitro were well correlated with the toxic intensities of compounds in in vivo toxicity data. Thus, this study showed the possibility that IATA-based read-across considering various information such as biological response could estimate differences in toxicity that couldn’t be predicted by structural similarity, and enhanced accuracy of the read-across conclusion.

References

Keywords
read-across; IATA; systemic toxicity; nephrotoxicity
A reduction in beta cell mass occurs during the progression of Type 2 diabetes (T2D) and has been attributed to net enhancement of the rate of beta cell death (1, 2). It is increasingly apparent however that changes in the identity of insulin producing beta cells may also be a contributory factor (3, 4). These changes in beta cell identity, which have been reported in rodent models of diabetes, are less well characterised in humans(3, 5).

We assessed the effects of lifestyle associated cellular stresses on beta cell identity and gene expression using a fully humanised culture system for human EndoC-βH1 beta cells. We found that approximately 5% of EndoC-βH1 cells exposed to these cellular stressors changed their identity, adopting a delta cell-like phenotype in vitro. Increases in the presence of the delta cell hormone, somatostatin, was also observed ex vivo in pancreas sections recovered at autopsy from donors with T1D or T2D (9.3% for T1D and 3% for T2D respectively compared with 1% in controls). Gene expression of key beta cell genes was also dysregulated in stressed EndoC-βH1 cells. These findings differ from those seen in rodent models of the disease, where changes in beta cell identity have shifted from a beta to alpha cell phenotype(3, 5), indicating the necessity of using human model systems. This study also showed that removal of the stressful stimulus normalised both beta cell identity and the pattern of gene expression. This suggests that reversible changes in beta-cell identity may occur during exposure to lifestyle associated cellular stressors in Type 2 Diabetes. These findings provide a possible mechanistic explanation for anecdotal reports of disease remission following lifestyle changes in Type 2 Diabetes.

References

Keywords
Diabetes; Human model; beta-cell
The development of non-animal alternatives in safety assessment is a priority area across multiple sectors. Read-across is increasingly being used, whereby information from data-rich (source) chemicals can be leveraged to infer information for data-poor (target) chemicals, provided that source and target chemicals are suitably “similar”. In order for any given chemical to elicit an effect, the chemical must possess intrinsic activity and be able to reach the relevant site of action at an appropriate concentration. Physiologically-Based Kinetic (PBK) models can be used to predict time-concentration profiles for chemicals in blood and/or organs so enabling a more accurate description of internal exposure that can inform safety-assessment decisions. PBK models require a wide range of data and are time-consuming to generate. As an increasing number of models have now been published in the literature, these can be used as templates for other chemicals of interest – as proposed by Lu et al (2016). Development of this concept requires information regarding the chemicals for which such models exist, and a method to identify which of these chemicals is “similar” to the target. This EPAA-funded project, seeks to address these issues by undertaking a systematic review of PBK models to determine their chemical space coverage and to develop a method by which appropriate “similar” chemicals can be identified. Results of the systematic review, which has generated a readily-searchable dataset of existing models, and the implications of source chemical selection in developing new PBK models are discussed.

Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency

References

Keywords
Physiologically-based kinetic (PBK) model; Read-across; Similarity
Constant efforts to develop more efficient Quantitative Structure-Activity Relationship ((Q)SAR) techniques and models aim to effectively improve drug discovery and predictive (eco)toxicology applications, among many others. The use of QSARs is also well-accepted by the OECD to characterize Molecular Initiating and Key Events (MIE, KE) of Adverse Outcome Pathways (AOPs), or as part of Integrated Approaches to Testing and Assessment (IATA) (OECD, 2016). Considering the potential applications of (Q)SARs, it was decided to explore their effective use, as a predictive method, within the AOPWiki, and the published OECD IATA Case Studies, also including the 12 Defined Approaches (DAs) for assessing Skin Sensitization (OECD, 2017). At time of writing, among the 1285 MIEs/KEs totally available in the AOP-Wiki, 946 have an empty “How it is measured and detected” section. Of the remaining 339 MIEs/KEs, only 11 were found to report computational applications as detection methods, such as SAR, molecular docking, in silico homology modelling, and a computational model simulating a biological environment. Whereas, a good implementation of (Q)SARs was noticed in the IATAs and DAs, since 11 of the 15 IATA and 7 of the 12 DA case studies embedded the use of in silico tools, such as the OECD (Q)SAR Toolbox, TIMES-SS, and others. Overall, this analysis shows that the use of (Q)SARs is still underutilised, especially for AOPs, despite the wealth of information and the models available in the literature. Indeed, further investigation of the literature explores the availability of existing (Q)SARs for the prediction of selected MIEs and KEs lacking measurement methods.

References

Keywords
(Q)SAR; AOP-Wiki; IATA; Defined Approaches
Hox genes are a family of highly conserved genes expressed in a colinear manner along the neural tube, defining the body plan and specificity of (neural) cells during development. Recent studies have shown that embryonic stem cells harbour a self-organising capacity that show colinear expression of Hox genes in vitro. In this study we show that the neural embryonic stem cell test (ESTn) also presents this typical wave of Hox gene expression. This may provide a useful read-out in ESTn for the effects of chemicals on early neural differentiation. Using our existing microarray data set of the first seven days of ESTn differentiation we could define the developmental domain of the test based on gene expression of morphogens, signalling proteins as well as Hox genes. During embryonic body formation in the first three days of differentiation, key signalling molecules for anterior-posterior patterning such as Fgf8 and Wnt3 were upregulated. By adding exogeneous retinoic acid for two days, a wave of Hox gene expression was unleashed, which presented a similar expression pattern as in vivo. After seven days of differentiation, Hox1-9 genes were expressed, indicative for the brain to thoracic region of the spinal cord. Exposure of ESTn to chemicals that affect early neural differentiation showed a typical regulation of Hox genes. For example, valproic acid, cyproconazole, hexaconazole and flusilazole upregulated Hox4 and 5 genes and downregulated Hox8 and 9 genes, while carbamazepine showed an opposite trend. These results suggest that the latter compound accelerated differentiation, while the former compounds inhibited differentiation. This study provides insight in the biological domain of ESTn and the effects of chemicals on early neural differentiation. This method may be applied to other stem cell-based neural in vitro systems and may be useful in a testing strategy for screening chemicals for their neurodevelopmental toxic potential.

References

Keywords
Embryonic stem cells; Neural differentiation; Developmental neurotoxicity; Hox genes; Biological domain
Discussions about improving animal welfare and experimental design are an everyday part of the lives of all who use or care for animals in research. Many suggestions for improvements are easily accessible in textbooks or scientific papers. However, these suggestions are often more general in nature. A number of discussion forums now exist, where specific refinements and new ideas can be shared. They allow discussions of techniques which do not warrant publishing as a scientific paper at that stage. However, many forums do not have easily searchable archives, so valuable ideas can easily be forgotten over time.

To bridge the gap between scientific papers and discussion groups, we have constructed a Refinement Wiki (https://norecopa.no/Wiki). We foresee a number of uses:

1. Rapid dissemination of refinement techniques where resources or interest in writing fullscale scientific papers are unavailable
2. As a hub where those investigating the effects of a potential refinement strategy in a multi-lab study can identify collaborators
3. Creation of pages encouraging colleagues to share experiences or develop new strategies to solve a problem

The contents of the Wiki are not curated. The quality of the Wiki is determined by registered bona fide members of the research animal community. No one else can add, delete or comment upon material.

The Wiki is an integral part of Norecopa's website: https://norecopa.no. Wiki content is retrievable from Norecopa's main search engine. In addition, the Wiki has its own search engine. We have written a simple instruction manual to keep the threshold for adding new content as low as possible.

We hope that this Wiki will help to accelerate the introduction of refinement methods. It is now up to the community to judge whether this initiative is worthwhile. Those interested in adding content to the Wiki may contact Adrian Smith (adrian.smith@norecopa.no).

References
https://norecopa.no/Wiki

Keywords
Wiki; Refinement; Norecopa
192 A NOVEL MICROFLUIDIC PLATFORM FOR PULMONARY NANOPARTICLE EXPOSURE

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Nanoparticles are a common byproduct of many modern technologies and industrial processes. Their toxicity, however, has not been sufficiently studied due to a lack of appropriate in vitro models. The lung is a major target organ for nanoparticles, as airborne particles are inhaled. Its barrier function determines the uptake of nanoparticles into the system and their resulting toxicity on other organs. Furthermore, their acute toxicity on lung tissue needs to be considered. Therefore, an innovative in vitro exposure system has been developed and is now combined with a microphysiological system that allows co-cultivation of several organ equivalents, thereby enabling elaborate toxicity studies in a relevant in vitro model. With the platform under development, lung equivalents can be subjected to repetitive nanoparticle exposure in an automated, selfsustained manner for at least 5 days. Thereby, toxic effects on both the lung and, as a secondary organ, the liver, can be examined.

Here, we present the concept and the layout of the combined exposure device with the microphysiological system. We further discuss the automated media exchange, which enables the autonomous cultivation of the microphysiological system. Additionally, we show data regarding the morphology, metabolism and marker expression of lung equivalents cultivated in the microphysiological system for at least 5 days in mono- and co-culture with liver equivalents.

The first results demonstrate that the holistic system under development is a promising tool that can be placed in nanoparticle exposure prone environments for safety assessments. This platform is designed to generate high-quality in vitro data predictive of nanoparticle safety in humans.

This project is funded by the German Federal Ministry for Education and Research, NanoINHAL Grant No. 03XP0226.

References

Keywords
The Exposure:Activity Ratio (EAR) is a tool in Next Generation Risk Assessment (NGRA) comparing internal exposure levels to in vitro/in silico derived biological activity data of compounds (Dent et al., 2019). With the Dietary Comparator Ratio (DCR), the EAR of exposure to a compound under consideration is compared to the EAR of an established safe level of human exposure to a comparator compound. Here, the EARs for safe human exposure to the comparator compounds 3,3-diindolylmethane (DIM), a food-borne constituent from Brussels sprouts, and the anti-androgenic drug bicalutamide (BIC) were defined to explore their potential in DCR-based risk assessment for human-realistic exposure scenarios to anti-androgenic compounds. To determine their EAR values, the free internal safe dose levels of the comparator compounds were surrogated to the lower confidence limit of the benchmark concentration with an extra 5% response (BMCL05) in the AR-CALUX assay, corrected for differences in in vitro and in vivo protein binding. The biological activity parameter was surrogated to the IC50 from the AR-CALUX assay. Results revealed BMCL05 values of 0.13 μM and 1.86 nM for DIM and BIC, respectively. The IC50 values of DIM and BIC were 1.28 and 0.17 μM, respectively. The corresponding EARs of respectively 0.10 and 0.01 for DIM and BIC were used in the DCR-based risk assessment. For DIM, this EAR provides a more realistic EAR than the previously derived EAR (1.12E-04) based on the intake of 50 gram Brussels sprouts (Dent et al., 2019). The EARs for the comparators were used to evaluate the safety of a series of realistic human exposure scenarios to known anti-androgens using the DCR approach. This study provides human-relevant comparator EARs for DCR-based risk assessment for exposure to anti-androgenic compounds. In future work, this animal test-free tool can be extended to other molecular initiating events combined with suited comparators.

References

Keywords
Next Generation Risk Assessment; Anti-androgens; Animal test-free tool; Exposure:Activity Ratio (EAR); Dietary Comparator Ratio (DCR)
Fish are important indicator species for contamination of the aquatic environment, thus millions of fish are sacrificed annually in toxicity experiments for chemical hazard assessment and effluent testing (Scholz et al., 2013). One alternative is the use of fish cell lines derived from rainbow trout, which have been employed in a variety of in vitro assays to determine e.g. acute toxicity of chemicals (Schirmer, 2006). Recently, bioimpedance monitoring of fish cell lines was established to follow the acute toxic response over time. Herein, cells are seeded on an electrode chip and resistance is measured noninvasively, reflecting the health status of the cells. A decrease in resistance is an indicator for loss of cell viability as can be elicited, for example, by exposure to chemicals (Tan and Schirmer, 2017). In the RAINBOWFLOW CHIP project, we employ this technique to establish time-resolved analysis of cell viability in response to chemical exposure under flow conditions. For this, we use an intestinal cell line of the rainbow trout (Oncorhynchus mykiss) – RTgutGC – which, similarly to the gill cell line, RTgill-W1 (Tanneberger et al., 2013), shows good correlation with fish in vivo LC50 results from acute toxicity tests (Schug et al., 2020). Yet, adherence of RTgutGC cells is superior to the RTgill-W1 cells, which is beneficial for use in impedance sensing chips. Moreover, while the gill is the first site of contact and primary uptake site for water-borne chemicals, the gut is the first tissue of contact for toxicants adhering to organic matter (including food), as is the case for hydrophobic chemicals. In the RAINBOWFLOW CHIP, the flow-through design leads to constant replenishment of the test substance, thus providing stable exposure concentrations, which is especially important for difficult-to-test (i.e., volatile and hydrophobic) chemicals that experience losses in the conventional static test set-up.

References

Keywords
fish acute toxicity; cell lines; impedance sensing; chemical toxicity testing; stable exposure concentrations
The persistence of vitreous fibres in lung tissue has an important influence on their potential to cause disease, with less persistent fibres being less harmful than those that are more durable. Current accepted methods for determining biopersistence under Nota Q of Annex VI of the Classification, Labelling and Packaging (CLP) Regulation require costly and time-consuming animal studies. In alignment with the three R’s principle, we aimed to develop an alternative in vitro acellular test system for reliable measurements of man-made vitreous fibre (MMVF) dissolution within biologically relevant fluids. Specifically, we investigated which parameters of the experimental design contributed to differences previously found within data sets in intra- and inter-laboratory comparisons (Guldberg et al., 1998). The testing strategy employed a flow-through system which has been used to measure the dissolution of historically relevant MMVFs including stone wool and glass wool (Sebastian et al., 2002). The parameters scrutinised included fluid pH and composition, flow rate, fluid maintenance, apparatus components and material loading conditions. In addition, data handling procedures relating to pre-experiment sample handling and post-experiment dissolution rate calculations were explored.

We have established that the composition and stability of the simulated biological fluid is essential in obtaining consistent results. As the system can run for an extended period of time it is vital that the chosen fluid does not precipitate or deviate from the desired pH. A number of fluids have been tested for their suitability in the application and regimented control and handling measures have been developed to ensure consistently. Likewise, it was determined that relationship between specific surface area of the material and fluid volume has a major impact on the calculated dissolution rate. Although experiments are still on-going, we have concluded that tight control of the studied parameters is necessary if the test system is to be reproducible.

References

Keywords
Dissolution; Biopersistence; Fibre; Simulated biological fluid
The gastrointestinal tract harbours a complex ecosystem comprised of trillions of microorganisms which have significant local and extra-intestinal effects on an organism’s health. The bi-directional interaction between the gut microbiota and the central nervous system has been coined the gut microbiota-brain axis. While growing evidence suggests the intestinal microbiome can affect an organism’s behaviour, to show causality and determine the extent of the contribution of the microbiota, fecal microbiota transplantation (FMT) must be performed. FMT is the administration of a solution of fecal matter from a donor into the intestinal tract of a recipient. Behavioural change in FMT recipients provides crucial evidence for the gut microbiota-brain axis and its influence on behaviour. Therefore, our group initiated a systematic review (SR) to evaluate the evidence that animal behaviour can be affected by FMT. An SR protocol was developed and published on Oct 21st 2019 on the SYRF (CAMARADES/NC3Rs Systematic Review Facility) online platform (http://syrf.org.uk/protocols/). Using the search strategy detailed in the protocol, a search of PubMed and Embase databases yielded 13,160 unique references. Two independent reviewers performed title and abstract screening and identified 552 references for the full text screening phase (ongoing). Of note, of these 552 references, 343 were review articles that were screened for references potentially missed in the original search. Our SR will give a qualitative overview of the use of FMT in behavioural animal studies, will investigate the prevalence of pseudoreplication, and aims to identify areas for improving experimental design needed to prove or disprove the role of the intestinal microbiota in modulating behavioural outcome measures. By identifying ways to improve the internal/external validity and reproducibility of FMT animal studies, less animal experiments need to be performed and clinical translation of preclinical animal studies will be improved.

References

Keywords

systematic review; behaviour; microbiota; fecal microbiota transplantation; animal studies
Botanical substances have been widely used for centuries in efforts to preserve and enhance human health and well-being. Safety evaluations of these substances are typically based on adverse event reports, historical textbook knowledge, and other published information, either scientific or anecdotal. For ingredients with insufficient safety data, animal models have often been used to investigate potential health risks prior to testing in a clinical setting. Today, a pressing need exists to define the appropriate levels of chemical characterization, identity standards, and safety evaluation required to support the safe use of these complex botanical substances. The Botanical Safety Consortium (BSC) is addressing this need by organizing an international effort to bring together scientific experts to create a botanical safety toolkit for botanical dietary ingredient assessments. The toxicological assessment must include an evaluation of genotoxicity potential, as genotoxicity is associated with a number of adverse human health effects that are not reliably predicted by spontaneous adverse event reporting. Established in vitro and in silico test methods are available and can support assessment of the genotoxicity of botanical ingredients. The BSC’s Genotoxicity Technical Working Group (TWG) is developing a pragmatic fit-for-purpose testing strategy for botanical ingredients. The BSC’s analytical and data analysis TWGs will support the Genotoxicity TWG in the characterization of samples of interest. A testing strategy will be developed, including the use of high-throughput screening technologies for the identification of potentially important chemical constituents in products already being marketed. The goal of the BSC is to develop predictive toxicology testing strategies for botanicals by integrating existing published data and the identified in silico and in vitro tools into a robust, comprehensive program that provides actionable safety data while minimizing the need for animal testing.

References

Keywords
Botanical substances; Botanical Safety Consortium; Genotoxicity testing; in vitro assays; in silico tools
The German Centre for the Protection of Laboratory Animals (Bf3R) launched 2019 the Animal Study Registry for preregistration of animal studies to increase transparency of bioscience research and to promote animal welfare (Bert et al. 2019). The reproducibility of results gained from animal experiments and their extrapolation to humans are intensively discussed. Meta-research has identified reporting bias, HARKing (Hypothesizing After the Results are Known) and p-hacking as main factors contributing to the irreproducibility of data. Flawed experimental design, e.g. lack of blinding and randomization, the non-differentiating between planned and unplanned statistical analyses as well as insufficient reporting of methods and raw data are also named as influencing factors.

Preregistration of preclinical studies including the statistical analysis plan before the data are collected has been proposed as a powerful countermeasure (Heinl et al. 2019). Animal Study Registry supports scientists in planning their study thoroughly by asking detailed questions concerning study design, methods, and statistics. The registry is designed for exploratory and confirmatory studies in the field of fundamental and preclinical research.

With the preregistration, more complete data about animal experiments will be preserved for the scientific community. As a consequence redundant experiments can be avoided: Null results or experiments that reveal methodological deficits are often not published, leading to a possible unnecessary repetition of the same experiment. Animal Study Registry also asks detailed questions concerning husbandry and animal characteristics, so researchers have to consider all factors influencing animal welfare and the experimental outcome before conducting the experiments. Animals can benefit from sharing experiences about housing conditions, handling, and refinement measures. Thereby, preregistration cannot not only improve the quality of research involving animals, but can also improve the wellbeing of laboratory animals.

References

Keywords
Preregistration; Transparency; Good research practices; Animal Study Registry
231 TRIPTOLIDE AND ATORVASTATIN SYNERGISTICALLY PROMOTE HEPATOTOXICITY IN CULTURED HEPATOCYTES AND FEMALE SPRAGUE-DAWLEY RATS BY INHIBITING PREGNANE X RECEPTOR-MEDIATED TRANSCRIPTIONAL ACTIVATION OF CYP3A4

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Triptolide (TP), an active component of Tripterygium wilfordii Hook. F, has been widely used in China for treating autoimmune and inflammatory diseases, and has also been validated by Western science and developed as a candidate anti-cancer treatment. However, the liver toxicity of TP has seriously hindered its use and development. Considering the major target regulation mechanism of TP is the suppression of global transcription regulated by RNAPII. This paper tries to reveal the molecular mechanism of TP-induced liver toxicity, focusing on the impact of TP on detoxification regulated by pregnane X receptor (PXR)-mediated transcriptional activation of hepatic microsomal enzymes, especially CY3A4, and its associated synergistic effect when co-administered with a CY3A4 substrate drug. The results showed that TP dose-dependently blocked transcriptional activation of CY3A4 in both hPXR and hPXR-CYP3A4 reporter cell lines, lowered the mRNA and protein expression of PXR target genes such as Cyp3a1, Cyp2b1, and mdr1, and inhibited the functional activity of Cyp3A in a time- and concentration-dependent manner in sandwich-cultured rat hepatocytes (SCRH) and female SpragueDawley rats. Furthermore, TP combined with atorvastatin (ATR), the substrate of CY3A4, synergistically enhanced hepatotoxicity in cultured HepG2 and SCRH cells (CI is 0.38 and 0.29, respectively), as well as in female Sprague-Dawley rats, with higher exposure levels of ATR and TP. These results clearly indicate that TP inhibits PXR-mediated transcriptional activation of CY3A4, leading to a blockade on the detoxification of itself and ATR, thereby promoting liver injury. This study provides an experimental basis for guiding the rational use of TP.

References

Keywords
Triptolide; Atorvastatin; Synergistic hepatotoxicity; Progesterone X receptor; RNAPII
Existing 3Rs Education and Training (E&T) courses cover animal research and animal testing. When it comes to alternative test methods, it is well admitted that validation studies and regulatory acceptance of alternative test methods are a long process but not the end of the journey to become part of routine use of the new technologies. Multiple initiatives exist to facilitate and improve the confidence of end-users, be a regulator, be a test provider or be a test performer. This diversity of end-users complexifies the needs and expectation for 3Rs E&T. Therefore specific solutions need to be provided accordingly. Most of the 3Rs E&T formats are already covered with theory thanks to webinar, massive open online course or lectures at university and practice with hands-on training or case studies. However, some gaps always exist, since it is almost impossible to be at the same pace as technology evolves. Currently most of the recognition and accreditation of such 3Rs E&T are mainly centralized by scientific societies and universities. Therefore there is limited opportunity for private sector and NGOs to feed in curricula in this field in spite of the multitude of initiatives and efforts. This talk intends to describe some of the accreditation mechanism and discuss ways to improve the effectiveness and recognition of 3Rs E&T courses coming from other sectors.

References

Keywords
3Rs; education; training; accreditation
In recent years, it has been reported that proteins are absorbed into the rough skin and cause skin sensitization. However, there is no established in vitro skin sensitization test for proteins. Therefore, establishment of a test for evaluating skin sensitization potential of proteins is expected. Here, we developed an in vitro skin sensitization test for proteins by applying human Cell Line Activation Test (h-CLAT), an in vitro skin sensitization test of chemical substances.

First, human monocytic leukemia cell line THP-1 cells were treated with phorbol 12-myristate 13-acetate (PMA) for 72 h to differentiate. Then differentiated THP-1 cells were exposed with proteins for 24 h and the expression of CD54 and CD86 were measured by flow cytometry and real-time PCR. In this study, lysozyme from egg white (LYZ) and ovalbumin from egg white (OVA) were used as positive controls, and human serum albumin (HSA) was used as a negative control. Both LYZ and OVA drastically increased the expression of CD54 and CD86 but HSA did not. Furthermore, the relationship between the expression of CD54 and CD86 the uptake of the test substances into the differentiated THP-1 were investigated using flow cytometry and fluorescent microscopy. As a result, it was found that the uptake amount of HSA was significantly smaller than those of LYZ and OVA. In conclusion, it was suggested that the use of differentiated THP-1 make it possible to evaluate skin sensitization potential of proteins. The other autologous proteins and low-immunogenic xenoproteins will be examined. In the presentation, development of skin immune models consisting of differentiated THP-1 cells and an artificial skin model were also demonstrated.

References

Keywords
h-CLAT (human Cell Line Activation Test); protein; skin sensitization; differentiated THP-1
The kidney’s excretory function is crucial in drug development, as it dictates drug clearance and reabsorption. Furthermore, nephrotoxicity of candidate drugs is one of the major reasons for drug attrition. Therefore, an accurate kidney model for Multi-OrganChip applications could revolutionize drug trials by providing a relevant in vitro platform. Emulating the kidney’s distinct functions accurately, however, requires a two-fold approach combining glomerular filtration and tubular reabsorption. The HUMIMIC Chip4 harbors a glomerular and a tubular compartment, which together form the interface between the separate surrogate blood circuit and the excretory circuit. The surrogate blood circuit comprises three additional organ compartments, which could e.g. contain liver and intestine equivalents for ADME studies.

In this study, iPSC-derived glomerular and tubular organ equivalents are generated and co-cultured in the Chip4. Using iPSC-derived organ equivalents allows the creation of an autologous chip, which has major implications for future studies, as it enables the incorporation of an immune system equivalent. The employed podocytes exhibit typical podocyte morphology and express key markers. Furthermore, they are capable of forming a closed barrier, as observed by TEER measurements. For the tubular model, kidney organoids are employed. These organoids contain mostly proximal and distal tubule epithelial cells and few podocytes, endothelial and interstitial cells, as confirmed by immunohistochemistry staining and gene expression analysis. When dissociated and seeded onto a permeable membrane, the cells form a barrier. The developed glomerular and tubular models can be co-cultured within the HUMIMIC Chip4 for several days whilst maintaining their morphology and marker expression.

Taken all together, the developed kidney-on-a-chip constitutes a potent tool for advanced in vitro drug trials. It is designed to generate high-quality in vitro data predictive of renal drug clearance, reabsorption and nephrotoxicity in humans. The HUMIMIC Chip4 was developed under EU-H2020 “EU-ToxRisk”-Flagship project, Grant Agreement No 681002.

References

Keywords
Heated Tobacco Products (HTPs) HTP aerosols contain lower levels of toxicants compared to smoke from combustible cigarette which are a cause of heart disease in smokers. This study compared the cardiotoxicity potential (cTP) of HTP aerosols compared to cigarette smoke extract using Stemina’s Cardio quickPredict™ assay. This assay uses human induced pluripotent stem cell-derived cardiomyocytes to predict cTP based on changes to the ratios of cell viability and 4 key cellular metabolism markers.

Aerosol extracts from two commercially available HTPs (termed A and B) and smoke extract from a reference cigarette (1R6F), were generated using a Vitrocell VC10 smoking machine. 1R6F smoke (56 puffs) and HTP (120 puffs) were bubbled into 30mls of PBS. All products were smoked using the ISO intense regime.

Extracts of 1R6F elicited a metabolic response indicative of potential cardiotoxicity at concentrations > 0.6%; whilst HTP extracts elicited a metabolic response of cardiotoxicity between 3.3 – 7.0%. Imperial Brands Pulze® HTP (product A) was 10-fold less toxic than 1R6F under the conditions of test.

HTPs did not exhibit the same metabolic response as 1R6F, as the shape of the doseresponse curves for both HTPs were significantly different from 1R6F (extra-sum-ofsquare F test, p<0.0001) under the conditions of test.

These data indicate that HTP aerosols have less cardiotoxicity potential when compared to cigarette smoke from 1R6F.

References

Keywords
Heated Tobacco Product (HTP) ; Carditoxicity; Cardiomyocytes; 1R6F reference cigarette; in vitro
In Read Across, untargeted and targeted NAMs can help to strengthen the identification of suitable substances for read across, increasing confidence in the analogue identification and NOEL used as a POD for the risk assessment. NAMs have also been used to inform on relative potency of analogue mode of action and to predict internal exposure in both human and animal studies allowing for a risk assessment approach based on internal exposures of the human versus the animal study. This ‘next generation Read Across’ approach is presented here, and illustrated by two case studies.
In order for any chemical (pharmaceutical, cosmetic, environmental pollutant etc) to elicit an effect on the body it must possess inherent activity and reach the site of action in sufficient concentration. Physiologically-based kinetic (PBK) models can be used to describe the concentration-time profile of a chemical at relevant sites within the body, they are flexible and adaptable to different species, life stages, routes of exposure and dosing scenarios. Historically, generating data for PBK models was intensive in animal use, leading to a drive to develop alternatives methods. One important method to reduce testing is leveraging existing data and applying this knowledge to other chemicals, species, exposure scenarios etc. Many PBK models have been published in the literature for a range of chemicals; in order to apply the knowledge from these models, it is first essential to be able to reproduce the model. To date, there has been little consistency in the way in which such models are recorded and considerable variation in the level of detail supplied. In this study two software systems (Matlab ODE solvers, Mathworks and PK-Sim, Open Systems Pharmacology) were used in assessing the reproducibility of a published PBK model for atenolol. Factors such as the model description, accessibility and interpretation of parameters from the published model as well as usability and flexibility of the modelling software used to reconstruct the model were all considered. Several issues were identified from the model information provided in the publication and recommendations made to assist future model development and reporting, particularly with respect to the potential of using such models as templates for other chemicals of interest.

References

Keywords
physiologically-based kinetic (PBK) models; reproducibility; MATLAB; PKSim
For better or for worse, society has been transformed by social media (Britton et al, Nature Chemistry, 2019). Twitter - a social microblogging platform created in 2006 - claims 321 million active users in 2018. Approximately 500 million tweets are tweeted each day. Thanks to the development of a specific twitter application, more than 700,000 tweets were collected from October 2014 to March 2020 based on the following hashtags: #animaltesting OR #animalfreetesting OR #animalfreetests OR #animalexperiments OR #3Rs OR #3R OR #BeCrueltyFree OR #endanimaltesting OR #stopanimaltesting OR #stopvivisection. Based on this unprecedented analysis, the authors were able to 1) identify the absolute number of users as well as its variation over the last seven years, 2) the popular tweets 3) extracting new hashtags from pre-defined ones 4) differentiate users that are “preaching to the choir” or “singing from the rooftop” (Cote and Darling, FACETS, 2019). Moreover, a Twitter sentiment analysis in R-language was performed resulting in classification emotion content of the tweets ranging from anger to surprise or joy with a majority of fear. Furthermore, elasticsearch was now included to further refine the database analysis. Thanks to this weight of evidence, the author argues that by communicating on social media, peers are able to interact further and revolutionise the way science is shared and spread (Glausiusz, Nature, 2019).

References

Keywords
twitter; social media
ECHA (European Chemicals Agency), EFSA (European Food Safety Authority) and EMA (European Medicines Agency) are related to the Committee on Environment, Public Health and Food Safety (ENVI) at the European Parliament (EP). All three agencies play an important role in collecting safety data for all manufactured goods that are introduced to the EU market. In all sectors represented by the agencies, similar types of data (toxicological endpoints) are collected, but they differ in format, transparency and confidentiality level assigned to them. Such differences are not due to legal requirements, but they are mostly linked to EU Agency internal policies. The lack of harmonization has dire consequences for the implementation costs of EU regulations, for the performance of the different industry sectors and for the excessive/redundant use of animals for safety testing. Moreover, the efficacy of the agencies themselves is reduced. This oral presentation intends to provide an overview of the current big data initiatives (e.g. OpenFoodTox [1]) at the EU agency level, collaborative activities at stakeholder level (e.g. AMBIT [2], Big data for better health outcomes [3]) or at European Commission level (e.g. DG ENV [4]). Last but not least the author will describe the adoption of a pilot project funded by the EP on “Feasibility on a common open platform on chemical safety data [5]” currently led by the European Commission. The goals of the pilot project are to facilitate seamless sharing of data between authorities and provide public access to researchers, regulators, industry and the citizen at large. This will promote: a) transparency and trust in EU decision making, b) research and data analytics, c) innovation d) less animal testing & more predictive toxicology, and e) better regulatory decision making and informed consumer choices.

References

Keywords
echa, efsa, ema; data access; data format; transparency
Next Generation Risk Assessment (NGRA) is an exposure and hypothesis-driven approach that integrates new approach methodologies (NAMs) to assure human safety without animal data. This work focuses on the development of an NGRA approach for inhalation exposures using hypothetical case studies of a film-forming polymer in personal care products (e.g. antiperspirants) and a silane in cleaning products.

Impairment of mucociliary clearance, lung fibrosis and lung surfactant inhibition were identified as the relevant endpoints for the most common consumer exposure scenarios (e.g. daily use of an antiperspirant). To investigate these endpoints, two cell models were selected for in vitro testing: the MucilAir™-HF cell model (Epithelix) and the EpiAlveolar™ cell model (MatTek). In addition to the two case study chemicals another 16 benchmark chemicals were selected either due to their well-known effects in the specific areas of the lung, history of safe use and/or due to chemical or physical similarities to the case study chemicals.

Consumer habits and practices were used to derive an airborne concentration (mg/m3) for each chemical and exposure scenario, which was then transformed into deposited mass in the bronchial and alveolar region (µg/cm2) using MPPDv2.8. Cells were then exposed to the predicted concentrations that reflected daily realistic exposures for up to 12 days and different endpoints (e.g. TEER, LDH, cytokines, histology, cilia beating frequency, mucociliary clearance, mitochondrial toxicity, etc) were measured at 4 different timepoints. Preliminary results indicate that the alveolar model was more sensitive to some of the pro-inflammatory benchmark substances tested. Polyhexamethylene guanidine phosphate for example induced a mild inflammatory response in the MucilAir™-HF system over the 12 days’ treatment while inducing significant cytotoxicity in the EpiAlveolar™ cell model after only 4 days of exposure.

References

**Keywords**
inhalation exposures; new approach methodologies; next generation risk assessment; in vitro models
Next Generation Risk Assessment (NGRA) is defined as an exposure-led, hypothesis-driven risk assessment approach that integrates New Approach Methodologies (NAMs) to assure safety without animal testing. Within NGRA there is an ongoing need to develop robust and relevant assays that can be used to characterize bioactivity of chemicals at human-relevant exposures (Dent et al. 2018). This type of approach does not aim to identify a specific adverse outcome or pathology but rather aims to be protective of human health by estimating an exposure at which no biological response is expected (Friedman et al. 2019; Wetmore et al. 2015).

The objective of this work was to develop and evaluate a cellular stress response panel that could form part of an early tier testing strategy. This panel consisted of 36 biomarkers representing mitochondrial toxicity, cell stress and cell health, measured predominantly using high content imaging (Hatherell S. et al. 2020). To evaluate the suitability of the panel for NGRA, data were generated using two sets of benchmark chemicals: 1) chemicals that at defined human exposures are known to cause adverse systemic effects due to cellular stress in a proportion of exposed individuals; 2) chemicals that at relevant human exposures have not been associated with adverse systemic effects related to cellular stress.

A Bayesian model was developed to quantify the evidence for a biological response, and if present, a credibility range for the estimated point of departure (PoD) was determined. PoDs were compared with the plasma Cmax associated with the typical substance exposures and indicated a clear differentiation between ‘low’ risk and ‘high’ risk chemical exposure scenarios.

The results presented in this work show that the cellular stress panel can be used, together with other new approach methodologies, to identify chemical exposures that are protective of consumer health.

References


Keywords

next generation risk assessment; cell stress pathways; in vitro models; Bayesian model ; in vitro point of departure
269 PROPOSAL OF A NEW APPLICABILITY DOMAIN OF VITRIGEL-EIT (EYE IRRITANCY TEST) METHOD UTILIZING THE PH LEVEL AND LIGHT ABSORBANCE OF TEST CHEMICAL PREPARATIONS

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Introduction: The Vitrigel-EIT method is a test method that determines the presence or absence of eye irritation with high sensitivity by analyzing the time-dependent changes of trans-epithelial electrical resistance values for 3 minutes after exposing a test chemical preparation (i.e. solution or suspension) to a human corneal epithelium model fabricated in a collagen vitrigel membrane chamber and was registered as TG494 of OECD in 2019. However, acidic chemicals that have a pH level of 5 or less in test chemical preparations and solids are excluded from the applicability domain. In this study, we aimed to establish a new applicability domain so that this test method can be applied chemicals not only liquids but also solids that are dissolved or uniformly suspended in the culture medium.

Methods: Total 137 test chemicals (94 liquids and 43 solids) were tested by the VitirgelEIT method and their judgments were compared with the GHS classification. According to the TG494, 89 test chemicals were predicted after excluding 9 acidic chemicals and 39 solids. In a new applicability domain, 96 test chemicals were predicted after excluding 9 acidic chemicals and 32 chemicals with the absolute differences over 0.1 in the light absorbance of test chemical preparation at 0 and 3 minutes after mixing. The light absorbance was measured by a UV/VIS spectrophotometer.

Results, Discussion: The sensitivity, specificity and accuracy under the original applicability domain in TG494 were 95%, 67% and 80%, respectively. Meanwhile, those under the new applicability domain were 96%, 65% and 82%, respectively. These data demonstrated that the new applicability domain improved the number of applicable chemicals and also the predictability. These results suggest that the Vitirgel-EIT method can be used for not only liquids but also solids by setting the new applicability domain utilizing the pH level and light absorbance of test chemical preparations.

References

Keywords
collagen vitrigel membrane; corneal epithelium; eye irritation test; HCE-T cells; transepithelial electrical resistance
Next Generation Risk Assessment (NGRA) is an exposure-led, hypothesis-driven approach that integrates New Approach Methodologies (NAMs) to assure safety without the use of animal testing. Over recent years several theoretical frameworks depicting a tiered and iterative approach to conducting a NGRA have been published [Berggren et al, 2017; Dent et al, 2018], although there is a lack of examples of implementation of these frameworks. In this study we conducted a hypothetical safety assessment of 0.1% coumarin in a face cream and body lotion using only NAMs to inform a safety decision, focusing on the potential for systemic toxicity. Internal exposure estimates were generated using a physiologically-based kinetic (PBK) model for dermally applied coumarin [Moxon et al, 2020]. In vitro points of departure [PoDs] were generated from assays that investigated the potential of coumarin to bind to pharmacologically active receptors (Eurofins Safety44 screen); cause immunomodulatory effects (BioMap Diversity 8 Panel); affect key cellular stress pathways [Hatherell S et al, 2020] and high-throughput transcriptomics in multiple cell lines. In silico alerts for genotoxicity were followed up using the in vitro ToxTracker assay [Hendriks et al., 2016].

A risk assessment decision was made by comparing the generated in vitro PoDs to the estimated internal exposure (plasma Cmax) and calculating a margin of safety (MoS) distribution. The MoS (5th percentile) for both face cream and body lotion exposure scenarios were greater than 100. Coumarin can be concluded to be non-genotoxic, does not bind to any of the 44 targets and does not show immunomodulatory effects at consumer relevant exposures.

While this case study demonstrates the capability of using NAMs to make safety decisions about inclusion of coumarin in cosmetic products, confidence in the applicability of this approach to other chemicals and products will only come through sharing the experiences of other case studies.

References

Keywords
NGRA; Systemic; Case Study
Animal models are 78% accurate in determining whether drugs will alter contractility of the human heart. Cardiomyocytes from human induced pluripotent stem cells (hiPSCCsMs) are increasingly recognized as valuable for determining the effects of drugs on ion channels but they do not always accurately predict contractile responses of the human heart. This is in part attributable to their immaturity but the sensitivity of measurement tools may also be limiting. Here, we benchmarked our hiPSC-CM based microphysiological models through a blinded drug study. Furthermore, we develop a method for systematic validation of drug-induced changes in the cardiac excitation-contraction coupling.

To evaluate the suitability of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) for predictive safety pharmacology, we quantified changes in contractility, voltage and Ca2+ handling simultaneously in 2D monolayers with our inhouse developed Triple Transient Measurement (TTM) system (van Meer et al., 2019). Furthermore, we developed an hypothesis-based statistical algorithm that identifies mechanisms of action. Subsequently, we evaluated a set of (blinded) drugs with known positive, negative or neutral inotropic effects.

Using the TTM system we were able to identify 78% of the expected drug-induced effects accurately in our in vitro model. Furthermore, we were able to pick up over 80% of the mechanistic changes in excitation-contraction coupling. While we will continue to strive for a 100% accuracy, these results indicate hiPSC-CM based microphysiological systems can be a serious alternative to certain animal models currently used.

Acknowledgments
We gratefully thank all researchers contributing in the CRACKIT InPulse project, especially Ana Krotenberg, Umber Saleem, Puspita Katili, Nurul Mohd Yusof, Ingra Mannhardt, Tessa de Korte, Marijn Vlaming, Karen McGlynn, Jessica Nebel, Anthony Bahinski, Kate Harris, Eric Rossman, Xiaoping Xu, Francis Burton, Godfrey Smith, Peter Clements and Arne Hansen. We thank NC3Rs for financial support, Ncardia for providing hiPSC-cardiomyocytes and GlaxoSmithKline for providing test compounds.

References
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Keywords
induced pluripotent stem cells; Cardiac modelling; cardiomyocytes; drug testing; microphysiological systems
279 RETROSPECTIVE EVALUATION OF ANIMAL AND NON-ANIMAL MODELLING: EFFICACY-RELATED EXAMPLES INFORMING ORGANISATIONAL PRACTICES AND DRIVING IMPROVEMENTS

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Research into clinical phase, efficacy-related attrition in the Pharmaceuticals sector has been partly linked in publications and analyst reports to an erosion of confidence in animal studies as a predictive modelling platform (Harrison, 2016). GSK is working to optimise the translational relevance of preclinical modelling strategies and improve future delivery of successful medicines to patients. Specifically, the GSK Animal Research Strategy team has supported this by leading efforts to leverage retrospective knowledge, from animal and non-animal models and by sharing learnings with stakeholders to continuously improve modelling practices in the organisation.

Efficacy-focused case examples will be shown from small molecules and biologics that reached clinical phases (predominantly phase II) and have been evaluated retrospectively via After Action Reviews (AARs) for preclinical-clinical concordance. Through integration of biology/pathobiology and “pillars of pharmacology” evidence (focusing on animal and non-animal models) and by relating this to clinical trial designs and outcomes, these reviews highlighted several themes for continuous improvement. These included biological and technical aspects, increasing alignment preclinically to clinical intent, and differences between endpoints measured and hypotheses articulated. This work also highlighted opportunities to integrate information across disciplines and solved perceived challenges in re-using historical information and appreciating past decisions.

The approach taken in the AARs resulted in a robust process to access and evaluate relevant content from projects retrospectively. The experiences from the AAR subset are being applied to inform and improve current practice, shape ongoing strategy to continually improve animal research, advance nonanimal considerations, story-tell potential challenges to discovery leaders and to shape organisational practices around decision-making and data capture.

References

Keywords
Efficacy; Animal model; Non-animal model; Research; Strategy
Fetal bovine serum (FBS) is a supplement widely used in cell and tissue culture to enhance cell growth and division, despite its many scientific disadvantages being widely discussed in the scientific community (van der Valk et al, 2018). Over and above, ethical and legal considerations should play a more substantial role in the discussion on the use of FBS. This is due to the fact that FBS is derived from the blood of bovine fetuses after their removal from the slaughtered dam. Fetal blood is harvested by cardiac puncture. This is usually performed without stunning or anaesthesia of the fetus, resulting in massive ethical and animal welfare concerns, as potential pain and suffering can not be excluded (van der Valk et al, 2004). But ethical considerations should not only include the fetuses but start as early as with the dams, as transportation of pregnant cows can cause distress and suffering and can trigger (premature) birth and even abort, especially in the late stages of gestation.

Moreover, blood harvesting for FBS production lacks binding regulations, resulting in a legal grey area and therefore opens the door for mistreatment or even fraud to the detriment of animals, scientists and patients. However, alternatives to FBS do exist and further use and development of ethical acceptable substitutes should be promoted. In our presentation we argue that instead of justifying FBS collection as a necessary evil and continuing to use a product that is questionable for a variety of reasons, the way forward should be a substantial change that starts from the way we treat farm animals and spans the replacement of FBS by alternatives that are more humane and scientifically sound.

References

Keywords
Fetal Bovine Serum; suffering; blood harvesting; ethics; legal objections
Background - Considerable interest exists in the potential of in vitro studies to address questions related to clinical use of drugs and the pathobiology of tumours (NC3Rs, 2020). Agreement is, however, required on how to assess quality, quantity and human relevance of such studies alongside adequate reporting (Hartung et al., 2019). The SAToRI-BTR (Systematic Approach To Review of In vitro methods in Brain Tumour Research) project focuses on seeking consensus as to how brain tumour studies using in vitro methods should be evaluated.

Objectives – To identify criteria for evaluating quality and human relevance of in vitro brain tumour studies; to assess the acceptability of such criteria to senior scientists in the field.

Methods – Potential criteria for evaluation were identified through: an online survey of brain tumour researchers; interviews with scientists, clinicians, regulators, and journal editors; analysis of relevant reports, documents and published studies. A preliminary set of criteria for quality appraisal was compiled through content analysis. In stage two, the criteria were reviewed by an expert panel (Delphi process).

Results – Methods for and quality of review of in vitro studies were found to be highly variable with a need for improved reporting standards. 129 preliminary criteria were identified; duplicate and highly context-specific items were removed, resulting in 48 criteria for review by a panel of senior researchers from 9 countries. 38 criteria reached consensus, resulting in a provisional checklist for appraisal of in vitro studies in brain tumour research.

Conclusion – Through a systematic process of collating criteria and subjecting these to expert review, SAToRI-BTR has resulted in preliminary guidance for appraisal of in vitro brain tumour studies. Further development of this guidance, including investigating strategies for adaptation and dissemination across different sub-fields of brain tumour research, as well as the wider in vitro field, is planned.

References
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Keywords
in vitro methods; quality appraisal; human relevance; brain tumours; evaluation
Since March 2013 the use of animal testing for cosmetic ingredients has been banned in the EU. To assess systemic toxicity of new cosmetic ingredients, read-across approaches based on structural analogy are one of the next generation risk assessment approaches currently used. However, structural analogies are not sufficient to ensure similar toxicological behavior and are limited to ingredients with a defined structure. Advances in omics technologies have allowed the emergence of public databases containing full genome transcriptomic profiles of thousands of compounds at many different biological conditions (concentration, time and biological system). By comparing transcriptomic fingerprints, such databases can support read-across approaches based on biological activity analogy.

In this context, a digital tool integrating transcriptomic profiles from Drug Matrix, Open Tg-Gates (Igarashi et al., 2015) and Connectivity Map was developed, allowing 1) to assess similarity between compounds and 2) to investigate the main biological pathways targeted by each compound. Using Hallmark gene sets (MSigDB), Gene set enrichment analysis (GSEA, Subramanian et al) was applied to calculate Normalized Expression Scores. Pairwise similarity scores were then computed from the GSEA results using Pearson’s correlation. In addition, wind-rose plots of implicated pathways are constructed to help characterize the mechanisms of actions (MOAs) and allow chemicals grouping based on their transcriptomic fingerprints.

High biological similarity scores were observed for compounds with similar structure and for compounds that were not previously identified as analogs by conventional readacross approaches. This tool could be useful to enhance structural based read across approaches and to assess analogy between chemicals with no defined structure. Next steps will consist of 1) further characterizing observed differences/similarities between compounds towards a better understanding of compound’s MoAs and 2) developing and evaluating a classifier including results obtained with this digital scoring tool.

**References**


**Keywords**
293 REMOVE ATT, TABST & LABST. HOW FAR AWAY ARE WE TO GLOBAL HARMONIZATION FOR THOSE SAFETY TESTS?

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Many institutions and organizations have been working independently or jointly to waive or remove those obsolete safety testing from the production and batch release testing for human (ATT) and veterinary vaccines (TABST, LABST). How far away are we from the global elimination of those tests? What is the role of international organizations in promoting the change towards animal-free safety testing? Humane Society International is presenting its work (Viviani et al., 2020) and roadmap to engage industry and regulators in countries like South Korea, Brazil, Argentina, Russia (and others), to promote this important change that reduces the burden on animals, reduce the final costs of the vaccines, and accelerates their release time to the market and to the people.

References


Keywords

Safety test; Vaccines; Deletion; Regulatory Alignment
The GOLIATH project focusses on one of the most urgent regulatory needs in EDC testing, namely the lack of methods for testing EDCs that disrupt metabolism – chemicals collectively referred to as ‘metabolism disrupting compounds’ (MDCs). MDCs are natural and anthropogenic chemicals that have the ability to promote metabolic changes that can ultimately result in obesity, diabetes and/or fatty liver in humans. GOLIATH will generate the world’s first integrated approach to testing and assessment (IATA) specifically tailored to MDCs. With a focus on the main cellular targets of metabolic disruption – hepatocytes, pancreatic endocrine cells, myocytes and adipocytes – GOLIATH will develop new and optimise existing methods that span the entire adverse outcome pathway (AOP) spectrum. GOLIATH will provide key information by which mode of action MDCs disrupt pathways by incorporating multi-omics, and translating results from in vitro and in vivo assays to adverse metabolic health outcomes in humans at real life exposures. Given the importance of international acceptance of the developed test methods for regulatory use, GOLIATH will link with ongoing initiatives of the OECD for test method (pre-)validation, IATA and AOP development. With a consortium comprised of world-leading experts, GOLIATH will be pivotal in the development of an internationally harmonised strategy for testing MDCs, with the ultimate aim of slowing the worldwide rise in metabolic disorders that have reached ‘Goliathan’ proportions.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement GOLIATH No. 825489.

References

Keywords
metabolic disrupting chemicals; new approach methodologies; integrated assessment; adverse outcome pathway
In the last two decades, a series of projects aiming towards the establishment of regenerative medicine technologies for skin injuries in Costa Rica have been developed. Joint efforts have been made between different state hospitals and universities, among which the Costa Rican Institute of Technology stands out as a protagonist.

Some of those projects have been limited to animal-cell culture studies, while others have required the use of animal models to verify the potential of cell therapies, in preclinical assays. This research has allowed the establishment of protocols designed to evaluate the wound regeneration rate and quality in various murine models, which in turn has allowed their refinement. To evaluate the regeneration potential of Adipose-derived Stromal Cells (ASC) seeded in a biological scaffold, this skin injury murine model was used.

One squared centimeter acute full-thickness skin wound was produced in the interscapular area of adult male Balb-C mice, and four different treatments were applied as follows: a) ASC in saline solution, B) ASC seeded in a biological scaffold (agarose), C) agarose without cells, D) a commercial agent for wound healing as a positive control and D) saline solution as a negative control. The animals were assessed and the injuries measured on a daily basis until the wound was completely closed. After that, samples were taken and a histological evaluation was performed.

Preliminary tests have shown that ASC seeded in an agarose scaffold tend to reduce the wound faster during the first week compared to ASC in saline solution, although complete closure was obtained around day 12 in both treatments. Other treatments showed lower regeneration rates compared to ASC treatments.

These experiments constitute some of steps taken in Costa Rica towards the use of cellular therapies at a clinical level.

References

Keywords
Full-thickness injury; Agarose; Biological scaffold; Adipose Stromal Cells; Murine model
307 SKIN SENSITIZATION AND IRRITATION ANALYSIS OF MULTI-HERB EXTRACTIONS USING DPRA, H-CLAT AND CLINICAL CLOSED PATCH METHODS

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The natural plant extracts in Asia have been studied and applied on human skin for thousands of years because of their moisturizing, anti-aging, antimicrobial and anti-inflammatory characteristics. Focusing on the AOP pathway involved in sensitization, DPRA (Direct Peptide Reaction Assay), h-CLAT (human Cell Line Activation Test) and KeratinoSens were set up to analyze chemicals. There are few literatures revealing the sensitization and irritation of plant mixtures using these methods. We firstly chose the DPRA and h-CLAT methods to test four natural mixtures which extracted from three plants such as Glycyrrhiza Inflata, Sophora Flavescens, Scutellaria Baicalensis (SGS) and Vitex trifolia, Gentiana scabra, Polygonum multiflorum (VGP). Only one mixture, VGP, showed positive results in both DPRA (100% dosage) and h-CLAT assays (0.1% to 0.3% dosage). In order to find the allergen, we continued to analyze the components of VGP, and finally found that tween-20, an additive solvent, showed positive results in both models. Interestingly, VGP at 10% dosage had no adverse reactions in the clinical closed patch test for 24 h. Furthermore the sensitization of VGP will be verified with clinical model and gene analysis related to skin sensitization in keratinocytes by RT-qPCR.

References

Keywords
DPRA; h-CLAT; skin sensitization; multi-herb extraction
IDENTIFICATION OF APPROPRIATE METHODS FOR SEVERITY ASSESSMENT IN A WIDELY USED MOUSE MODEL OF ACUTE COLITIS

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INTRODUCTION: According to the EU Directive 2010/63, recognizing and minimizing pain, suffering, and harm of laboratory animals is a legal requirement and essential for both scientific and ethical reasons. To detect signs of diminished well-being, it is crucial to choose sensitive parameters which allow the required classification into mild, moderate, or severe experimental severity. We compared and assessed the respective merits of different behavioral and physiological methods for severity assessment in laboratory mice in a model of chemically induced, acute colitis in an effort to refine this model.

METHODS: 12 week old female C57BL/6J mice were exposed to 0.0 %, 1.5 %, or 2.5 % dextran sulfate sodium (DSS) via drinking water for five consecutive days. Over the course of disease, we compared species-specific burrowing behavior and a composite pain score with physiological parameters like body weight, clinical scoring, corticosterone levels, and fecal occult blood. RESULTS: The acute colitis had an observable impact on burrowing behavior, the composite pain score, and other physiological parameters. With the lower dose of 1.5 % DSS, the burrowing tests and the composite pain score were superior in detecting disease severity, whereas body weight remained stable. No impact on corticosterone was detectable and fecal occult blood did not correlate with colitis severity.

CONCLUSION: Physiological parameters and clinical scoring should always be monitored in severity assessment. Nevertheless, behavioral tests will provide a substantial added value to the refinement. Changes in burrowing behavior reflected pain that was not evident in clinical scoring. Additionally, the composite pain score detected impairment of animals earlier in the course of colitis than clinical scoring or body weight.

References

Keywords
Welfare; Behavior; Colitis; C57BL/6J
To ensure the safety of new ingredients when used in cosmetic products, development of New Alternative Methods (NAMs) addressing Acute Oral Toxicity (AOT) and Repeated Dose Toxicity (RDT) has become essential since the animal testing ban. In this context, we propose a new approach that could feed the Next Generation Risk Assessment including in silico approaches as well as in vitro models to support decision-making. For AOT, we developed an in silico profiler and a multiparametric approach for the oral LD50 prediction. RDT assessment was evaluated using a panel of traditional and emerging technologies from transcriptomic analysis to tissue specific models. The approach was assessed on a set of compounds without in vivo AOT alerts including, well-known chemicals (Amiodarone, Acetaminophen), a negative control (Mannitol) and one hair dye with an unfavorable SCCS’ opinion due to genotoxicity alerts. It is also known to cause in vivo liver and heart histopathological alterations in rodents after oral repeated dosing from legacy studies. The LD50 prediction correctly identified non acute toxic compounds (3/4). No toxicity was observed for Mannitol whereas Amiodarone and Acetaminophen showed in vitro mitochondrial alterations described as one of their mechanism of action involved in the chemical–induced hepatotoxicity in vivo. Finally, a strong decrease in albumin secretion in the 3D-liver spheroid assay, and a decrease of cardiomyocytes’ conduction velocity and contraction forces were observed in vitro for the hair dye. This new approach provided relevant elements contributing to hazard characterization for the 4 test articles, which must be assessed on a larger set of compounds to better evaluate the value of NAMs in the assessment of systemic toxicity. Next steps will consist of 1) enriching the approach for reproductive toxicants assessment, and 2) integrating the kinetics to increase the relevance of the data in realistic use conditions.

References

Keywords
Tetanus vaccines are prepared from chemically inactivated tetanus neurotoxin (TeNT). This detoxified material is called tetanus toxoid. Safety tests are prescribed for each toxoid batch to make sure that it has been sufficiently detoxified. According to the European Pharmacopoeia, these safety tests have to be performed as toxicity tests in guinea pigs. One of these prescribed tests addresses the irreversibility of the inactivation: In order to demonstrate that there is no "reversion to toxicity" during storage, the toxoid has to be stored at 37°C for six weeks before being tested in guinea pigs. We have investigated the relevance of this irreversibility test: Comprehensive literature research and communications with some vaccine manufacturers revealed no convincing evidence that any reversion events have ever been observed during the production of tetanus vaccines at all. Moreover, using the binding and cleavage (BINACLE) assay as an in vitro method for the detection of active TeNT (Behrensdorf-Nicol et al., 2013), we could show that the toxin rapidly loses its activity at 37°C. Consequently, active TeNT molecules that may potentially arise in a tetanus toxoid owing to reversion events will no longer be detectable after the 6-week storage period, anyhow.

Based on these findings, we concluded that the prescribed test for irreversibility has no relevance for the safety of tetanus vaccines (Behrensdorf-Nicol and Krämer, 2019). We presented our findings to the Expert Groups of the European Pharmacopoeia Commission to stimulate discussions on the possible deletion of this animal test. These discussions have ultimately led to the decision that the test for "irreversibility of toxoid" will be deleted from the Pharmacopoeia monographs for tetanus vaccines for human and veterinary use in the near future.

References

Keywords
tetanus vaccines; test for irreversibility of toxoid; European Pharmacopoeia
The Threshold of Toxicological Concern (TTC) is a pragmatic and conservative tool for the risk assessment of substances. It is based on the principle of establishing a human exposure threshold value for all chemicals, below which there is a very low probability of a possible risk to human health. Such threshold values may be identified for many substances on the basis of their chemical structures and the known toxicity of chemicals sharing similar structural characteristics. The TTC concept has been internationally accepted and used in a wide range of regulatory contexts. The human exposure threshold values have been originally derived from oral toxicity data on cancer and noncancer toxicity endpoints (Munro et al., 1996). This database has been substantially enlarged by the COSMOS database, an enhanced oral non-cancer TTC dataset on a larger chemical domain, thereby resulting in a new, transparent and public TTC database which also includes 552 cosmetics-related chemicals (Yang et al., 2017). The 5th percentile point of departure value for each Cramer Class was determined, from which human exposure TTC values have been derived. The COSMOS-plus-Munro federated dataset provided TTC values of 46, 6.2 and 2.3 µg/kg bw/day for the Cramer Classes I, II and III, respectively. Overall, the TTC is accepted by regulatory authorities and most scientific committees, and there is broad application potential for use in safety assessments of cosmetic ingredients. Cosmetics Europe has prepared several case studies which demonstrate that the TTC approach is a sufficiently conservative approach to safeguard the consumer. Overall, the TTC concept is useful to avoid animal testing and successfully evaluates the safety of cosmetic ingredients for which the consumer exposure is low.

References

Keywords
TTC; cosmetic ingredients; safety assessment; low exposure
Botulinum neurotoxins (BoNTs) are highly potent bacterial toxins inducing a flaccid paralysis. The muscle-relaxing effects of the serotypes BoNT/A and BoNT/B are exploited in clinical and aesthetic medicine to treat a broad spectrum of disorders associated with muscle overactivity. In order to avoid toxic side effects, the potency of each BoNT batch has to be precisely determined. The “gold standard” method for this purpose is a test measuring the lethal toxin dose (LD50) in mice, which causes severe distress for the test animals. Although some approved alternative methods exist, none of them is applicable to all relevant BoNT products and freely available for all potential users.

We have developed a method for measuring the activity of BoNT/A and BoNT/B in vitro based on the two most important specific characteristics of these toxins, namely their receptor-binding and proteolytic properties (Wild et al., 2016; Behrensdorf-Nicol et al., 2018). In-house characterization studies demonstrated that this BoNT BINACLE (binding and cleavage) assay is highly sensitive and applicable to all approved BoNT products, thus meeting the basic prerequisites to serve as an alternative to the LD50-based animal tests. It was further shown that the method can be straightforwardly transferred to other laboratories. Currently, an international collaborative study is ongoing to promote acceptance of the BINACLE assay by BoNT manufacturers and regulatory authorities. In future, the BINACLE assay could allow animal-free activity determinations of BoNT products, and could thus lead to a noticeable reduction in animal numbers.

Funded by the German Federal Ministry of Education and Research (BMBF projects no. 031A210 and 031L0148) and the German Research Foundation (DFG).

References

Keywords
botulinum neurotoxins; activity testing; BINACLE (binding and cleavage) assay
325 DEVELOPMENT OF A GUT-ON-A-CHIP DEVICE TO DUALLY PERFUSE HUMAN TISSUE BIOPSIES.

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Introduction/Aims: Culturing anaerobic microbiota together with oxygen dependent gut epithelial cells is a major challenge for studying host-microbe interactions ex vivo. To overcome this, Cherry Biotech is developing a gut-on-a-chip model, tailored for use within the GROWTH European Industrial Doctorate consortium but also broadly usable. The poster will include the design (while preserving IPR), validation data and early biological experiments.

Methods: End-user interviews within the GROWTH consortium were conducted to determine the most pressing need for a new device in light of the range of existing models (Dawson et al., 2016; Jalili-Firoozinezhad et al., 2019). A multiwell based fluidic culture unit was designed and will be prototyped by combining different polymeric materials. Mechanical validation will be carried out using CubiX Mark I (Cherry Biotech). Biological validation will be carried out within the GROWTH consortium.

Results: From end-user interviews, we identified that recreating the physiological oxygen gradient and extending the lifespan of tissue sections are the key objectives. Thus, we are developing a dually perfused gut-on-a-chip system able to hold tissue sections as small as 1mm³ biopsies. Oxygenated media is perfused basolaterally using the CubiX platform, while the apical compartment is kept in an anoxic environment to reproduce physiological hypoxia. The culture unit consists of two manifolds fitted for standard 24well culture plate. The lower manifold isolates basolateral flow and supports the intestinal tissue, while the upper manifold seals the system and the apical laminar flow, as well as provides optical access for microscopy. The culture unit can be used in conjunction with biopsies, larger resected material and cell-seeded membranes.

Discussion/Conclusion: Here we present a gut-on-a-chip model grounded in the specific research needs not yet met by other 3D cell culture techniques. Further testing will be required to ensure its functionality.

Funding: H2020-GROWTH-EID, Grant Agreement ID: 814168

References

Keywords
organ-on-a-chip; microphysiological control; Horizon 2020; applied biosciences
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In the last 5 years, initiatives on decision-making supported by New Approach Methodologies (NAMs) have made progress and delivered several pragmatic tools. In addition to the capability building effort, other challenges related to increasing confidence and fostering the implementation of these approaches globally are being addressed. The Cosmetics Europe Long Range Science Strategy (LRSS) is an initiative that aims to develop human-relevant safety assessment approaches for cosmetic ingredients. Case studies are the backbone of the science strategy. They offer the opportunity to proof check the validity of the approaches being developed, while highlighting strengths and limitations of the NAMs used and gaps which warrant further effort. For systemic toxicity, different types of safety assessment are addressed within the LRSS, including the threshold of toxicological concern (TTC), development of an internal TTC (iTTC), next-generation read-across and ab initio approaches. We have conducted case studies based on caffeine and propylparaben, which illustrate the use of NAM-based toxicodynamic information combined with toxicokinetics for systemic toxicity assessment. The safety assessment is exposure-led, hypothesis-driven and builds on a Next Generation Risk Assessment (NGRA) workflow. One feature of the NGRA is to derive margins of internal exposure using bioactivity data from NAMs in combination with exposure. It is hoped that the learnings from these case studies will be leveraged to elaborate guidance documents on applying NGRA.

References

Keywords
Next generation risk assessment; Exposure-led; Hypothesis driven; Cosmetics Europe; Margin of safety
Animals that are notably useful for humans/human health like laboratory animals and as a (negative) consequence, are denied a species-appropriate life, are particularly gladly supported by animal welfarists. Adopters are therefore usually happy when rehoming them and willing to give a lot in return. Generally, high demands are placed on rehoming laboratory animals (e.g. specific expertise, suitable housing conditions, well-planned procedures). Rehoming concepts must be species-specific including different requirements for the donor, intermediary organisations/persons as well as new owners. Aspects relating to animal health, prior castration, transport equipment, species-specific knowhow, rehoming experience, animal-friendly husbandry etc. must be taken into account. New animal owners must be carefully selected, while ensuring high standards of housing conditions, species knowledge, sufficient time as well as species-appropriate husbandry conditions. In addition, it must be ensured when rehoming through animal welfare organisations that acceptance for control services (before and after the animals are handed over) as well as signing protection treaties is given.

Successful rehoming projects require sensitivity, good communication and cooperation from all parties involved. Last but not least, financial considerations and agreements are also required. Currently rehoming projects are financed privately mainly by animal welfare organisations. The adopters also make substantial contributions. Sometimes also researchers contribute with private funds.

From an animal welfare point of view, rehoming-projects must ensure, in the sense of a comprehensive culture of care concept, the whereabouts of laboratory animals after experimentation are well thought out and rehoming is taken into account whenever possible. Rehoming funding must be given space, ideally already proactively together with the submission of the application for the animal experiment project. Private funding should be seen very most as initial funding and be replaced by an adequate research budget.

In this talk I will present you our concept and experiences of rehoming laboratory rats, mice and rabbits.

References

Keywords
rehoming rodents; rehoming laboratory animals; 3Rs; culture of care ; animal welfare organisation
347 DEVELOPMENT OF A HUMAN BONE-ON-A-CHIP TO MODEL INTRAMEMBRANOUS OSSIFICATION IN BASIC SCIENCE AND TOXICOLOGY

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Bone is surprisingly dynamic and harbors a variety of cell types. Unlike other tissues, it is constantly renewed by a coordinated interaction of bone-forming (osteoblasts) and bone-resorbing cells in a process called remodeling. It is thus challenging to recreate bone and its developmental steps in vitro. Hitherto, most of the current models focus on aspects relevant only within a certain biological context and do not consider all key features of bone like mechanical stress, hypoxia, the composition of the extracellular matrix and its different cell populations [Scheinpflug et al., 2018]. Consequently, animal models are still the gold standard to explore bone biology and pathology, although it becomes more and more evident that species-specific differences in physiology hamper the translation of results obtained from animal models to humans [Knight, 2007].

In our project, we aim to establish a 3D human in vitro model to simulate bone development in a more physiologic manner. Therefore, we combine a 3D aggregate, generated from primary osteoblasts, with an in-house designed micro-physiological system, able to regulate oxygen saturation and mechanical load, to create a “bone-on-a-chip”. We established two protocols for aggregate generation. One approach is based on bio-assembly strategies, starting with osteoblast spheroids that are subsequently fused, while the other approach uses 3D printing for organoid generation. Our data demonstrate that both aggregate types exhibit typical osteogenic features. In addition to mineralized matrix components, an elevated alkaline phosphatase activity could be determined for bio-assembled samples. In printed aggregates, mechanical loading within the micro-physiological system induces osteogenesis when compared to unloaded samples as determined by an extracellular increase in inorganic phosphate.

The proposed strategy will enable the implementation of a developmental model for human ossification. In the long term, the “bone-on-a-chip”, should provide a meaningful alternative to reduce and replace animal testing in basic research and toxicology.

References

Keywords
Bone-on-a-chip; Osteoblasts; 3D aggregate; Microphysiological platform; Alternative method
Physiologically-Based Kinetics (PBK) modelling is an integral part of the tool set used in Next Generation Risk Assessment of ingredients in consumer products (Moxon, et al., 2020). Accurate predictions of the exposure allow for comparison to biological effects, and an understanding of the risk to consumers. But providing confidence in these exposure predictions without the use of in vivo data for validation can prove difficult. This work proposes and outlines the use of a PBK framework, with application to a number of case study chemicals (caffeine, coumarin and sulforaphane) in hypothetical products.

The framework highlights how confidence in PBK model can be increased through an iterative process, and by using sensitivity analysis to identify parameters which will have a large influence on the model output. The use of Monte Carlo analysis allows for population variability and parameter uncertainty to be integrated into the study, and a distribution of Cmax values calculated, instead of a point estimate. The case studies show that the framework can be applied to a variety of products whose main route of exposure is transdermal, and in cases where the product is left on the skin (e.g., face cream and body lotion) or rinsed-off (e.g., shampoo and kitchen cleaner). The work highlights the need for running the simulation for sufficient amounts of time during repeat exposure to ensure that steady-state has been reached (up to 20 days of repeat exposure in some cases). Validation of the models against published clinical trial information shows that they predict within 3-fold of the observed C_max results, without the use of animal/human data.

References

Keywords
next generation risk assessment; physiologically-based kinetic modelling; uncertainty analysis; sensitivity analysis; in silico first
Improving reproducibility in preclinical studies will require many individuals to take action. But how is this achievable when we all work in different environments and have varying levels of knowledge, experience, habits, pressures and support?

One practical tool that can help is a checklist. They break complex tasks down into an ordered sequence of steps that transforms understanding and makes consistent results achievable. Checklists clarify what is required to: help us be organised; motivate us to take action and complete tasks; improve efficiency and reduce stress by supporting us to make less mistakes; improve self-confidence and support individuals to implement good practice. They are a valuable tool that puts everyone on the same level, without judgement or presumptions.

What checklists can’t do is: make individuals read or implement them, provide the answers or replace the thought processes that individuals need to go through when planning, conducting, analysing or communicating their research. These aspects are influenced by the local research culture and the individuals within it.

In this talk I will share with you my personal checklist for responsible animal research with links to freely available tools (MERIDIAN, 2020), resources (PREPARE, 2018) and guidance (UKRIO, 2019; UKRN, 2020) to help incorporate it into our daily research practices. I will also share what I believe to be key points to consider in relation to how good training and support programmes can influence local research cultures (Insight blogs, 2020).

References

Keywords
Animal Research; Checklist; Research Culture; Reproducibility
Next Generation Risk Assessment (NGRA) is an exposure-led, hypothesis-driven approach integrating new approach methodologies (NAMs) to ensure the safety of consumer products without animal data. We have applied an ab initio tiered framework based upon ICCR principles [Berggren et al., 2017; Dent et al., 2018] to a hypothetical skin allergy risk assessment, where 0.1% coumarin is used in a face cream. For the purpose of evaluating the use of NAMs, existing animal and human data for coumarin were excluded.

Initially, applied dose exposure estimates for coumarin were used to determine the extent of skin exposure and in silico chemistry predictions (ToxTree, OECD Toolbox) were used in conjunction with expert opinion to establish skin allergy potential. Then the skin allergy risk assessment was conducted using DPRA (OECD TG 442C), KeratinoSens™ (OECD TG 442D), h-CLAT (OECD TG 442E) and U-Sens™ (OECD TG 442E) data were generated and a point of departure (PoD) derived from our Skin Allergy Risk Assessment (SARA) model/defined approach (DA, Reynolds et al., 2019). By evaluating the SARA DA prediction for 0.1% coumarin in a face cream against other skin allergy risk benchmarks we can conclude that there is a low risk of inducing skin allergy under this exposure scenario.

References

Keywords
Skin Sensitization; Next Generation Risk Assessment (NGRA); Defined Approach (DA); New Approach Methologies (NAMs)
The assessment of the human relevance of toxicities observed in animal assays still represents a major challenge for various areas of toxicology. Rodents, which are widely used for regulatory toxicity testing, are particularly sensitive to perturbations of the thyroid homeostasis. Evaluation of the species-specificity of adverse effects on the thyroid gland is key for the regulatory acceptance of a risk assessment. Thyroid toxicity may result from direct effects on the thyroid gland or be mediated e.g. by inducing liver biotransformation. Microfluidic microphysiological systems (MPS) represent a promising approach to reproduce physiological and toxicological interactions of target organs which previously required animal studies or clinical trials. However, adoption of MPS by the pharmaceutical or agrochemical industry is still slow, mainly driven by a lack of qualified assays to predict the safety of drugs and chemicals. Here, we present an interconnection of three-dimensional organ models representing two important target organs, liver and thyroid, of both human and rat origin in a commercially available multi-organ-chip platform. The structural and functional integrity of both organoids could reproducibly be demonstrated for at least 14 days, i.e. by steady glucose consumption, low LDH release, immunohistochemistry of thyroid follicles and liver spheroids, and organ-specific functional readouts like albumin secretion, urea production and thyroid hormone release by liver spheroids and thyroid follicles, respectively. Finally, perturbation of the hepatic thyroid hormone catabolism was demonstrated by acceleration of thyroid hormone glucuronidation following treatment with reference inducers like beta-naphthoflavone. Thus, we show for the first time a functional model of the hepatic-thyroid axis as single in vitro assay for two different species. These two chip-based models represent a major step towards an improved assessment of potential species similarities/differences of certain toxicity findings observed in rodents with significant contributions to the 3R principles.

References

Keywords
microphysiological systems; liver; thyroid; toxicity
On average, Virbac Australia sells 32 million doses of livestock vaccines per year. Those vaccine doses in turn protect about 29 million animals (cattle and sheep) per year. Every batch of vaccine manufactured and sold by Virbac Australia undergoes stringent tests to ensure their safety, efficacy and quality. Most of these tests, especially tests for product release (potency testing), are performed according to international regulatory guidelines, such as the British or European Pharmacopoeia or the USDA’s guidelines as laid down in Code of Federal Regulations 9. These guidelines unfortunately prescribe the use of significant numbers of laboratory animals. In line with the 3R principles to replace, reduce and refine animal use, Virbac Australia is committed to reducing (and eventually eliminating entirely) the number of animals used in the manufacture and release of vaccines. Virbac has an ongoing program to develop, validate, register and implement in vitro assays to replace our current animal-based tests. This presentation focuses on the first phase of Virbac’s in vitro program, which involves the development, validation and implementation of immunoassays as replacements for product potency tests requiring LD50-type assays. The in vivo potency tests to be replaced by immunoassays are: i) mice serum neutralisation tests, which evaluates the antibody response of vaccinated rabbits in mice by assessing the dilution of rabbit serum which can protect mice from lethal amounts of toxin; and ii) guinea pig challenge, where vaccinated guinea pigs are challenged with virulent cultures of Clostridium chauvoei. The latter is a prescribed product batch release potency test for Blackleg vaccine. We believe Virbac will be the first Australian manufacturer to locally register and use a non-LD50-type assay to release Blackleg vaccine. Here we describe the development, validation and discussions with the Australian Pesticides and Veterinary Medicines Authority (APVMA) leading to registration.

References

Keywords
Clostridial; Vaccine; 3R; in-vitro; Potency-Test
375 MULTI-CENTER VALIDATION OF EPISKIN™ MICRONUCLEUS ASSAY: A 3D APPROACH FOR THE ASSESSMENT OF GENOTOXICITY POTENTIAL

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The establishment of in vitro genotoxicity assessment using 3D skin models becomes essential for the safety evaluation of new chemical with topical exposure to overcome the limitations of 2D assays. Cosmetics Europe established a 3D micronucleus assay using EpiDerm™ model. To meet the increasing testing requests especially for Asian needs, in China, a novel in vitro human reconstructed skin micronucleus assay has been developed using locally produced Episkin™ model (Chen Lizao et al., 2020). The Episkin™ Micronucleus Assay showed good predictivity and reproducibility during the internal validation. In this study, a formal multi-center validation was conducted in China with 3 laboratories. 28 reference chemicals were selected by Cosmetic Europe Genotoxicity Taskforce with different physicochemical properties and coded by the third party organization. 14 of them were blind tested in all three laboratories, then the other 14 were randomly assigned to the labs. Independent statistical expert performed the decoding, data analysis and released the report. Auditor with GLP credential checked the document managements and quality management systems of each participating organization. The report of the first 14 chemicals has been released. Data of all batches met quality criterion described in SOP, which indicated the stability of model production and scientists’ operation. Classification results showed high sensitivity (90.5%) and good specificity (80.9%) for its predictive capability of genotoxicity potential. Altogether, these results showed that micronucleus assay using reconstructed skin model offered promising tool for the genotoxicity assessment of chemicals applied on skin. The availability and validity of the Episkin™ Micronucleus Assay are expected to contribute to the in vitro genotoxicity testing strategy to follow up on positive results from standard 2D in vitro assays. This validation study will strongly support the commitment to develop alternative methods to animal testing and the safety assessment needs for industry and regulatory purpose.

References

Keywords
Reconstructed human epidermis; EpiSkin™; Genotoxicity; Micronucleus assay; Multicenter validation
387 BASIC STUDIES OF CYTOTOXICITY ASSAY BY USING CULTURED PLANT CELLS

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[Introduction]
The use of cultured plant cells has several advantages over the use of cultured animal cells as an alternative to animal experiments, including simplified culture conditions and reduced culture costs. We have previously studied in vitro cytotoxicity test conditions using T87 cells, which are cultured cells derived from the representative model plant Arabidopsis thaliana. However, it was difficult to quantitatively compare cytotoxicity data obtained from T87 cells with those obtained from animal cells due to the formation of cell aggregates by T87 cells during culture. Therefore, in the present study, we attempted to conduct cytotoxicity tests using protoplasts isolated from T87 cell aggregates and also attempted to isolate protoplasts from tobacco-derived BY-2 cells using the same method (Yamada, H. et al., 2014). We then used these T87- and BY-2-derived protoplasts to establish a basic technology for in vitro cytotoxicity testing using plant-derived cultured cells.

[Materials and methods]
The optimal experimental conditions for establishing a cytotoxicity test method using the protoplasts of T87 and BY-2 cells were examined using various detergents as cosmetic materials (Itagaki, H. et al., 1998). Specifically, we examined the cell culture conditions for exposure to the test substance, the exposure time of the test substance, and the method for detecting living cells.

[Results and discussion]
The findings of this study suggest that the use of protoplasts will make it possible to quantitatively compare in vitro test results obtained using cultured plant and animal cells. Furthermore, the detergent exposure test showed that the addition of serum albumin to an experimental system of cultured plant cells reduced the toxicity of the detergent to protoplasts. Thus, it appears that the presence of serum proteins had a significant effect on the evaluation of the cytotoxicity of detergents.

References

Keywords
cytotoxicity; in vitro assay; plant cells; protoplast
Nanomaterials (NMs) are widely used. Ensuring their safe use requires adequate environmental and human health risk assessment. Besides toxicity data, this includes a systematic physico-chemical characterization of the NMs’ fate during their life cycle, covering the stages of raw material production and processing, product use and waste removal. Chronic exposure of plants, animals and humans to low-level NM concentrations remains a challenge for in-situ characterization in complex matrices, in vivo or in vitro, with current technologies. A major drawback is the lack of standardized and systematic analysis approaches, and possibility for data correlation. Therefore, we aim to develop a new integrated instrument, called the nanoparticle SCOPE (npSCOPE), for accurate and reproducible physico-chemical characterization of NMs in their pristine format and embedded in complex matrices, such as biological tissues and cells, food matrices or soils. In addition, we aim to develop and optimize dedicated workflows for cryo-chemical fixation of different sample types, as well as for multi-modal imaging and correlative data analysis.

To benchmark and validate the npSCOPE performance, we have selected different representative test cases, including products and scenarios relevant for oral, dermal, respiratory, and environmental exposure. For each test case we have defined generic products (e.g. basic soup and standard soil matrix) spiked with relevant NMs (from the list of Au, Ag, Si-Ag, TiO2, Si-Al-TiO2, ZnO, PLGA, PLGA-Au), and NM-exposed samples derived from standard in vitro and in vivo toxicity tests. We present here characterization data on selected, fixed samples obtained by stand-alone, benchmarking techniques (SEM, TEM, HIM-SIMS, Raman, Enhanced darkfield hyperspectral imaging) and the npSCOPE (STIM, SEM). These include TEM data of chemically fixed and resin embedded human buccal epithelial TR146 cells exposed to TiO2, cryo-SEM images of standard soil spiked with Au, and HIM-SIMS and npSCOPE-derived STIM and SE data on Daphnia exposed to TiO2.

References
This work was financed by the European Commission under grant agreement N° 720964 – H2020 npSCOPE project.

Keywords
IN VITRO EVALUATION OF GENOTOXICITY AND IRRITATION POTENTIAL OF EYE DROPS CONTAINING AQUEOUS PLANT EXTRACTS

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According to Committee on Herbal Medicinal Products (HMPC) Guidelines, the genotoxic potential of herbal preparations shall be assessed since genotoxic effects cannot be completely ruled out based on pharmacovigilance and long-standing use only. Also, in a product specific risk assessment further gaps in non-clinical data e.g. local tolerance testing for the final pharmaceutical product should be taken into account. Following the R3 principles, there is a drive to omit in vivo testing whilst meeting the requirements of safety evaluation. Although in vitro methods accepted by OECD are available, these are mainly applied to single chemical substances. Yet herbal preparations are multicomponent mixtures, reaching an even higher level of complexity when used in combinations. Therefore the study aimed to assess the applicability of in vitro tests to single plant extracts as well as combinations thereof contained in eye drops produced according to current pharmaceutical standards. Aqueous extracts from Atropa belladonna L., Echinacea pallida (Nutt.) Nutt., Mercurialis perennis L., Euphrasia officinalis L. and an aqueous dilution of Rosae aetheroleum were tested for their mutagenic potential in an in vitro bacterial reverse mutation assay following OECD standards. Extracts did not cause gene mutations and are thus considered to be nonmutagenic. To evaluate the eye irritation potential of eye drops containing combinations of the aqueous extracts listed above, an in vitro ocular irritation assay (OECD 492) using the EpiOcular™ human tissue model was conducted. Results classified the eye drops as “non-irritant” in accordance with UN GHS “No Category”. The applicability of both in vitro assays to more complex multicomponent mixtures is considered to be given, as no nonspecific reactions with the test items were observed and all criteria of validity were met. Therefore, this study should encourage the use of in vitro methods for safety assessment of herbal medicinal products.

References

Keywords
mutagenic potential; eye irritation; aqueous plant extract; preclinical safety assessment; reconstructed human cornea-like epithelium
CHEMICALLY DEFINED CELL CULTURE MEDIA – A CONTRIBUTION TO ADDRESS THE REPRODUCIBILITY CRISIS IN BIOMEDICAL SCIENCES

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The use of fetal bovine serum (FBS) in cell culture media has different drawbacks. The harvesting of FBS from bovine fetuses after slaughter of the pregnant parent (dam) raises ethical and legal concerns. From a pure scientific point of view the use of FBS is unacceptable since regional differences in type and concentration of ingredients exist (Baker, 2016, van der Valk et al., 2010). Hence, a non-definable quality of FBS undermines data reliability and decrease or even prevent experimental reproducibility. However, the use of chemically defined cell culture media is still scarce.

The introduction of chemically defined cell culture media is a contribution to fight the reproducibility crisis in the biomedical sciences and an approach to address animal welfare concerns. Usage of chemically defined medium will eliminate some unknowns in cell culture experiments. A procedure to develop serum free media was introduced by van der Valk and colleagues (van der Valk et al., 2010). In the presented work, a detailed recipe to prepare a defined DMEM / Ham’s F12 + ITS cell culture medium is given. This medium has proven to work in our laboratory for L929 and Caco-2 cell lines in combination with a certain plastic ware (Greiner Bio-One, Advanced-TC). To prepare the chemically defined cell culture medium (DMEM /F12+ITS) mix 50% DMEM (e.g. Sigma Aldrich, D5648) and 50% Ham’s F12 (e.g. Sigma Aldrich, N6760), add 14.7 mmol/l NaCl, 20.9 mmol/l NaCHO3 and 5 ml/l ITS (Sigma Aldrich, I3146) (cellasys, 2019). The medium was developed for cell culture in a 5% CO2 incubator. The presented method was employed to develop a chemically defined cell-based assay for cytotoxicity determination according to ISO10993-5 (Wiest, 2017), furnishing a first proof of its applicability as an alternative to cell culture media containing FBS.

References

Keywords
Chemically defined media; Fetal bovine serum; Reproducibility crisis; L929; Caco-2
The Dutch government has established a Transition Programme for Innovation without the use of animals (TPI). The TPI programme is bringing people and organizations together in order to accelerate innovation towards better science with less animals.

Utrecht University, the University Medical Centre Utrecht and the University of Applied Sciences Utrecht have decided to actively embrace the challenge and have recently founded a joint TPI Utrecht working group, with ambassadors representative of the different research fields. The aim of TPI Utrecht is to effectively support and further boost the transition.

TPI Utrecht has the ambition to create a safe environment for all parties involved, that all strive for scientific excellence. We recognize that diversity makes us stronger, and therefore seek out inclusion of differences. We insist on a culture of respect, and recognize that words and actions matter. Communication is a central element in all our activities.

TPI Utrecht will have a dense agenda of inspirational sessions driven by our ambassadors of innovation; connection with the several research groups will create out-of-the box ideas and new opportunities for grant application geared to achieve better human and animal health. We strongly believe in innovation of education: we will work on new technology and curricula.

In our presentation, we will share our strategy, our short term and long terms plans, and our accomplished and ongoing activities. We will talk about our struggles and seek for feedback and collaboration.

References

Keywords
Transition; Animal-free; Innovation; Acceleration; Collaboration
Animal experiments are the current standard for (developmental) neurotoxicity (D)NT testing, but due to their high resource needs, we currently only have information for a very small number of compounds on their (D)NT potential. Because of this lack of knowledge and known species differences between rodents and humans, there is a need for alternative testing methods assessing (D)NT potential of chemicals in a faster, cheaper and more human-relevant way. Here, we expand our method portfolio with neurally-induced human induced pluripotent stem cells (hiPSCs) for setting up test method that assess disturbance of neuronal network formation as well as acute neurotoxicity.

For this purpose, we compared three different adherent neural induction protocols for inducing hiPSCs into the neuro-ectodermal lineage and obtaining human induced NPCs (hiNPCs) by qPCR and FACS analyses. All protocols act via inhibition of the SMAD signaling pathway, yet two of them are modified from published protocols, while one is a commercially available kit (Stemcell Technologies™). After 2D neural induction, single cells were transformed into 3D neurospheres by using either AggreWells™ or a shaking platform. Subsequently, differentiation was performed either in 3D in the sphere itself or adherent on an extracellular matrix. The resulting neural networks were then analyzed with immunocytochemistry and microelectrode arrays (MEAs). Here we show that induced hiNPCs down-regulate stem cell markers present in hiPSC and express neural markers as well as markers for specific brain regions using qPCR and FACS analyses. All neural networks stained positive for βIII-tubulin (neurons) and S100β/GFAP (astrocytes). Comparative results of the different differentiation methods concerning cell function and activity on MEAs will be provided.

The value of hiNPC-derived neuronal networks for studying (D)NT in vitro will be assessed by compound testing on the networks in the future.

References

Keywords
human induced pluripotent stem cells (hiPSCs); human induced neural progenitor cells (hiNPCs); neurotoxicity testing; microelectrode arrays (MEAs); neural network formation
The human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) provides the source of human CMs for cell therapy, drug testing, and disease modeling. However, the function and structure of hiPSC-CMs are similar to immature fetal CMs than mature adult CMs. There are many successful methods that have been developed for the maturation of hiPSC-CMs, such as long-term cultures for >100 days, electrical and/or mechanical stimulation, co-culture with non-CMs, etc. Because CMs evolve in a mechanically active environments generated by spontaneous contraction, the exploring mechanical cue for the maturation of hiPSC-CMs may thus suggest a strong biomimicking rationale for producing functional (or spontaneously beating) myocardial tissue.

Therefore, this study aims to elucidate the effects of mechanical stress on the maturation of hiPSC-CMs and its underlying mechanism. In this study, we found that cyclic stretching induced the electric maturation of hiPSC-CMs, and the increased cell size, and the decreased circularity. Cyclic stretching also increases the ion channel expression, which is important to cardiac action potentials and function. In addition, the RNAseq analysis demonstrated several key molecules related to the maturation of hiPSCCMs. These results provide a new insight to the relationship of ion channel and maturation-related molecules during the physical stimulation of iPSC-CMs.

[Acknowledgements]
This work was supported by the Technology Innovation Program funded by the Basic Science Research Program funded by the Ministry of Education (NRF2018R1D1A1B07047733). In addition, this work was also supported by the Ministry of Food & Drug Safety of Korea (19172MFDS168).

References

Keywords
Human induced pluripotent stem cells-derived cardiomyocytes; Cardiomyocytes maturation; Mechanical stimulus; Cardiac electrophysiology; Gene expression
Charité, the university hospital in Berlin, is a large faculty that conducts about 50,000 animal experiments per year for biomedical research. Since 2018, it actively strengthens the in-house implementation of the 3Rs by the foundation of Charité3R. This newly established faculty body supports 3R research and education by research funding, workshops and courses. The measures are accompanied by communication and outreach activities. Up to now, 6 calls for 3R research projects have been published. For these, 77 proposals have been submitted and evaluated by independent peer-reviews. As a result, 29 projects have been funded with a total of 3.4 € Mio. The calls focus on refinement, innovative high risk research ideas and other 3R approaches that are rarely funded by public funding agencies. In this way, Charité 3R aims to incentivise the change towards alternative methods and draw the attention to the relevance of refinement and reduction for high quality biomedical research results. To disseminate 3R knowledge within young researchers, Charité 3R provides 3R courses for PhD students. In addition, Design Thinking Workshops for the creation of new 3R approaches are organized for PhD students and postdocs. Outreach activities focus on public events and transparent communication on 3R research.

Taken together, these measures strongly support awareness, recognition and implementation of the 3Rs at Charité. Future perspectives comprise the expansion of these activities by institute overarching collaborations with external partners, thereby enhancing the opportunities, impact and knowledge transfer of 3R developments. https://charite3r.charite.de/en/

References

Keywords
3R centre; 3R research funding; 3R education; 3R communication
The tasks of Animal Welfare Bodies are defined by the European Union's (Directive 2010/63/EU of the European Parliament and of the Council; Article 26 and 27) and national legislations (in Hungary the Decree 40/2013 of the Hungarian Government; Article 39). One of the duties of Animal Welfare Bodies' is the supervising of the authorization of scientific projects at the institutional level. The Animal Welfare Body of the University of Debrecen (named Committee of Animal Welfare) is participating actively during the application process for scientific projects.

To acquaint the researchers with the project application procedure, at the University of Debrecen in the Laboratory Animal Science and Welfare Courses' was introduced new topics as follows: in Function Specific (Prerequisite) Modules the "Design of procedures and projects (Level 1 and 2)" as lectures and in the Additional Tasks Specific Modules the "Project evaluation" as seminars. These topics can help the participants to familiarize themselves with current legislation, with the application form, and the project application procedures. Moreover, the researchers can learn how to design a project, how to apply the principles of 3R's during animal experiments, how to approach the severity assessment, and how to perform the retrospective analysis of the experiment.

We would like to present with examples and statistical data on how the EU/63/2010 Directive changes the attitude of the researchers to apply the objectives of animal welfare during their scientific work.

References

Keywords

3R; Animal Welfare Bodies; Project application; Directive 2010/63/EU
In many research fields, experiments still substantially rely on animal models. Routine procedures such as frequent handling and injections are performed according to the research aims with little consideration of the impact on the animals, despite the influence on study outcome and data quality. Therefore, this study monitored a preclinical experiment by assessing voluntary wheel running as a measure of general well-being. The study was hereby aiming to identify impacts potentially being missed by conventionally applied methods to enable refinement of the procedures in future.

The study comprised a laparotomy for pancreatic cancer cell injection and a subsequent phase with daily intraperitoneal injections of chemotherapy and vehicle substances. After laparotomy, minimal body weight reductions, slightly elevated clinical scores but significantly decreased voluntary wheel running (VWR) behaviour were observed. Following the repeated intraperitoneal injections, body weight was reduced in response to the chemotherapy, but not after vehicle treatment, while the activity-related behaviour was decreased in both cases. Wheel running behaviour further revealed differences between the substances and altered nightly activity patterns. By assessing voluntary wheel running, this study demonstrated a high impact of the repeated injections and differences between substance effects on well-being. Moreover, it revealed VWR as a more sensitive indicator of impairment, strongly suggesting the need for multimodal severity assessment and refinement of experimental protocols.

However, differences in tumour growth between the treatment groups could not be determined. This might be due to the high impact of the procedures uncovered in this study, as exaggerated stress responses are potentially confounding factors in preclinical studies.

References

Keywords
voluntary wheel running; severity assessment; pancreatic adenocarcinoma; galloflavin; chemotherapy
Societal concern for animal welfare and scientific concerns about the predictive power of animal models are driving forces for the development of animal-free approaches for the safety testing of chemicals. Despite the many interesting innovations, a paradigm shift towards an assessment of human health risks fully based on animal-free approaches is not foreseen within the next decade. To accelerate the use of animal-free innovations in the EU, it has multiple advantages to work bottom-up towards a new safety assessment paradigm and to aim their development at better meeting the current regulatory needs. The question then is what these needs are and what regulatory questions need to be answered? In relation to chemical safety assessment, management and communication, a large number of different questions can be posed that may require the development of different tools and assessment strategies (Bos et al., 2020). For instance, what basic information is needed within the context of the following areas of chemical safety assessment in the EU: 1) classification, labelling and packaging, 2) the derivation of health-based guidance values and product limits, 3) risk assessments of exposure situations of concern and 4) addressing specific topics of societal concern. Agreements on the level of detail and uncertainty, robustness, predictive value, reproducibility and validation are a prerequisite to develop tools that can be trusted and that will be legally binding. A challenging aspect is to go beyond the present regulatory requirements and to develop animal-free innovations that can address exposure scenarios that vary in exposure route, intensity (dose, concentration), duration and frequency, including shortterm and long-term high exposures, following e.g., accidental release. To develop innovations that are fit-for-purpose, a multi-stakeholder collaboration is needed already in the development phase of animal-free innovations, where regulators can inform on the regulatory needs and the criteria for acceptance.

References

Keywords
safety assessment; regulatory needs; animal-free innovations; implementation
Assessment of developmental and reproductive toxicity (DART) uses a large number of mammals. It is particularly in this field of toxicology that alternative test methods that follow the principles of the 3Rs (Replacement, Reduction, and Refinement) are highly warranted, to reduce the amount of animal testing. There are many efforts to design alternate assays, whose results are very encouraging. However, a challenge remains in the interpretation of the data. DART is very complex, involving many diverse biological processes and interconnected adverse outcome networks. It is now possible to address this challenge with the recent developments in molecular biology, omics datasets, database interconnectivity, and data science.

We have designed a user-friendly web-based platform that enables complex data integration. For example, the interface can be used starting from chemical(s), via (predicted) DART phenotypes, to adverse outcome pathway, and reversely: from an adverse outcome pathway towards DART phenotypes. Molecular fingerprints are used to compute compound similarity, which allows the comparison of toxicity profiles between similar compounds. Additionally, the interface contains DART phenotypic endpoint data for a wide variety of test systems, to compare OECD DART test data with 3R test methodologies (zebrafish, C. elegans, slime mold, and cell-based assays). Cross-species phenotype-to-pathway analysis of toxicity endpoints allows the prediction of evolutionarily conserved pathways affected by compounds.

The use of the interface enables the selection of relevant biological tests for DART assessment and supports the prioritization of chemicals and adverse outcome effects for further investigation. By integrating years of scientific research and knowledge that is available in public resources, we aim to boost the acceptance of alternatives to mammalian DART testing. We thank the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) CRACK IT Challenges programme for funding this work.

References

Keywords

Developmental and Reproductive Toxicity (DART); In silico assessment; 3R test methodologies; Phenotype-to-pathway analysis; CRACK IT Innovation Platform
Benzyl Salicylate (BS) is a chemical manufactured up to 10 000 tonnes/year for a wide set of uses including consumers products, notably cosmetics. Concern about its endocrine disruption (ED) potential has been raised based on significant in-vitro estrogen activity (comptox.epa.org). In rodent uterotrophic bioassays performed on immature animals, the uterine weights were significantly increased in animals exposed orally to BS for 3 days (Zang et. al., 2012). However, these results are not enough to conclude on reprotoxic effects on adults. Since no in-vivo reprotoxicity studies on young adults are available for BS that could confirm this ED related effect, there is currently not enough experimental evidence to conclude on the ED potential of BS.

The aim of this poster is to determine whether our screening battery method is consistent with the preliminary results described above and if so, to provide a mechanistic interpretation. BS was screened using 30 in-silico prediction models (including our internal ED SAR) for ED related endpoints (Anderson et al., 2018), i.e., binding to estrogen (ER), androgen (AR) and thyroid receptors (TR), as well as models which screen for effects mediated via these receptors.

BS was inside the applicability domain (AD) for 4 AR models, 9 ER models and out of AD for the 9 TR models used for screening. 2/4 AR models (binding and antagonism) and 3/10 ER models (binding and agonism) triggered an alert for BS. Therefore, our conclusion is that BS triggers alerts for both AR and ER. Based on these results BS should not be listed as a substance of low priority for ED and therefore is recommended for higher tier studies.

Currently, our team is working on the expansion of the ED SAR to refine this consensus by including Docking and Dynamics Molecular Modelling for ER, AR, TR and other targets.

References

Keywords
in-silico, endocrine disruption, safety assessment
In as much as West European and North American countries forge ahead in discovering, validating and practicing alternative methods the situation in developing countries, especially Asia, is not anywhere near. Adoption of alternative methods discovered and validated elsewhere and contribution to discovery of newer methods for international harmonization by these countries can be greatly motivated and encouraged through establishment of national societies. In Asia, Japan, through its JSAAE and JacVAM, stands a unique exemption. 3R/Alternatives Societies have come up in South Korea, China, India and Sri Lanka but national priorities being different the alternatives scenario is not encouraging. The JSSAE has taken the initiative of a federation of these societies. Nevertheless, international harmonization at the global level such that the countries which are less endowed are pushed forward by those that are better endowed, will be rewarding.

References

Keywords
Alternatives; National societies; 3Rs Centers
A variety of in vitro assays exist to examine the genotoxic potential of compounds, however the application to real-life exposure is questioned. We show that 3D tissue models can be effectively combined with an in vitro animal-free genotoxicity screen, overcoming insoluble formulations and with dosing that mimics real-life application. This system allows investigation into whether potential genotoxic compounds can pass through a reconstructed skin barrier, and if so, remain genotoxic following exposure to skin metabolic enzymes. Furthermore, use of the animal-free BlueScreen test allows for identification of all 3 classes of genotoxins; mutagens, clastogens and aneugens (Etter, S 2015; Hughes, C et al 2012).

In summary, we created a co-culture system consisting of TK6 cells and EpiDerm tissue models. PPD (a suspected genotoxic compound in some hair dyes) was added to the apical side of the tissue models or the cells directly, for 48hrs. EpiDerm tissues were rinsed after 50-minutes to mimic real-life exposure of hair dyes on the scalp and returned to the co-culture system until 48hrs post dosing. TK6 cells were collected 48hrs post dosing and quantified for genotoxicity (luminescence) and cytotoxicity (fluorescence) measurements. Results show that the EpiDerm tissue models and TK6 cells can be combined to detect genotoxicity. PPD penetrated the 3D tissue layer and elicited an increased luminescence and decreased fluorescence signal in TK6 cells, even after a 50-minute rinsing procedure. Furthermore, we tested the genotoxic potential of a commercially available hair-dye formulation and this showed that the addition of a 3D tissue model reduced the cytotoxic and genotoxic effects upon the TK6 cells. This suggested that the skin barrier could be effective at preventing a proportion of the genotoxic substance from being absorbed, or that it is detoxified by the skin’s metabolic enzymes, providing a more relevant model for genotoxicity assessment of skincare formulations.

References

Keywords
Genotoxicity; Cosmetic; Co-culture; Safety; in vitro
442 IN VITRO TO IN VIVO EXTRAPOLATION FOR DEVELOPMENTAL TOXICITY POTENCY OF VALPROIC ACID ANALOGUES

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To support implementation of alternatives to animal testing for regulatory decisionmaking for developmental toxicity, several case studies have been developed under the European Union ToxRisk project. One case study is to investigate the teratogenic potency of valproic acid (VPA) analogues, which has been tested with the devTOX quickPredict assay (devTOXqP), a human induced pluripotent stem cell (iPSC)-based assay. Previous work showed that the potency ranking from devTOXqP was consistent with observed developmental toxicity potency in vivo. In this study, we applied in vitro to in vivo extrapolation (IVIVE) to evaluate the impact of pharmacokinetics (PK) and different modeling approaches on predicting relevant external exposure from in vitro developmental toxicity potential concentrations derived from the devTOXqP assay. We used several PK models, including an open-source one-compartment model, an open-source physiologically-based PK (PBPK) model, and both open-source and commercial pregnancy-specific PBPK models. The IVIVE analysis estimated equivalent administered doses (EADs) that would result in maternal or fetal blood concentrations equivalent to the in vitro developmental toxicity potential concentrations. The estimated EADs were compared to published lowest effect levels (LELs) from rat in vivo developmental toxicity studies and clinical doses in humans. For the five VPA analogues with LEL data, at least one PK model produced an EAD within 1.5-fold of the rat LEL range. All the models produced EADs within 4-fold of the rat LEL range for three of the five analogues. The close agreement between EADs and in vivo rat LELs suggests that the devTOXqP assay and IVIVE approaches are suitable for quantitatively predicting in vivo developmental toxicity potential at relevant concentrations. This study highlights the importance of PK considerations in assessing a chemical’s developmental toxicity potency based on in vitro assays. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

References

Keywords
in vitro to in vivo extrapolation; pharmacokinetics; physiologically based pharmacokinetics; reproductive and developmental toxicology; in vitro and alternatives
Existing in vitro tests for skin irritation were historically developed for analysis of harsh chemicals and do not have the sensitivity required to resolve differences in irritation potential between ultra-mild surfactants or cosmetic ingredients. Furthermore, validation of these historical tests used in vivo animal data (Spielmann H et al., 2007), which can be over-predictive of human irritation, when compared to human patch tests (Jírová D et al., 2010).

Therefore, we developed a new test, adapted from a skin irritation ET50 test. By using 3D reconstructed tissue models, skincare formulations can be dosed mimicking real-life application. Additionally, by extending the dosing time to 48 hours from 18 hours, subtle differences between mild and ultra-mild formulations can be quantified, that would not have been distinguished in the original method. These products can then be ranked by order of irritation potential (and therefore, mildness).

We demonstrate the predictive capacity of the in vitro XtraMild test by directly validating it with human patch test data. In a series of blind-coded tests, the XtraMild in vitro assay accurately predicted the rank order of a series of different surfactants and mild skincare formulations, to all the rank orders obtained from the human patch test clinical scores. We have generated a benchmarking database validated against human, not animal, data, which is a critical part of providing a valuable test for the industry, and allows results to be interpreted in the context of typical industry ranges for a specific product type across the personal care spectrum.

This test provides an effective pre-screen for formulations and ingredients relevant to the personal care industry. By using this test in the initial stages of product development, it can identify the mildest ingredient combinations, therefore reducing the number of patch test volunteers required and their risk of developing an adverse irritation reaction.

References

Keywords
mildness; safety; validation; irritation; in vitro
444 APPLICATION OF NEWLY VALIDATED ROUTE-SPECIFIC IN VITRO GENOTOXICITY ASSAYS TO SUPPORT THE SAFETY ASSESSMENT OF COSMETIC INGREDIENTS

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New in vitro skin models have been established as follow up assays to improve the prediction of potential genotoxicity of cosmetic ingredients in the absence of in vivo data. The reconstructed skin micronucleus test (RSMN) and RS Comet assays are now validated and have been accepted into the OECD test guideline development program. Here, we demonstrate their application to safety assessment by conducting a case study based on the oxidative hair dye, 4-nitro-1,2-phenylenediamine (B24). The strategy is based on an endpoint-triggered follow up of positive results from the Ames and in vitro micronucleus (MNvit) 2-test battery. For topically applied chemicals, the RSMN assay is recommended for MNvit positive chemicals; whereas, Ames positives should be tested in the RS Comet assay. B24 was positive in the Ames assay but negative in the MNvit assay; therefore, it was tested coded in the RS Comet assay. In experiment 1, B24 was non-cytotoxic and did not induce DNA breaks in keratinocytes or fibroblasts up to the lowest precipitating dose (50 mg/cm²). In experiment 2, in the presence of the repair inhibitor, aphidicolin, there was no statistical increase in %tail DNA that exceeded the historical controls up to 50 mg/cm². B24 was concluded to be negative, which is in accordance with negative results in the HPRT mammalian cell gene mutation assay. The conclusion from this case study is that while B24 causes mutagenicity in bacteria, it is not genotoxic in mammalian cells or in a higher tier assay using human skin equivalents. The RS Comet assay allows an assessment under the relevant exposed conditions i.e. topically, and in a test system containing relevant metabolizing enzymes. Further case studies are under way to evaluate other scenarios from the testing strategy.

Funded by Cosmetics Europe and the German Ministry for Research and Education.

References

Keywords
reconstructed skin micronucleus test (RSMN); reconstructed skin comet test (RS Comet); 3D skin models; cosmetic ingredients; follow up testing
The demand for therapeutic antitoxins is global, as are concerns about their availability, production, and efficacy (Chippaux, 2017). Most currently available antitoxins are derived from the blood of immunised animals, often equines. Equine-derived antitoxins include treatments for snakebites, dog bites, and bacterial infections such as diphtheria. These are commercially prepared by repeatedly exposing horses, donkeys, or mules to repeated doses of a toxin, causing their immune systems to produce large quantities of antibodies against the toxin. For the animals, this process can cause local and systemic adverse effects, including injection-site oedema, thrombosis, phlebitis, abscesses, fistulas, and fibrosis (World Health Organization, 2018). The resulting drugs have well-documented quality, efficacy, and safety issues, including hypersensitivity reactions and serum sickness. Maintaining reliable stockpiles for quick transportation to patients in need of treatment is also challenging (Both et al, 2014).

Fortunately, recombinant human antitoxins can be developed and produced in cell culture without the use of animals (Wenzel et al, 2020). Several recombinant antibody-based therapeutics have been approved for clinical use, and more are in development.

This presentation will discuss the limitations of animal-derived antibody products, the condition of the equines on factory farms in India, and our work with the Indian government to replace equines in the production of therapeutic antitoxins. Additionally, we will summarise the use of the phage display approach to generating recombinant human antitoxins without immunising and bleeding animals. We will also describe the PETA International Science Consortium’s current projects for the development of recombinant human monoclonal antibodies that neutralise the diphtheria toxin and black widow spider venom.

References

Keywords
Recombinant human antitoxins ; Equine Sera; Diphtheria ; Snake bites ; Phage Display
A main argument for the use of animals in biomedical experiments is often related to their genetic similarity to humans. Genomic cross-species comparisons estimate that over 85% of human genes have homologs (orthologous) in mice, the most widely used vertebrate animals in experiments. However, this high number is often misleading as many important differences in the structure, expression, posttranscriptional and posttranslational modifications and functions of these orthologs and their products are disregarded. Here, we address these differences and critically assess the suitability of animals in biomedical genetic studies.

As a start, determining the exact number of orthologs between humans and mice is challenged by the fact that both the number of human genes as well as the definition of “gene” itself are a matter of debate. In this respect, the role of noncoding RNAs and untranscribed DNA regions is often neglected although many of them have important regulatory functions are responsible for most of the sequence divergence between mice and humans. Furthermore, discrepancies of gene expression and alternative splicing each affect at least 10–20% of human–mouse ortholog pairs and significant variability is observed also in orthologous gene isoforms, copy number and function (Gharib and Robinson-Rechavi, 2011). Finally, the collective effects of these differences often add up to divergent phenotypes in mice and humans, for example, one study shows that over 20% of human essential genes have nonessential orthologs in mice (Liao and Zhang, 2008). In conclusion, we argue that the abundant genetic differences between mice and humans lead to a low predictive value of mouse-derived data, highlighting that mice and other animal species are unsuitable for genetic and biomedical research.

References

Keywords
genetics; translatability; orthologs; genetic differences; genes
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Intestinal stem cells reside in the intestinal crypts and are able to differentiate into all mucosal cells present in the intestine, e.g. Paneth, Goblet, Enteroendocrine cells and Enterocytes. In this study, we culture these stem cells, allowing us to form in vitro miniguts which can be used to assess the production and regulation of antibacterial peptides (AMPs) such as Reg3B and Reg3G.

Reg3B and Reg3G are produced in the small intestine by Paneth cells. Both Reg3 proteins are involved in keeping the intestinal mucus layer free from invading bacteria and lack of these proteins is associated with intestinal inflammation. Although it has been suggested that Reg3G expression can be directly induced in Paneth cells by LPS or indirectly through the IL23-IL22 axis, we show that the dendritic cell- or macrophage-derived cytokines IL6 and TNF can stimulate T cells to produce IL22, which will subsequently induce expression of Reg3B and Reg3G in Paneth cells. In addition, we show that IL22 enhances mucus production, indicating the importance of IL22 in the different aspects of intestinal barrier function and defense against intestinal bacteria. Our data shows the applicability of mini-guts or organoids as an animal-free model to study AMP production as an important component in the first line of microbial defense.

References

Keywords
intestinal organoids; antimicrobial defense; mucus formation; inflammation; cytokines
468 ALTERNATIVE METHOD TO CULTURE INTESTINAL ORGANOIDS WITHOUT LOSS OF BIOLOGICAL FUNCTIONALITY

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Over the last years, organoids have become increasingly popular in research as they resemble the composition and functionality of organs, making the organoid model an ideal alternative to in vivo testing. Intestinal organoids are derived from freshly isolated intestinal epithelial crypts that harbor Lgr5-expressing stem cells. A method for longterm culture of 3D murine intestinal organoids was first developed by Sato et al. Several essential factors to maintain basic crypt physiology were described: Wnt/R-spondin1 to activate the Wnt signaling pathway, Noggin as a BMP4 inhibitor and EGF to stimulate proliferation. For structural support and biochemical cues, organoids are cultured in Matrigel matrix.

In search for reduction of time and costs in culturing intestinal organoids, we screened several factors to replace one or more of the components used by Sato et al. This screening has led to the composition of a new culture medium. We developed and thoroughly tested our new culture medium and designed a standard operating procedure (SOP) for long-term maintenance of intestinal organoids. To standardize our method, the SOP was performed in three separate laboratories, in which a vial of murine ileum organoids from liquid nitrogen was thawed, expanded and maintained for 3 weeks, with weekly passaging. The viability of the organoids was assessed by observing morphology. In addition, the expression of IL22-induced gene expression of the anti-microbial peptide Reg3G was determined by exposing organoids to IL22 for 24 hours each week, followed by RNA isolation.

Results show that three laboratories were able to culture viable and healthy murine intestinal organoids with the newly developed culture medium and SOP. As expected, IL22 stimulation consistently induced Reg3G expression in the organoids. These results indicate that the developed SOP to culture murine intestinal organoids can be transferred to independent laboratories and can be used for long-term culture of intestinal organoids.

References

Keywords
Organoid culture; Standard operating procedure; Standardization
Concern over the potential for environmental chemicals to perturb hormone systems has led to the development and implementation of a number of OECD Test Guidelines for the screening and testing of endocrine disrupting chemicals. Although a number of methodologies have been developed to interrogate reproductive steroids, incorporation of test methods to evaluate disruptors of thyroid hormone signalling pathways has been limited, owing largely to the complexity of the thyroid system. Research efforts are funded by the European and International funding programmes to support the necessary development of new methods and approaches in this particular field to complement the information gaps identified. Furthermore, the European Commission Joint Research Centre’s European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) has compiled a number of Thyroid Hormone Disruption (THD) in vitro methods with validation potential, based on the OECD scoping paper (OECD, 2014) and with input from THD meetings and workshops. This large set of 17 methods has been used as the starting point for validation of selected in vitro THD methods for the identification of modulators of thyroid hormone signalling. Central to the validation exercise is the selection of the chemical validation set. A chemical expert group proposed a list of 51 chemicals that has been reduced to a final set of 30 chemicals informed by the use of chemoinformatics tools and Artificial Intelligence based (AI) tools, i.e. machine learning and text analytics. The symposium will illustrate how global collaboration, harmonisation, interdisciplinary efforts and increasing common awareness of common agreed regulatory information needs can deliver methods and approaches responding to current regulatory challenges for identifying human thyroid disruptors.

References

Keywords
in vitro; thyroid; validation; artificial intelligence; mechanism
The U.S. Environmental Protection Agency (EPA) uses estimates of dermal absorption to assess the potential for systemic toxicity to occur through skin contact. To calculate a chemical-specific dermal absorption factor (DAF), EPA uses data from in vivo rat, in vitro rat, and in vitro human dermal absorption studies, commonly known as the “triple pack”. To assess the feasibility of using only in vitro data to estimate the DAF, we conducted a retrospective analysis of agrochemical triple pack reports completed between 2003 and 2019. For each chemical, we calculated the ratio of in vitro to in vivo dermal absorption to determine if such an approach would be sufficiently protective (i.e., in vitro DAF ≥ DAF derived from in vivo studies or the triple pack). We also analyzed ratios of concentration and time-matched absorption values from the in vitro human: in vitro rat studies to determine differences in permeability between human and rat skin. 30 triple packs were analyzed using concentration-matched results. For at least 80% of those chemicals, these ratios were greater than 1, indicating in vitro estimates were higher than in vivo estimates. For chemicals with ratios less than 1, no single parameter (physical-chemical properties, assay conditions, etc.) could be identified as responsible for this finding. As expected, the in vitro human: in vitro rat ratios were less than 1 for 29 of 30 chemicals, indicating that the dermal absorption in rat skin is greater than in human skin. This analysis confirmed that, in general, a DAF derived solely from in vitro data would be more protective than the triple pack DAF. More work would be needed to understand the mechanism underlying those few cases where the in vivo absorption exceeded the in vitro absorption. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

References

Keywords
dermal absorption; pesticides; risk assessment; triple pack
The need for models of human corneal epithelia in pharmacology and toxicology fields leads to the development of three-dimensional (3D) corneal models. SkinEthic HCE is a reconstructed human corneal epithelia model produced by EPISKIN from cultivation of transformed human corneal epithelial cells on a polycarbonate membrane at the air-liquid interface in a chemically defined medium. This 3D construct resembles the stratified cellular organization of human corneal epithelium: a monolayer of columnar basal cells covered by intermediate cells similar to in vivo corneal wing cells and a nonkeratinized superficial layer of flattened cells. Transmission electronic microscopy (TEM) imaging showed in all layers a well-developed cytoskeletal network and cells-cells connection with numerous desmosomes and, in the cells of the basal layer, anchoring hemi-desmosomes connecting the construct to the support. Scanning electron microscopy (SEM) surface analysis of SkinEthic HCE revealed structures resembling to the microvilli observed in vivo. These structures appear to be coated with a sort of mucus forming large plaques suggesting presence of a superficial glyocalyx layer as observed on human cornea. RT-qPCR analysis for mucin genes MUC1, MUC4, MUC5B, MUC5C and MUC16 on SkinEthic HCE confirmed expression of these genes excepted for MUC4. Immunohistochemistry of the membrane-anchored mucin, MUC1, showed a suprabasal location compatible with a glyocalix-like accumulation. Cells of the suprabasal layer express also keratin 3, a human cornea-specific differentiation marker. Barrier function of the SkinEthic HCE model can be measured by ET50 and the observation by immunohistochemistry of occludin protein in all layers is in agreement with existence of dense tight junction network. This work has showed some parameters similarities between SkinEthic HCE model and the in vivo situation in human. It confirms suitability of 3D construct to model human corneal tissue as an alternative to animal use.

References

Keywords
transcriptomics; tissue engineering; human cornea; mucins; barrier function
Computational modeling can be used to design effective nonanimal approaches, if grounded in reliable experimental data. We have developed a set of computational models to predict eye irritation and corrosion. We compiled and curated a database of in vivo eye irritation studies from scientific literature and stakeholder-provided data. The database contains over 500 unique substances, including many mixtures, tested at different concentrations. Substances were categorized according to Globally Harmonized System (GHS) and U.S. Environmental Protection Agency (EPA) hazard classifications. Two modeling approaches were used to predict classification of mixtures. A conventional approach provided predictions based on the chemical structure of the most prominent component of the mixture. A mixture-based approach accounted for all components in the substance by weighted feature averaging. Accuracy rates (assessed by the area under the receiver operator characteristic curve) for undiluted test substances were 71.79% and 72.80% for the conventional and mixture-based models, respectively. Classification rates (by balanced accuracy) were 67-76% for both models. Accuracy rates for substances diluted to 10% in the conventional and mixture-based models were 89.91% and 91.92%, respectively. Classification rates for both models were 86-87%. A strong trend between a substance’s pH and activity was observed. Our results suggest that these models are useful for screening compounds for eye irritation potential. Future efforts to increase the models’ utility will focus on expanding their applicability domains and using the models in conjunction with other input variables (e.g., in vitro data) to establish a defined approach for eye irritation testing. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

**References**

**Keywords**

eye irritation; eye corrosion; machine learning; Draize; integrated testing strategy
The 3Rs (replace reduce refine animal testing) principles, a scientific and ethical driven design of experiments, were established in 1959 and have since been adopted widely, particularly in Europe with the European Directive 2010/63/EU.

At GSK, animal studies, which are conducted with high standards of humane care and treatment, represent a small but vital part of our procedures in the release and development of vaccines. While GSK continues to work toward an era of non-animal based research and development, we remain committed to acting ethically and practicing good animal welfare when animal use is still required. Whenever possible, an in vivo test is replaced by an in vitro test. When alternative is not scientifically available, in vivo test are still required by authorities for batch release to demonstrate the consistency. However, while waiting for sounded alternative, special care and effort to reduce the number of animals used for release testing is always a priority.

An illustration of this is the reduction of guinea pigs used in the assay for Tetanus and Diphtheria toxoid adsorbed vaccine for humane use by seroneutralisation. An in-depth evaluation of the test performance and the Minimum Requirements of the US authorities to perform the assay for product marketed on US market has permitted to propose a reduction for each lot from 6 to 4 guinea pigs in the first phase of the assay and a reduction from 4 to 2 animals in the second phase of the assay.

After formal regulatory acceptance of the change granted in 2019, it is estimated that 600 guinea pigs are saved every year.

References

Keywords
Quality Control; Potency; Reduction
For decades, in vivo studies have served as the benchmark against which new approach methodologies (NAMs) have been compared. Data from animal test methods are inherently variable, even when tests are conducted according to accepted test guidelines. Characterizing this variability is critical to set appropriate expectations for NAM performance and establish confidence in NAMs. We assessed the reproducibility of two in vivo acute toxicity methods that are required for testing of pesticide technical active ingredients and formulations by the U.S. Environmental Protection Agency (EPA): the rat acute oral lethality test and the rabbit skin irritation test. Datasets were compiled for each endpoint and comprehensively curated. Regulatory hazard classifications (United Nations Globally Harmonized System and EPA) were derived when possible. The range of results for chemicals with multiple independent study reports per assay was evaluated, as was the probability of obtaining the same regulatory classification for a substance tested multiple times. Where possible, we evaluated a variety of physicochemical properties and study protocol elements to determine if there were any trends among the chemicals exhibiting the greatest variability in results. Overall, the observed variability could not be attributed to methodological or physicochemical properties for either test method. These analyses help provide much needed context not only to assess “gold standard” reference test methods, but also to aid in setting expectations for NAM performance. The resulting datasets provide a resource for development and evaluation of alternatives to in vivo tests. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

References

Keywords

new approach methodology; variability; rabbit; replacement
484 PERSPECTIVES OF CHINA’S FUTURE SAFETY EVALUATION SCIENCES TOWARDS NON-ANIMAL TESTS

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With the recognition of the importance of 3Rs (Replacement, Reduction, and Refinement) in science and regulation, China was seen gradual shifting toward 21st-toxicity testing paradigm. Over the last decades, significant efforts have been made by the governmental regulatory agencies to promote the development and application of alternative methods in China for the risk assessment of various products. Since 2016, six alternatives methods were adopted in the Safety and technical standards for cosmetics (STSC) (2015 version). Safety assessment approach was increasingly introduced and adopted to exempt redundant toxicology tests since 2014. The upcoming Cosmetics Supervision and Administration Regulation (endorsed by China’s State Council in Jan 2020) and Administration of Filing for Non-Special Use Cosmetics (Drafted in May 2019) might further streamline the filing process and underpin the application of modern toxicology toolbox. Moreover, the newly effective Group Standard Management Regulations (release in Jan 2019) was expected to encourage industrial engagement with more international regulatory toxicology practice.

Continuous academic efforts have also pushed the boundaries forward. The Society of Toxicological Alternatives and Translational Toxicology under the Chinese Society of Toxicology and the Society of Toxicity Testing and Alternative Methods under the Chinese Environmental Mutagen Society established in 2014 have provided a scientific communications interface for government, enterprises, and academia via national/Asian conferences, training, and other activities. Over 100 million of national scientific funds were invested in alternatives since 2014, including drug, food, environmental chemicals, etc. In 2018, alternative development was included in the latest national strategical document released by the China Association for Science and Technology. Standardization work was also initiated to promote the harmonization. Monographs addressed 21st-century toxicity, AOP and In Vitro Toxicology were published or to be published. It’s foreseen that multidisciplinary and cross-regional collaboration will further promote the translation of modern toxicology in China.

References

Keywords
non-animal tests; alternative; toxicology
Acute toxicity is a key human health endpoint for safety assessment, but no validated in vitro test is currently available. This poses a challenge in regulatory submissions for cosmetic ingredients, which must avoid animal testing in many parts of the world. A new test using human dermal fibroblasts in xeno-free culture was established as a preliminary prediction of the acute toxicity of cosmetic ingredients in vitro, to support a Weight of Evidence (WoE) approach to risk assessment. Cell membrane damage was used as an endpoint to generate an in vitro IC50 value (concentration causing 50% cell death) to replace the in vivo LD50 (concentration causing death in 50% of the animals). A preliminary prediction model was developed to correlate IC50 values with GHS classes for acute oral toxicity. Five ingredients routinely used in the cosmetic industry were used to set up and establish reproducibility of the test (Barker-Treasure et al., 2015). Based on these results, the test was extended to 20 chemicals representing key categories of cosmetic ingredients (surfactants, preservatives, colourants, emulsifiers and fragrances). Good correlation was obtained as compared to GHS categories (65% correct classification). The test has been used by leading chemical companies and cosmetic ingredient suppliers to support weight-of-evidence approaches to safety assessment in EU REACH submissions. Further development continues, to incorporate metabolism into the test system and broaden the dataset of reference chemicals to other industry sectors.

References

Keywords
Acute Toxicity; Weight of Evidence; Cosmetic; xeno-free; in vitro
Testing of cosmetics and their ingredients on animals has been banned for 7 years in Europe, yet in vitro testing still makes widespread use of animal derivatives including serum, antibodies and tissue extracts. Most cell culture, even using human-derived cell lines, uses foetal bovine serum (FBS) derived from blood extracted by cardiac puncture of living, unanaesthetised, calf foetuses. As well as being detrimental to animals, these additives can reduce the reproducibility of tests and compromise their relevance to human physiology. Here we provide some guidance on how to define truly animal-free in vitro testing and describe some of the principles we follow. We also introduce our scale for animal-free testing, which allows the ‘animal-free’ status of methods to be clearly labelled. We have developed the scale by defining 7 clear categories of tests, with an aim to help industry move towards Level 7 as the most scientifically and ethically preferred approach. Level 1: in vivo live animal testing. Level 2: in vitro with test components that involved live animal suffering (eg FBS). Level 3: in vitro with test components that required “humane” animal sacrifice (eg rat liver extract). Level 4: in vitro with test components that are waste products of the meat industry. (eg gelatin; bovine cornea). Level 5: in vitro with test components that have previously been exposed to animal product (eg human cell lines previously cultured in FBS; some human tissue models). Level 6: in vitro animal-product-free with test components derived ethically from humans. Level 7: in vitro animal-product-free, fully defined. The scale can provide useful context for goal-setting in moving towards animal-product-free and defined in vitro systems. It is also a valuable tool for stakeholders to communicate accurately and transparently about how ‘ethical’ their testing is – an important factor in the global trend towards vegan products.

References

Keywords
In vitro; Animal-free; FBS; Animal-product-free; Ethics
511 CHEMICALLY-SELECTIVE AND LABEL-FREE CHARCATERISATION OF PANCREAS ORGANOIDS INSIDE HYDROGEL MATRICES

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Within the growing field of alternative approaches to animal testing, the implementation of in vitro cell cultures in 2D and 3D environments has experienced a surge in popularity over the last decades. The cultivation of three-dimensional cell culture systems, especially organoids, constitutes a promising approach in different fields of life sciences and is gaining interest with regard to clinical applications and personalised medicine. Although the cultivation of complex cellular systems is evolving steadily, their analysis is stagnating, relying on the visual inspection of organoid size and development via light microscopy. While analysing the expression of specific marker molecules via immunofluorescence staining is a reliable approach to determine the identity of organoids, the fixation and staining procedures of 3D cellular structures in their extracellular matrix (ECM) produces several issues and artefacts. Further, the interaction of the cells with the ECM during organoid expansion and development is completely neglected in established analysis procedures. In order to develop a comprehensive understanding of organoids non-invasively during their development, confocal Raman microscopy was used in this study to characterise pancreas organoids in different ECMs. The label-free and chemically-selective technique was implemented to monitor the interaction of cells with two synthetic hydrogels in order to assess their suitability to replace commercial Matrigel®. Using confocal Raman imaging, the contact-free analysis of pancreas cells within the ECM could be achieved, successfully showing the distribution of nucleic acids, protein- and lipid-rich regions within the cell. Further, the expansion mechanism and interaction of whole organoids with the ECM was investigated, monitoring the chemical composition of the surrounding environment as well as the lumen of the organoid in detailed imaging and higher throughput approaches. These results provide a glimpse into the possibilities of confocal Raman microscopy as an innovative technology to revolutionise the analysis of 3D cell culture systems.

References

Keywords
Confocal Raman Microscopy; Pancreas Organoids; Non-invasive analysis; Chemicallyselective imaging
In collaboration with pioneering Chinese scientists across regulatory laboratories, research institutes and universities, Unilever organised a scientific workshop on non-animal approaches for chemical safety in Shanghai on 10th and 11th April 2019. Like the rest of world, significant scientific progress has been made over the last decade in China on non-animal approaches to chemical safety. In particular, the use of new approach methodologies (NAMs) for making consumer safety risk assessment decisions was reviewed in light of recent advances in this area as summarised by the International Cooperation on Cosmetics Regulation (ICCR, Dent et al, 2018). The first day of the workshop reviewed developments in in vitro models in China (including organ-on-a-chip technology and 3D bio-printing) as well as advances in omics technologies (including dose-dependent transcriptomic approaches for screening and prediction of chemical toxicity) and computational modelling. There was also discussion of regulatory developments and approval of alternative methods in China (including recent adoption of OECD non-animal methods into Chinese technical guidance). The workshop then focussed on the future of non-animal approaches for chemical safety in China with breakout groups identifying needs for the future in 5 defined areas (1) in vitro models (2) in silico models (3) regulatory science (4) education and training and (5) application in next generation risk assessment. Several opportunities were identified to accelerate the scientific and regulatory advances in adoption of non-animal approaches for chemical safety in China including opportunities through global international partnerships with global scientific programmes.

References

Keywords
NGRA; China; NAM; Risk Assessment
Human iPSCs are a promising tool to improve toxicity evaluation without making use of animals. Within the European in3 project (MSCA-ITN ETN n°721975), different in vitro models (e.g. brain, kidney, lung, liver and vasculature) have been derived from the same iPSCs and used to assess the response to a common set of compounds.

The blood brain barrier (BBB), essential for brain homeostasis, limits the access of substances from the vascular circulation to the brain. Therefore, knowledge on compound distribution through the BBB is essential to assess neurotoxicity. In addition, compounds can also exert indirect neurotoxic effects at the level of the BBB, resulting in unwanted effects on neurons by compromising brain homeostasis or affecting the passage of compounds to the brain.

Multiple iPSC differentiation protocols for brain like endothelial cells (BLECs) have been evaluated based on their efficiency and BLECs characteristics: presence of endothelial markers, the formation of a tight barrier and the presence of functional efflux pumps (e.g.: ABCB1 and ABCG2).

The selected protocol was used to evaluate the effects of in3 compounds at the level of the BBB in comparison with the other in vitro models within the in3 project as well as the distribution of these compounds through the BBB.

References

Keywords
iPSC; Blood Brain Barrier; Neurotoxicity; in3 ITN
Meningococcal disease is caused by a gram-negative diplococcus, Neisseria meningitidis. 4CMenB is a Meningococcal group B vaccine indicated for active immunization to prevent invasive disease caused by serogroup B.

According to the European Pharmacopoeia and the Code of Federal Regulation, the release process established a series of quality control tests to guarantee its potency, purity, safety and pyrogenicity. The following in vivo tests have been part of the release testing panel: (RPT) Rabbit Pyrogen Test; (ATT/GST) Abnormal Toxicity/General Safety Test; (MDRP) Multi Dilution Relative Potency.

In accordance with the company’s commitment to 3Rs principles, we focused on the waiver of those tests without any added values (Reduction), and on moving testing process from in vivo to in vitro (Replacement), with the final purpose to achieve the complete replacement of animals use.

From 2010, European Pharmacopoeia allows use of MAT (Monocyte Activation Test) to replace RPT, and in 2013, GSK started collaboration with NIBSC for development of this assay. MAT test is now licensed to release our vaccine in almost all markets. Approval by CBER of removal of the GST for US market was also possible due to demonstrated consistency of the manufacturing process and due to the FDA decision to remove the test.

Finally, we are about to request for approval an in-house developed IVRP (In Vitro Relative Potency) assay. This ELISA test is intended to substitute the currently approved in vivo potency assay. IVRP method has been successfully validated and will be submitted soon to Health Authorities worldwide.

Although the set-up and validation of the new in vitro methods have been particularly demanding, GSK has maintained focus on its 3Rs program and we are confident that in due time we will be able to obtain the final approval for the remaining in vitro methods, to complete successfully our journey.

**References**
- DIRECTIVE 2010/63/EU on the protection of animals used for scientific purposes.;
- European Pharmacopoeia (Ph. Eur.); Chapter 2.6.8 – Pyrogens; Chapter 2.6.9 - Abnormal toxicity; Chapter 2.6.30 - Monocyte-activation test.;
- US Pharmacopoeia, Chapter 151; PYROGEN TEST.;
- NIBSC: [https://www.nibsc.org/about_us/latest_news/animal_free_.aspx](https://www.nibsc.org/about_us/latest_news/animal_free_.aspx)

**Keywords**
Meningitidis; Vaccine; Release; 3Rs
Pharmaceuticals worldwide are transitioning from the use of the rabbit pyrogen test (RPT) for batch release of parenteral drugs, or for risk assessments where bacterial endotoxin tests (BET) such as the limulus amebocyte lysate (LAL) are used. This is in accordance with revisions made within the European Pharmacopeia, now starting to be enforced for all producers who wish to distribute across Europe. Beyond the law, the omission of tests for non-endotoxin pyrogens (NEPs) carries significant risks for patient safety – specifically where biological based products are involved.

Unlike LAL or rFC (solely endotoxin assays), the monocyte activation test (MAT) is an in vitro pyrogen test that detects and quantifies the full span of both endotoxin and nonendotoxin contaminants in parenteral pharmaceutical and medical products (Solati et al. 2015). Setting itself apart from the RPT, the MAT’s use of cryopreserved, human peripheral blood mononuclear cells (PBMCs) make it the first human-specific pyrogenicity assay – delivering high reactivity, sensitivity and reproducibility. By way of an enzymelinked immunosorbent assay (ELISA), the presence of IL-6 cytokines is used as a read out for the MAT. This is well fitting as IL-6 is produced by the PBMCs upon the stimulation of both endotoxin and non-endotoxin contaminants.

Importantly, MAT succeeds as a pyrogenicity assay for blood transfusion products and medical devices – where RPT and LAL have each proven problematic or insufficient. In the case of medical devices for example, LAL and RPT are based on testing rinsing solutions, though neither test can adequately reflect the surface contamination if pyrogens are not solubilized. Only the MAT is able to test the device by direct incubation with human blood cells – making it the secure pyrogen test for pharmaceutical products and other medical devices.

References
Solati et al. 2015. An improved monocyte activation test using cryopreserved pooled human mononuclear cells. 0(0) 1–8. DOI: 10.1177/1753425915583365

Keywords
In Vitro Test Pyrogen test ; Monocyte Activation Test; Animal Free Test; Cryopreserved, Human Peripheral Blood Mononuclear Cells (PBMCs) ; Endotoxin
Exposure-based approaches and hypothesis-driven data generation form the basis of nonanimal cosmetic safety assessments. These approaches are often described as New Approach Methodologies (NAMs) and can include computational models and human-relevant in vitro assays which are applied in combination to provide information on ingredient hazard and risk assessment.

Established approaches such as physiologically based kinetic (PBK) modelling, exposure-based waiving, predictive chemistry, History of Safe Use approaches and internationally accepted (OECD) in vitro methods enable the risk assessment of many ingredients in the context of how individuals are exposed to them within normal use. However, for more novel, higher exposure materials bespoke assays are required to understand biological effects from both a targeted perspective to ensure identification of the most sensitive effects, and broader screening approaches to ensure adequate biological coverage. The ability to confidently determine adequate margins of safety are essential to ensure the protection of consumers.

In this session, we have heard how external exposure is assessed, how PBK modelling informs internal exposure estimates, and have seen examples of NAMs for cosmetic safety. Here we present end-to-end case studies based on a non-animal risk assessment framework. In-use exposure scenarios will be used to illustrate how NAMs are applied in a hypothesis-driven ab initio approach to assure the safety of ingredients in consumer goods. Consideration will be given to assumptions and sources of uncertainty in the risk assessment and how they contribute to decision making.

References

Keywords
NGRA; NAM; cosmetics; decision making
Animal testing on cosmetics was banned in Europe in 2013, with similar bans following in other regions, but most “non-animal” methods still use a variety of animal-derived components and therefore cannot be described as truly animal-free. One major concern is the ongoing, widespread use of foetal bovine serum (FBS) in cell culture, even for human cell lines. Since FBS is derived from blood extracted by cardiac puncture of living, unanaesthetised calf foetuses, it has been questioned whether this process should be reclassified as a live animal procedure (van der Valk et al, 2017). At XCellR8, we have developed a 7-point scale that classifies test methods according to the level of animal suffering involved. Level 1: in vivo testing. Level 2: in vitro with test components that involve live animal suffering (eg FBS). Level 3: in vitro with test components that require “humane” animal sacrifice (eg rat liver extract). Level 4: in vitro with test components that are waste products of the meat industry. (eg gelatin; bovine cornea). Level 5: in vitro with test components that have previously been exposed to animal product (eg human cell lines originally derived in FBS; some human tissue models). Level 6: in vitro animal-product-free with test components derived ethically from humans. Level 7: in vitro animal-product-free, fully defined. Any cell culture method using FBS is classified as Level 2 – only one level up from animal testing – due to the suffering involved. While Level 7 tests are desirable both scientifically and ethically, this isn’t always possible using currently available culture systems. However, even small changes, such as a switch from FBS to human serum, can elevate a test from Level 2 to Level 5/6. We will look at examples where this approach has been used to adapt skin sensitisation tests accepted at OECD level.

References

Keywords
Animal product free; In vitro; Cell culture; FBS; Ethics
ASSURING CONFIDENCE IN NEW APPROACH METHODOLOGIES (NAMS) – A DATA GOVERNANCE CASE STUDY

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Chemical safety assessment is undergoing a paradigm shift from assessments based on a structured and defined animal testing approach to more bespoke, ab initio problem solving using non-animal technologies. Next Generation Risk Assessments (NGRA) are exposure led and apply human-relevant, toxicity pathway-based in vitro approaches coupled with in silico models. These approaches are often described as New Approach Methodologies (NAMs) with the selection of the most appropriate being driven by the hypothesis being tested. Good Laboratory Practice (GLP) has traditionally been the internationally accepted standard for generation of experimental data in support of studies conducted to assure the safety of chemicals. However, many of the NAMs used to support an NGRA are not commonly or readily performed to GLP or due to their bespoke nature are not conducive to such a regulated structure (e.g. ‘omics panels and computational modelling). For modelling, limited accepted guidance exists and certainly there are no regulatory accepted frameworks analogous to GLP against which compliance can be claimed (1,2). Recent publications and workshops on the topic (3,4) have highlighted the need for a more flexible and robust approach to be taken to the generation of information using NAMs. With this, the emphasis shifts to the experimentalists and modellers to ensure that data integrity is upheld to high standards through peer review, accreditation schemes, governance frameworks and electronic systems.

Ensuring that experiments are planned, performed, monitored, recorded, reported and retained is essential for providing assurance of data quality through a transparent approach to allow decisions to be reproducible. Here we use an example NGRA case study (coumarin, 5) in a consumer goods context to illustrate how a combination of data governance approaches can build confidence in safety decision making using NAMs.

References

Keywords
NGRA; NAM; governance
There is an increasing acceptance of the role in vitro assays can play in assuring consumer safety, particularly as part of Next Generation Risk Assessment (NGRA) (Baltazar et al., 2020). NGRA is an exposure-led, hypothesis driven approach integrating new approach methodologies (NAMs) to ensure chemical safety without the use of animal testing. There is also a growing desire to remove animal products from these in vitro assays to make them more scientifically robust and human-relevant. For example, the use of foetal bovine serum (FBS) and animal-derived antibodies can introduce a lot of batch-to-batch variability potentially resulting in experimental quality (e.g. contamination of FBS; specificity of antibodies) and reproducibility issues (Baker et al., 2016; van de Valk et al., 2018). Additionally, it is more frequently becoming recognised that knowledge of all the constituents of the cell culture medium used and their influence on cellular processes are important for improved reproducibility (Hirsch & Schildknecht, 2019). Therefore, ideally chemically-defined media would be used to culture human cells for in vitro assays to eliminate any remaining scientific quality issues resulting from use of animal- or human-derived components (van der Valk et al., 2010) although this is technically very challenging. Here we will describe some of the challenges, opportunities and potential options for replacing animal-derived products in in vitro systems.

References

Keywords
NGRA; NAM; chemically-defined media; in vitro; consumer goods
The Portuguese Transparency Agreement was initiative initiated by the Portuguese scientific community in collaboration with the European Animal Research Association (EARA) to promote a more consistent approach to communicating the scientific, ethical and moral justifications for animal research in Portugal. The signatories have committed to proving open and transparent information about the research involving animals and the standards of animal care and welfare carried out in the institution. The signatories only use animals models in research when there are not validated alternative methods. Signing the Portuguese Transparency Agreement has given the organisations the opportunity of building a collective institutional approach to improving openness and transparency with the public. Has helped to commit the researchers to be prepared to provide information to the media and the general public on the conditions under which animal research is carried out and how the results obtained. Has brought scientists to build up an engagement in a dialogue with the society to improve the level of understanding the reasons why animals are still needed for biomedical research. The next steps will be presented and discussed.

References

Keywords
Openess; Transparency; Communication; Agreement
In as much as West European and North American countries forge ahead in discovering, validating and practicing alternative methods the situation in developing countries, especially Asia, is not anywhere near. Adoption of alternative methods discovered and validated elsewhere and contribution to discovery of newer methods for international harmonization by these countries can be greatly motivated and encouraged through establishment of national societies. In Asia, Japan, through its JSAAE and JacVAM, stands a unique exemption. 3R/Alternatives Societies have come up in South Korea, China, India and Sri Lanka but national priorities being different the alternatives scenario is not encouraging. The JSSAE has taken the initiative of a federation of these societies. Nevertheless, international harmonization at the global level such that the countries which are less endowed are pushed forward by those that are better endowed, will be rewarding.

References

Keywords
Alternatives ; India; Indian society
The OECD Guidance Document, Good In Vitro Method Practices (GIVIMP), coordinated by the European Commission Joint Research Centre's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM), provides a globally harmonised framework of technical and quality practices to help ensure that the overall development and implementation of in vitro methods is of scientific integrity and of the highest quality possible. While the guidance is intended for all OECD member states and encompasses a wide range of audiences including method developers, test system and method suppliers, validation bodies and end users, there has also been great interest from regions where in vitro methods are starting to take root. In recent years, the Chinese government has been working closely with the scientific community to review methods and accept data from new approach methodologies including in vitro and in silico testing approaches. This has resulted in updated regulations, particularly policies regarding cosmetics. Collaborative efforts between industry and the Institute for In Vitro Sciences (IIVS, Gaithersburg, USA) have focused on the transfer of several OECD Test Guideline methods to government laboratories in China and have supported the creation of an operational in vitro toxicology testing laboratory within the Zhejiang Institute for Food and Drug Control (Hangzhou, China). BASF SE (Ludwigshafen, Germany) and IIVS have partnered to introduce a cell based in vitro skin sensitization test, LuSens-Assay (OECD442D), into China using the principles of GIVIMP as a standard. This case study exemplifies the practical way in which the GIVIMP guidance can assist interested parties in the development, transfer and establishment of in vitro approaches.

References

Keywords
China; Good In Vitro method Practices; LuSens; global collaboration; harmonization
In vitro micronucleus (MN) analysis is extremely important within the regulatory agency scenario, based on Guideline OECD 487 (2016), the results obtained following its guidelines and good laboratory practice (GLP) procedures determine whether the test items are genotoxic or not. This analysis should be performed in several methods, including conventional microscopy (Rodrigues, M.A. et al., 2018). On the other hand, the automated methods result in increased sensitivity and, combined with their rapidity. Such details are interesting when it is said that it is related to production in a Contract Research Organization (CRO). In this sense, our laboratory verified the positive response criterion in relation to vehicle control (VC) between the two methodologies (microscopy vs flow cytometry) in order to ensure the results safety. For this, 10 assays were performed under three conditions (short exposure with and without metabolic activation and long exposure without metabolic activation) using the CHO-K1 cell line based on OECD 487 (2016). The MN analysis was using microscopy and flow cytometry, in which it was used Microflow in vitro (Litron) commercial kit. Our results showed a 6-fold increase in the MN amount in relation to VC when microscopy was used. As expected, flow cytometry demonstrated an increase in the detection MN capacity, about 15-fold, since it is possible to discriminate it from nucleus according to the fluorescence intensity (Bryce, S.M. et al., 2013). In addition to the high sensitivity, this methodology requires less material than conventional reading, being an excellent technology and alternative, in front of our need for fast and reproducible results.

References

Keywords
Micronucleus assay; CHO-K1; Flow cytometry; Genotoxicity; Chemical genotoxins
PROVIDING MEANINGFUL IN-CAGE RESOURCES FOR MICE: CONSIDERATIONS FOR NESTING MATERIAL AND A CARDBOARD SEMI-CAGE DIVIDER FOR IMPROVING MOUSE WELFARE

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Within laboratory cages, space and resources are limited, and mice are unable to choose their cage mates. Providing mice with useful in-cage resources may help increase mouse control over their environment, as well as increase psychological and physical stimulation, and performance of motivated behaviours such as nest building. We examined the provision of shredded paper pucks and cardboard semi-cage dividers as manipulable resources for mice to modify and increase control over their environment. Initially, a pilot study was conducted with C57BL/6 male and female mice (n=27 mice/sex, n=9 cages/sex). An association was seen between the shredded paper puck and tissue, and reduced chasing, increased material manipulation, and protecting female mice from barbering compared to when shredded paper and a tissue were provided (P<0.04). A larger study additionally examining mouse behaviour and when provided with a cardboard semi-cage divider. Group-housed male C57BL/6 and CD-1 mice were randomized to one of 4 groups: 1) tissue only (control; CD-1 n= 10, C57BL/6 n= 10), 2) tissue + shredded paper puck (CD-1 n= 10, C57BL/6 n= 9), 3) divider + tissue (CD-1 n= 8, C C57BL/6 n= 9), 4) divider + tissue + shredded paper puck (CD-1 n= 10, C57BL/6 n= 9). Overall, the results indicated strain differences in the way mice chose to use the dividers. Male CD-1 mice preferred to collapse and rip the cardboard divider into pieces for supplementary use (i.e., nest material, tunnel and hut-like structures), while male C57BL/6 mice kept the dividers up-right and built their nests within a semi-divided area. Overall, our results suggest that the shredded paper pucks are useful, time-consuming and manipulable resource, particularly for C57BL/6 mice of both sexes, and a cardboard partial-cage divider may help male mice increase control over their environment.

References

Keywords
laboratory mouse housing; laboratory animal welfare; mouse behaviour
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3Rs Centre was founded in 2019 at the Faculty of Medicine, University of Colombo to promote and facilitate alternative models to replace animals use (Replacement) in education (skills development) and research. This reduces the number of animals (Reduction) bred and use for research purposes leading to reduction of animal suffering (Refinement). Necessary measures were already taken to introduce several alternative models for research and skills development.

References

Keywords
3R principle; Alternatives; Sri Lanka
The first guidance on Good Cell Culture Practice (GCCP) dates back to 2005 (Coecke et al. 2005). With the availability of human induced pluripotent (2006) stem cells, the potential applications of human cell culture models have been greatly broadened. When using stem cells and stem-cell-derived models in research, product development, testing and manufacture of biotechnology products and cell-based medicines, it remains critical to include aspects of quality assurance. As such, the original set of GCCP principles of best practice can be used as a basis to assure good cell and tissue practices and conditions when working with stem cell derived test systems in simple set-ups or in very technological advanced formats (Pamies et al., 2017). GCCP and its next generation principles (GCCP 2.0) are intended as guidance to obtain reliable and relevant study data. Applying GCCP as part of overall Good In Vitro Method Practices (GIVIMP, OECD 2018) by the global life science community is leading to more harmonization of in vitro method related processes and procedures. Researchers are key players to ensure use of such best scientific and quality practices and are ideal ambassadors to use them in the novel generation of stem cell and tissue–based methods. The European Commission Joint Research Centre’s European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) is working on developing and validating innovative, mechanistic methods and approaches based on current harmonized scientific and quality standards complying with the GCCP principles and the GIVIMP guidance document (OECD, 2018) and is crowd-sourcing for introducing stem cells and stem-cell-derived methods into the required regulatory test batteries. Key instruments to disseminate globally harmonized good cell, tissue and method practices are published principles and guidance, conferences, face-to-face meetings, training, webinars, templates (Krebs et al., 2019), reporting and evaluation tools (e.g. http://www.scirap.org/) and e-learning training materials.

References
Keywords
GCCP; stem cells; harmonization; quality assurance; in vitro
563 INTEGRATED STRATEGY FOR EYE IRRITATION ASSESSMENT OF AGROCHEMICAL FORMULATIONS

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For the registration of agrochemical formulations, acute eye toxicity assessment is required by regulatory agencies. The Draize rabbit eye test (OECD TG 405) has worldwide acceptance to assess eye irritation, even it has been increasingly questioned. The test distinguishes four categories considering reversible and non-reversible ocular lesions according to UN GHS Categories 1 (severe eye damage), 2A and 2B (reversible eye damage) and No Category (minimal effects).

Bovine Corneal Opacity and Permeability (BCOP) and Short Time Exposure (STE) are methods for identifying Cat. 1 and No Cat. products, according to OECD TG 437 and TG 491 respectively. The limitation of both methods is to classifying in the middle-range categories (2A and 2B). The aim of this work was to integrate the results of different methodologies to identify these categories.

The BCOP and STE methods were used to test 17 pesticides manufactured by ATANOR SCA. These products had been previously classified in categories 1, 2A, 2B or No Cat. using the Draize eye irritation test.

By the BCOP test, liquid products were tested neat and at 10% and solids at 10%. By STE test, all formulations were tested at 5 and 0.05%.

It is known that BCOP and STE methods have low false negatives to classify No-Cat. products. The STE also has low false positives to classify Cat I. Taking into account the sensitivity and specificity of each method, we have distinguished the products into three categories: 1, 2A+2B and No Cat. with an accuracy of 76.5% (13 of 17). The remaining 23.5% corresponds to products whose damage was over-estimated with respect to the in vivo test; there were no cases of low-estimation. The accuracy could improve with the corneal histopathological analysis data that is still in progress.

Finally, we successfully established an in house strategy for testing agrochemicals eye irritation.

References


Keywords
eye damage; SIRC cells; pesticides
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Introduction: PAH is characterized by an increased blood pressure in the small pulmonary arteries (PAP), which induces cardiac impairments leading to a poor prognosis. Despite the development of several preclinical models of PAH, treatments are often complex and remain ineffective. This study focuses on the refinement of preclinical models which represent a major issue to slow or prevent the progress of PAH in human.

Methods: Pulmonary artery catheterization is the gold standard measurement of PAP, that requires an invasive terminal surgery. Echocardiography is known as a reference examination in human cardiovascular diseases with several advantages: It is a noninvasive technic that allows the measurement of many cardiovascular parameters, such as Pulmonary Arterial Acceleration Time (PAAT), Cardiac Output (CO) and the right ventricular area (RVEDA). In addition, echocardiography allows longitudinal studies that provide key information on the development of PAH.

Results: In PAH rats, a significant decrease of PAAT has been shown over time (Jones et al. (2002)). Thus, a 25% decrease was observed after 14 days and was emphasized after 30 days (~40%). A more recent study has shown a correlation between echocardiographic and hemodynamic parameters in different models of PAH. Indeed, a significant correlation was observed between PAAT and right ventricular systolic pressure in a rat model of PAH (r²=0.5037; p<0.001) (Zhu et al. (2019)).

Conclusion: Combining echocardiography with the PAP measurement provides a major refinement procedure to improve the PAH comprehension. Finally, this method respect 3R by improving procedures refinement and by reducing the animals number used in echocardiographic longitudinal studies.

References

Keywords
Refinement; PAH; Echocardiography
A FULL THICKNESS LONG TERM SKIN EQUIVALENT ALLOWS REPEATED TESTING OF COSMETICS TO EVALUATE EFFICACY AND SAFETY

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Introduction: Most commercial 3D in vitro epithelial model systems are currently limited by their relatively short longevity that ranges from 3-5 days meaning that long-term or repeat-dose chemical testing over prolonged periods is not possible. There is currently no safe cost-effective way to assess the allergenicity of novel compounds. The Patch Test creates patient discomfort and can trigger anaphylactic shock. Objectives: This study aims to develop a safe and robust predictive method for allergenicity testing of cosmetic compounds and cosmetics. Materials and Methods: Normal human dermal fibroblasts (HDF) were isolated from biopsies and cultured to generate their own native connective tissue scaffolds. After the 28-day development of fibroblast-derived matrices (FDM), hTERT-1 skin keratinocytes were seeded on to the surface.

Results: Tissue engineered Fibroblast derived matrices (FDM) produced a self-assembled organised, collagen fibre meshwork with a thickness over 100 μm. This results in models histologically resembling human skin tissue and displayed classical expression of AE/13, collagen type iv and E-cadherin. These equivalents remained viable for at least 42 days in culture as observed by metabolism assay. Immune cells (MUTZ-3 or THP-1) have been added in order to predict allergic potential of cosmetic ingredients. Several contact sensitizers and UVB effect have been tested upon repeated exposure. We have also tested whether antioxidant can reverse the detrimental effect of repeated exposure to sensitizers and UVB by measuring cell viability and transepidermal electric resistance (TEER). Intercellular communication was also observed by live cell imaging in a non invasive manner.

Conclusion: This novel skin equivalent provides physiologically relevant, flexible yet robust in vitro tools for use in pharmacological and cosmetic chemical toxicity testing, modelling disease processes and drug absorption studies. It allows repeated testing and study immune response in a non invasive manner.

References

Keywords
Tissue Engineering; Long term skin equivalent; Repeated Exposure ; Sensitizers; UVB
THE CHANGING FACE OF CHEMICALS LEGISLATION IN INDIA: OPPORTUNITIES TO MINIMISE TESTING ON ANIMALS

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A new legislative framework for chemicals in India has long been anticipated (Chemical Watch, 2019) and, earlier this year, the Indian Ministry of Chemicals and Fertilizers released the draft Chemicals (Management & Safety) Rules, 20XX (2020). The draft Rules describe a multifaceted programme for identifying and managing the risks associated with the use of chemicals. They include provisions for the creation of a national chemical inventory; the registration, prioritisation, and restriction of chemicals imported into or manufactured in India; the implementation of a hazard-communication system; and a safety/chemical accident prevention program.

Modernisation of the chemicals legislation provides India with an opportunity to use and promote the most scientifically advanced testing methods while saving resources and sparing the lives of countless animals. International chemicals legislation, such as the Registration, Evaluation, Authorisation and Restriction of Chemicals regulation in the European Union (Regulation (EC) No 1907/2006; European Chemicals Agency, 2017) and the Toxic Substances Control Act (2019) in the US include requirements to avoid the use of animal tests wherever possible, and India must implement similar requirements to ensure it is aligned with international standards. Therefore, PETA India made recommendations to the Indian government highlighting opportunities for using reliable and relevant regulatory testing approaches while minimising the use of animals. These recommendations include, for example, the mandatory use of validated non-animal methods, testing on animals only as a last resort, promotion of a “risk-based assessment” approach, and mandatory in vivo data sharing. PETA India continues to work with the Indian government to ensure the new chemicals legislation is the most scientifically advanced in the world. This presentation provides the blueprint that will ensure better protection of human health and the environment while promoting the use of reliable, relevant non-animal methods.

References

Keywords
Chemicals; Regulatory; non-animal; Testing
TOWARDS IN VITRO-IN VIVO CORRELATIONS OF RESPIRATORY TOXICITY CAUSED BY NANOMATERIALS

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In the literature, in vitro/in vivo correlations of respiratory toxicity caused by nanomaterials (NM) lead mostly to conflicting results; only few toxicological studies on NM have shown positive correlations between in vitro and in vivo inflammation (neutrophils in mouse/rat lungs). In vitro models with a high in vivo predictive power are urgently needed for financial and ethical reasons. In this presentation, I will first discuss important aspects for in vitro-in vivo correlations, such as: 1) cell type and species in mono- or co-cultures exposed in submerged or at the air-liquid interface; 2) physicochemical characterization of the NM to estimate the “real-sedimented dose”; 3) statistical tests used. Secondly, I will relate these aspects to a case study that aimed to correlate in vitro-in vivo pulmonary inflammation and genotoxicity caused by seven carbon nanotubes (CNT), with different sizes, shapes and surface modifications. In vivo, inflammation (cytology) and DNA damage (comet assay) were assessed in BAL cells of mice exposed by intratracheal instillation to 3 doses (low, medium or high) of CNT, and followed for 1 and 3 days post-exposure. In vitro, human epithelial cells, macrophages and fibroblasts, in mono- or co-culture, were exposed for 6 or 24 hours to 3 doses (low, medium or high) of the same CNT in submerged conditions or at the air-liquid interface. Inflammation (cytokines gene expression) and DNA damage (comet assay) were quantified. A scoring system, based on a categorization of effects into standard deviation units, was developed to correlate the DNA damage in vivo and in vitro, showing good predictivity and sensitivity. Inflammation was dependent on the CNT surface area in vivo, but it was highly dependent on cell type and sedimentation rate in vitro. Cell types and effective doses appear to be important parameters for the in vitro-in vivo correlation of CNT respiratory toxicity.

References

Keywords
nanotoxicology; in vitro-in vivo correlation; Respiratory Toxicity; carbon nanotubes
581 COMPARATIVE STUDY ON GENOTOXIC ACTIVITIES OF DIFFERENT COMPONENTS OF FINE PARTICULATE MATTER (PM2.5) USING IN VITRO BIOASSAYS

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Genotoxic activities PM2.5 samples were collected in winter and summer during the year 2016 from two locations of Shanghai (Pudong, Huinan) with urban and suburban characteristic respectively. The genotoxicities of total particulates (TP), organic component (OC) and water soluble fractions (WS) were estimated using bacterial genotoxicity test with five Salmonella typhimurium strains (TA98, TA100, TA97a, TA1535 and WP2), SOS Chromotest® assays with E. coli PQ37 and chromosome aberration test with Chinese hamster lung cell line (CHL). Direct genotoxicities in different levels were observed for both TP, WS and OC, and there were no obvious indirect genotoxicities. Statistically significant differences between TP, WS and OC were shown in all bioassays, genotoxicity of OC can be neglected compared to TP and WS, especially with relatively low exposure dose (≤10m³ atmospheric volume), although genotoxicity of OC increased with exposure time and dose, genotoxicity still lower than it caused by TP and WS. PM2.5 and its extracts in two locations identified significant seasonal differences, the genotoxicity in winter is higher than summer. Therefore, the genotoxicity in both winter and summer could not be neglected. No significant locational difference was shown in this study, indicated that the pollution in suburb is becoming serious. The genotoxicity of TP and WS were still in the similar level, indicating the main genotoxic component of PM2.5 in two locations were water soluble parts and their genotoxic activity may decreased by metabolism.

Acknowledgements: Financial support from the Natural Science Funds of Shanghai science and technology commission (Grant number: 14ZR1435500).

References

Keywords
583 INVESTIGATING THE EPITHELIAL BARRIER IN HUMAN SKIN 3D TISSUE MODELS WITH A NON-INVASIVE FLUORESCIN LEAKAGE ASSAY

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One of the main functions of the skin is to protect the body from external, potentially harmful influences. For biomedical research, 3D in vitro skin models of varying complexity have been generated. However, an intact barrier function is often not verified before the examination, which can lead to misinterpretation of the results obtained. To investigate this epithelial barrier in vitro, we use an innovative technique to create full skin 3D tissue models, namely cell crowns covered with a biological vascularised scaffold (BioVaSc-TERM®). In order to test the integrity of the barrier before using models in downstream applications, we are developing a non-invasive, indirect assay employing fluorescein as a marker molecule. Fluorescein, as a well-characterised validation substance, does not damage the cells when passing paracellularly through human epithelial barriers. In case the barrier is not properly developed, fluorescein leakage is increased, thus providing an indirect measurement of the tightness and, moreover, the functionality of the skin model.

In short, a biological decellularised matrix (SiSmuc) is reseeded with primary human fibroblasts and keratinocytes in a co-culture. On day 19 of development, model tightness is estimated by applying solely fluorescein to the skin model. The leakage into the basal compartment is measured time-dependently up to 1h after application. If the model is then judged to be intact, we apply three well-characterised substances on d21, which will later serve as reference substances. These are caffeine as non-irritant but penetrating, SDS as irritant and penetrating and HCl as corrosive substance. This approach might serve as a further replacement for the Draize skin irritation test (OECD Guideline 404) and a refinement for test methods based on reconstructed human epidermis (OECD Guideline 439) to a more natural full-skin model. Furthermore, we are working on additional non-invasive techniques for model characterisation, e.g. 2-photon microscopy or an optimised TEER-device.

References

Keywords
tissue engineering; human skin 3D tissue model; model epithelial barrier; fluorescein
Caco2 cell line models are a standard for intestinal drug absorption studies, whilst they have advantages (forming a largely impermeable polarised cell layer and expressing some intestinal transporters), they lack mucosal cells and have decreased paracellular transport - limiting their efficacy and predictive accuracy. Standard liver in vitro models are often based on immortalised cell lines (e.g. HepG2) that have reduced metabolic capacities in comparison to primary human hepatocytes (PHH). While PHH suffer from donor-to-donor variation and cultures only retain in vivo characteristics for a short time in standard culture. The effect of improving these in vitro assays may lead to more effective and rapid preclinical drug development. This study explores the use of human primary hepatocytes (PHH), 3D scaffolds and perfused MPS technology to study the process of human first pass metabolism in a single multi-organ in vitro platform. A perfused gut co-culture model was established by culturing Caco2 and HT29-MTX cells in an MPS platform. This model showed an increase in permeability whilst maintaining a tight functional barrier formation in comparison to standard Caco2. Aminopeptidase-N enzymatic activity was increased with perfused conditions and RNA expression analysis revealed an increase in genes involved in key metabolic pathways. To determine drug transport and metabolism together, perfused Caco2/HT29-MTX cocultures were combined with PHH in a multi organ MPS platform. PHH were cultured within the perfused platform or for comparison in a standard 24-well plates. Probe compounds were used to compare the drug transport and metabolism of both MPS and control static models. The absorption was aided by the addition of recirculating flow within the MPS and the absorbed compound was then metabolised by the PHH. This demonstrates that this gut-liver co-culture system can be used to model human first pass metabolism of xenobiotics.

References

Keywords
Housing and care programs for laboratory pigs have primarily been developed based on cost, convenience of staff, and health status of animals, but with limited consideration of comprehensive animal behavioral management (ABM) programs. Currently, there are many recommendations for improving the well-being of commercial pigs held on farm; however, there are few papers or recommendations specifically about enhancing the behavioral management of research pigs of different breeds. Capitalizing on 2019 as the lunar ‘Year of the Pig’, we formed a Pig Welfare Working Group with the goal of improving research pig behavioral management, with special consideration given to minipigs. Our primary areas of consideration were enhancing options for pigs to express natural behaviors such as rooting, chewing and foraging; providing comfortable housing with exercise and opportunities for social interaction; increasing use of operant conditioning techniques to better prepare pigs for studies; employing fear-free methods of handling, restraint, transportation, and conduct of technical procedures; and inserting periodic welfare assessments to ensure the program is suited to meet each pigs’ needs. While principally designed to implement within our facilities in the EU and North America, these guidelines for a research pig ABM program can be used as a best practices framework that other organizations and facilities can adopt and modify to meet their own program-specific needs.

References

Keywords
Behavior; Training; Pig; Refinement
Osteoarthritis is an invalidating disease characterised by gradual articular cartilage deterioration affecting 1.4 million Dutch and costing 18 billion € in 2018. The lack of translation power of current in-vivo and in-vitro models contributes to the estimated increase in treatment costs to 54 billion € in 2040. Consequently, the developed predictive model that accurately emulates the tissue’s microphysiology (e.g. organ-on-chip) is urgently needed. Articular cartilage is an avascularized structure comprising specialized cells (chondrocytes) experiencing both compression and shear stress generated through the sliding of the two bones. Our platform mimics the 3D structure of the tissue and incorporates multi-axial mechanical stimuli, as present in the joint (Paggi et al., 2020), emulating shear strain and physiological/hyper-physiological compression.

The platform, in polydimethylsiloxane, comprise three sections: a mechanical actuation chamber; a cell-hydrogel section; and a perfusion section, to provide nutrients. Human chondrocytes (hCHs) were cultured in agarose hydrogel within the platform using differentiation medium. The cell projected area deformation was determined by applying 7 individual pressures from the mechanical actuation system. Cell viability was evaluated for different mechanical stimulation conditions. Glycosaminoglycan production was assessed using Alcian blue and nuclear fast red on histology sections after 15 days of culture in the platform.

Cell area decrease is correlated to the cell location in the device with respect to the membrane and the applied pressure. The hCHs cultured with hyper-physiological compression (>20%) showed a significant decrease in cell viability (day 3), while under physiological conditions (<20%) they maintained their viability after 15 days of stimulation. hCHs were next cultured in static or dynamic conditions (compression or combination of compression and shear strain). Here, Glycosaminoglycan production demonstrated that a combination of compression and shear strain greatly enhance matrix formation.

This platform allows mimicking of healthy and hyper-physiological stimulation, which is instrumental in studying disease progression and drug uptake and response.

References

Keywords
organ-on-chip; mechanical stimulation; protein production
The synovial membrane is a soft tissue vital for maintaining joint homeostasis. In chronically inflamed joints, this tissue responds with an abnormal proliferation of the fibroblasts, infiltration of immune cells and ultimately hyper-plasticity of the synovial membrane surface. Furthermore, in pathological conditions, synovial fibroblasts (SF) combined with macrophages secrete factors stimulating cartilage degradation. This study aims to create a synovial membrane–on-chip recapitulating the native structure of the tissue under physiological conditions.

The organ-on-chip model consists of a porous poly(dimethylsiloxane) PDMS membrane (3µm thick; with pore diameter 5µm) separating two fluidic layers, mimicking the intraarticular cavity and the blood vessels in the subintima. At both sides of the fluidic chambers, two mechanical actuation chambers are engineered each consisting of three individually addressable pressure-units. Vacuum (-350 mbar) can be applied to these six units independently, creating multiple stretching patterns of the cell-laden membrane. The vacuum is applied to the single pressure-units for 1 h at 0.3 Hz per day for up to 7 days. A monoculture of human SFs or a co-culture of SFs and macrophages was performed. Cell viability was evaluated using a live/dead assay at day 7 and 14.

SFs were cultured in static conditions for up to 14 days without a noticeable effect on their viability. Next, a dynamic co-culture with macrophages was performed. A confluent layer of both cell types was observed after 1 day of culture. Results of the mechanical stimulations with a multi-modal pattern showed reversible cell rearrangement and deformation, indicating cell response to stimulation.

Our platform is the first microfluidic model to study the effect of mechanical actuation on the synovium. The next steps will include studying the responses of the macrophagefibroblast co-culture to induced inflammation and combining this module to our previously published cartilage-on-chip (Paggi et al., 2020) platform to recreate the joint.

References

Keywords
organ-on-chip; mechanical stimulation; co-culture; synovial fibroblasts; macrophages
To date, the education and training of veterinarians, human doctors and researchers is still largely based on the use of live animals. Only in the Netherlands, yearly more than 22,000 animals are used for training and research.

Virtual reality (VR) and Augmented reality (AR) offer unlimited and standardized specimens for dissection. Introduction of this new technology aims to decrease the number of animal and human specimens used for teaching and training.

By substituting animals and humans with dynamic holographic 3D models (Avatar models), we aim to study and acquire anatomic, physiologic, pathologic knowledge of specific systems in a comparative way, while not being restrained by ethical concerns and huge costs. We will study anatomical phenotypes, diseases and mimic operations without being restricted by time or availability.

The student/researcher will manipulate 3-dimensional anatomical structures up close, from all angles. In addition, dynamic exploration will be supplemented with rich and immediate automated feedback further boosting applicable knowledge, retention, and motivation. The doctor of the future will be equipped with the knowledge necessary for everyday clinical practice, while specialists will improve surgical skills and researchers will be able to deepen and share their knowledge even further. Our 3D-avatar learning concepts are based on grounded educational theories and stimulate active and embodied learning.

We present a working example of a 3-dimensional avatar rat, using Microsoft HoloLens for vivid holographic projection. The student is able to view the avatar from different angles and to show/hide anatomical structures (e.g. bones, muscles, organs, nerves) in an interactive way, using intuitive gestures and voice control. We aim to implement this model within the Dutch Laboratory Animal Science course programs as a replacement to rodent anatomy dissection sessions. Importantly we will investigate how effective the developed tools are in order to reach assigned learning goals.

References

Keywords
Augmented Reality; Replacement; Education
Stereotaxic neurosurgery in laboratory animals is a demanding technique used in a wide variety of studies in Neurosciences. Allowing to position one or more optical, electrical, or chemical probes, this approach remains indispensable for exploring brain functions. To date however, stereotaxic surgery is not taught as a subject per se, but rather passed-on behind closed door in research laboratories, resulting in a variety of practices and success rates. There is therefore a need to harmonize practices and enhance neuroscientists’ abilities to explore the brain in a valuable and reproducible way.

Here we introduce an animal-free training on stereotaxic neurosurgery. The teacher/trainee ratio is 1:3 and, as a pre-requisite, trainees validate an online course on elementary concepts such as aseptic techniques, anesthesia and pain management, peroperative animal care, incisions and sutures (Vogt et al, 2011). The course covers the theoretical background of stereotaxy and focuses on techniques and surgical approaches to optimize spatial precision, while minimizing the risks of irreversible harm to the animal. Anatomy and functional organization of the brain are reminded, with a peculiar attention to the 3-dimensional arrangement of brain blood vessels and ventricles. Hands-on practice includes exercises to acquire an ease in the manipulation of a stereotaxic frame, micropositioners, rulers and verniers. Instead of real animals, trainees use realistic high-resolution simulation devices to measure stereotaxic coordinates of cranial landmarks and entry points, and safely prepare the skull for the insertion and fixation of a probe. Exercises are repeated as needed and the accurate placement of a probe can be checked promptly without the need of histology. Tools and supports are made available for trainees to maintain their skills once back to their laboratory.

Really designed with the 3Rs’ principle in mind, this course should contribute to promote more reproducible and compassionate approaches in animal research in Neuroscience.

References

Keywords
Alternative methods; Training program; Continuing education; Stereotactic neurosurgery; Simulation
609 ENGINEERING 3D VASCULARIZED HEART TISSUES USING HPSC DERIVED CARDIAC CELLS

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The main function of the heart is to pump the blood through the body, via contraction of cardiomyocytes (CMs) that constitute the cardiac tissue (Voorhees et al., 2015). The contraction force produced by the cardiac tissue is a factor that determines the maturity of the CMs. A higher contraction force indicates a higher degree of maturation of CMs, which is more representative of a functional human heart.

Two-dimensional (2D) models do not have the tissue structure and organization of the human heart. In contrast, 3D models have the advantage of resembling tissue organization, function and cell-cell interaction.

The current gold standard 3D cardiac in vitro model, validated by different scientific groups, is the engineering heart tissues (EHT) (Weinberger et al., 2017).

We have successfully developed a medium-throughput platform that fits in a 12 well plate, where we can produce 36 EHTs using human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs). The contraction force of the 3D cardiac tissues is analyzed with a custom-made software that identifies the displacement of flexible pillars during tissue contraction. Such pillar displacement is converted to force using the Young’s modulus of the material. This software automatically goes through the stack of folders with the recordings of the EHTs and provides 2 graphs: force of contraction and contraction speed. For generating cardiac EHTs we used a fluorescent Double Reporter of mRubyII-α-Actinin and GFP-NKX2.5 (DRRAGN) in hPSCs, which allows live recording of sarcomere movement and alignment of cardiac cells.

Here we used this advanced EHT platform for functional analysis of cardiac maturation, by comparing different culture media, electrical stimulation and co-culture of cardiac cells (including hPSC-derived fibroblasts and endothelial cells). These improved culture conditions for cardiac maturation using this EHT platform will be important for a higher predictability regarding human cardiac disease modeling and drug screening.

References

Keywords
Engineered heart tissue; Cardiac maturation; Contraction force
Pathogenic enterobacteria comprise a challenging group of infectious agents that can spark intestinal inflammatory disease. These bacteria constitute a re-emerging global health threat, often showing resilience towards antibiotic therapy. To prevent the infection, it is essential to better understand the initial steps of bacteria interaction with the host. Our work focuses on Salmonella Typhimurium (S.Tm), and how this pathogen interacts with the intestinal epithelium, which it invades and uses as replication niche. In the past, cellular and molecular host-pathogen interactions have been studied in simplistic culture settings, using tumor-derived cell lines, which poorly recapitulate the architecture and physiology of the mammalian gut mucosa. More physiologically relevant infection studies usually require in vivo experimental models. To facilitate host-pathogen interaction studies ex vivo, we adapted the recent developments of crypt-derived organoid culture technology, combined with defined media formulations to modulate epithelium lineage-specific cell differentiation. We generated organoid-derived 2D monolayers atop a loose collagen hydrogel and combined this with state-of-the-art live microscopy to study the cellular response during the first minutes of bacterial invasion. Striking differences were observed between cultured cell lines and our primary monolayer model across all stages of S.Tm infection, e.g. differences in cell viability and bacterial intracellular replication. A particularly notable observation was a dynamic response of monolayers within 10-20 min post-infection, which propagated across more than a thousand cells within minutes, resulting in tissue-scale contraction. Using S.Tm mutants and knockout/transgenic mouse organoids, we could decipher the cell components that sense S.Tm invasion and trigger the dynamic response. Moreover, microinjection of S.Tm in 3D-organoids confirmed the physiological relevance of the dynamic response, not observed in tumor-derived cell lines. Our platform of technologies paves the way for physiological studies of pathogen-host interactions at the cellular level in an ex vivo context and highlights a dynamic epithelial tissue response to bacteria invasion.

References

Keywords
organoid; intestinal epithelium; Salmonella; host-pathogen interaction
627 INVESTIGATION OF ABSORPTION PATHWAYS OF SMALL PLASTIC PARTICLES USING IN VITRO MODELS OF THE HUMAN SMALL INTESTINE

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[Background] Microplastics (MPs) found in oceans are not only concerned in terms of their environmental effects, but also of possible toxicities in humans. It was reported that MPs were detected in human feces in 2018 (Liebmann et al., 2018). However, the detailed route of entry of MPs into the body and their toxicity on humans have not been elucidated well, necessitating further investigations.

[Method] Caco-2 cells were cultured alone or together with HT29-MTX-E12 for providing with mucosal layers on cell culture inserts. In addition, Raji B cells were cultured below the inserts for induction of M cells from the Caco-2 cells (tri-culture). The cells in culture were exposed to fluorescent-labeled polystyrene particles having a size of 50, 100, or 500 nm for up to 72 hours. Thereafter, the amounts of particles that penetrated in the lower part of the insert and their accumulation levels in the cells were measured. Penetration pathway was examined by immunostaining of various cellular markers and the fluorescence of the MPs.

[Results and Discussion] In Caco-2 cells cultured alone, 50 and 100 nm MP particles penetrated through tight junctions (ZO-1) and accumulated intracellularly, but 500 nm particles showed almost no penetration and accumulation. Additionally, Mucosal layers produced by HT29-MTX-E12 cells suppressed the penetration of all the MPs, confirming the inhibition of MPs ingestion into humans. Interestingly, 500 nm MP particles penetrated in the tri-culture system, showing the possibility of M cell-mediated ingesting of larger particles. Furthermore, We observed that MP particles were also taken up by Raji B cells below the insert, raising some concerns possible interactions of ingested MPs with the immune system.

[Conclusion] (1) MP particles (< 100 nm) penetrate through tight junctions; (2) Intestinal mucin layer inhibits the uptake of MPs; (3) MPs (50-500 nm) containing larger sizes penetrate through M cells

References

Keywords
Microplastics; Nanoplastics; in vitro model; M cells; Absorption pathway prediction
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The clinical development of advanced human cell therapies suffers from a lack of adequate preclinical testing in laboratory animals. The informative value of such (humanized) animal trials are limited due to their phylogenetic distance to humans and, especially, their lack of a human immune system. Due to the histocompatibility mismatch between laboratory animals and the patient, challenges increase significantly once personalized regulatory T cell (Treg) therapy approaches for the prevention of transplant rejection are under evaluation. Adoptive transfer of Tregs is a therapeutic option to reshape intra-tissue immune imbalance in transplant patients. It aims at supporting long-term function of allografts by overcoming the challenge of undesired immune reaction by the recipient.

Here, we used the HUMIMIC® multi-organ-chip platform to establish a next-generation human in vitro assay for predictive preclinical testing of Treg products. The platform enables co-culture of various human organ models but lacks blood micro-capillary vessel structures covered with human endothelial cells.

For this purpose, we implemented a network of miniature vascularized channels in the organ compartments of the HUMIMIC® platform for two-organ co-culture exploring 3D printing tools and endothelial self-assembly processes. The organ models and endothelial cells were generated from iPSCs of two different individual HLA-tested healthy persons emulating the recipient and the donor background. Finally, we aimed to qualify a HUMIMIC® based next-generation transplant rejection assay to evaluate both, safety and efficacy of Treg products in a universal repeated dose long-term assay environment. Multi-organ-chip design and prototyping results are presented along with the results of iPSC-based differentiation of human endothelial cells, liver equivalents and kidney models for the establishment of the interconnected two-organ model. Furthermore, we present data on on-chip micro-vessel formation and co-culture over prolonged culture periods. Results will be discussed in the light of the assay potential to replace respective animal transplant models in use.

References

Keywords
Testing chemical compound toxicity is a regulatory requirement, but guidelines typically require extensive testing on large number of animals for approval. Current OECD guidelines are based on traditional methods that require extensive animal experimentation. Organizations face a challenge as the procedure of testing toxicity is time consuming and costly. In addition to that, traditional in vivo toxicity testing is often based on fixed set of assumptions or uncertainties, which fail to assist in related regulatory and policy decisions. These methods often lack information on how a toxin might impact genes at the cellular level or its effect in other species. Currently, there is a growing interest in computational methods which can integrate and analyse complex datasets to assist decision making for toxicity testing. Integrating this information would allow generating insight which a single set of experiments might fail to identify. This can then be combined into current tests in regulatory requirements, which might aid in reducing, replacing and refining (3Rs) animal testing. This not only improves the process itself, but offers more transparency and validity. By knowing which genes and proteins are conserved across species, the effect of a chemical in a specific species can be noninvasively evaluated. This can facilitate understanding a species susceptibility to a specific class of toxins. Molecular pathways play an important role in how a specific chemical might affect an organism. Understanding pathway conservation across species (human vs alternative test species) forms the basis for developing reliable pre-screening strategies. This is especially useful for reducing redundancy in toxicity testing of chemicals with large number of analogues.

In this pilot study, we focus on molecular pathways involved in developmental and reproductive toxicity to gain understanding on its conservation across species, with a broader focus on developing methods to advance the 3Rs in toxicity testing.

References

Keywords
pathway conservation; orthology mapping; 3Rs; toxicity testing; pre-screening strategy
645 ZINC OXIDE NANOPARTICLES DISRUPT AUTOPHAGY AND INDUCE CYTOTOXICITY IN HUMAN KERATINOCYTES BY PERTURBATION OF NRF2 PATHWAY

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The assessment of toxicity pathways perturbation has been increasingly applied to the modern toxicity in the 21st century. Nrf2 pathway is recognized as the most important cellular antioxidant pathway mediating antioxidant genes and phase II detoxification enzymes. Evaluation of Nrf2 pathway has been recently adopted by OECD as a new method for in vitro skin sensitization testing (Test No. 442D). However, the specific role of Nrf2 pathway in the skin injury is still not fully understood. Zinc oxide nanoparticles (ZnONPs) are one of the important and widely used metal oxide nanoparticles. Due to its outstanding performance in blocking ultraviolet rays, nearly 80% of ZnONPs is used in cosmetics and sunscreens. This study was aimed to investigate the role of Nrf2 pathway in ZnONPs-induced toxicity in human keratinocyte HaCaT cells. Our results indicate that ZnONPs induce cytotoxicity, ROS accumulation and mitochondrial membrane potential loss in a concentration-dependent manner. ZnONPs increased the expression of Nrf2 and autophagy-related proteins such as P62 and LC3 II/I at 200 μM for 6 h. Meanwhile, Nrf2KD cells were vulnerable to ZnONPs-induced autophagy and cell damage. Antioxidant genes were decreased in Nrf2-KD cells induced by ZnONPs, while ROS and LC3 II/I was higher in Nrf2-KD cells than in Scramble cells. Autophagosomes and autolysosomes also increased markedly with ZnONPs treatment in Nrf2-KD cells. We further demonstrated that the inhibition of autophagy by pharmacological inhibitors 3-MA could ameliorate ZnONPs-induced cell death. These results suggest that ZnONPs induce the generation of ROS and mitochondrial dysfunction. Excessive ROS leads to activating of adaptive antioxidant response and autophagy regulating mediated by Nrf2 pathway, but overly autophagy accumulation results in cell death. ZnONPs disrupt autophagy by perturbation of Nrf2 pathway then induce toxicity in human keratinocytes.

References

Keywords
Nrf2 pathway; zinc oxide nanoparticles; autophagy; mitochondria; dermal toxicity
Currently it is necessary to establish alternative testing methods for product safety assessments. However, alternative methods for developmental toxicity tests have not been well developed because of the complicated toxicological responses. Zebrafish early embryos are non-protected animals and one of the promising models for screening of common birth defects owing to conservation between their developmental programs and those of mammals, rapid development and transparency. Although conserved endpoints are necessary for accurate prediction of effects in mammals, little is known about crossspecies conservation of teratogenic responses between mammals and fishes. We focused on cleft palate, one of the most frequent birth defects and one for which it is difficult to evaluate chemicals’ potential effects using cell culture assays. We investigated a conserved mechanism of cleft palate between mammals and fishes. Zebrafish embryos were exposed to 12 teratogens that induce cleft palate in mammals. Palatal morphology and number of proliferative and apoptotic palatal cells were examined at 96 hpf. By impacting tWnt signaling pathway chemically, we investigated the involvement of canonical Wnt signaling, a key contributor to genetically induced orofacial clefts. (Reynolds et al., 2019). All 12 teratogens induced palatal defects in zebrafish embryos, with decreased proliferation and increased apoptosis in the palate. Wnt signaling was inhibited in these zebrafish, and the aberrant phenotypes were rescued at the cellular and molecular levels by Wnt agonist treatment. We identified conserved responses to teratogens between mammals and zebrafish: palatal malformation and regulation of proliferation/apoptosis via the Wnt signaling pathway. Our results suggest that our zebrafish embryo assay would be a suitable model for assessing chemical-induced cleft palate as well as being a screening tool for prediction of cleft palate in mammals. We will confirm the conserved key endpoints by a comprehensive analysis as a next step for accurate prediction of teratogenicity in mammals.

References

Keywords
Developmental toxicity; Zebrafish; Alternative method; Teratogenicity; Cleft palate
Valproic acid (VPA) is a frequently prescribed antiepileptic drug. VPA may cause liver toxicity and steatosis through mitochondrial dysfunctioning. Nevertheless, the mechanisms underlying these adverse effects are incompletely understood. We and others have previously studied the effect of VPA on gene expression and DNA methylation profiles in primary human hepatocytes (PHH). In this study, we aimed to link changes in gene expression to mitochondrial dysfunctioning by performing an RNA interference (RNAi) screening strategy. First, we predicted which transcription factors could explain the VPA-induced expression profiles. We identified 18 TFs that were constitutively activated during 3 days of VPA exposure. Next, we knocked down the expression of 16/18 TFs using lentiviral-based shRNAs, and determined their effect on mitochondrial dysfunctioning by measuring determine oxygen consumption rates (OCR). We found a dose-dependent decrease in OCR in PHH after 24, 48 and 72 hours of repetitive VPA administration. There was no acute effect of VPA administration on OCR, indicating the need for prolonged exposure. Knockdown of several TFs modified the response of PHH to VPA treatment after 72 hours. CEBPA was one of the prominent TFs of which the expression affected the cellular response to VPA. CEBPA knockdown increased basal and maximal respiration rates in PHH upon VPA exposure compared to control shRNAs. In order to identify CEBPA-dependent gene expression, RNA-seq was performed on CEBPA knockdown and control cells. Out of the 27 transcriptional targets initially used to identify CEBPA activation, we could confirm 23. Four of the previously identified transcriptional targets of CEBPA did not respond to CEBPA depletion, suggesting that these targets are not regulated by CEBPA in the cellular context of the PHH we used. Altogether, our study demonstrates that VPA-induced changes in gene expression can be causally linked to mitochondrial dysfunctioning in PHH.

References

Keywords
Valproic acid; RNAseq; RNAi; Mitochondrial dysfunction
To support the evaluation of non-animal approaches for skin sensitisation assessment, we collected data for over 2500 human predictive patch tests (HPPTs; human maximisation test and human repeated insult patch test) from more than 1500 publications. Each test was evaluated for reliability. Results from over 1900 tests considered to be sufficiently reliable were further analysed to better understand strengths and limitations of HPPT data and develop a strategy for using them to classify chemicals for their skin sensitisation potential under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). HPPTs are performed with single doses making identification of thresholds uncertain and difficult to use e.g. as reference data for test method validation. To overcome this challenge, classification criteria from the GHS were extended using a decision tree to partly resolve ambiguity in the results. If an individual chemical in the database had multiple discordant test results, a weight-of-evidence approach was used to obtain a single classification for the chemical. This classification approach was applied to a reference list of substances to support the evaluation of defined approaches (DAs) for skin sensitisation proposed for inclusion in a new OECD guideline. Classifications were compared with those based on LLNA data, and the predictivity of the evaluated DAs vs. HPPT and LLNA was characterised. The results of this exercise are presented alongside some learnings about limitations of HPPT data and deficiencies in the current GHS approach. The entire human skin sensitisation patch test database was made publicly available earlier this year and may assist in future evaluations of alternative skin sensitisation methods and development of new models. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C. The views expressed above do not necessarily represent the official positions of any federal agency.

References

Keywords
Skin sensitisation; Defined approaches; Human data; Classification and Labelling; Validation
Skin sensitization, one of three the biocompatibility endpoints required for all medical devices (MD), is still based on in vivo approaches (ISO 10993-10). The need for nonanimal methods relies not only on ethical reasons but also on scientific reasons as the accuracy of the commonly used animal tests (GPMT, Buehler test, LLNA) barely reach 70% compared to human data. In 2012, OECD proposed an Adverse Outcome Pathway (AOP) for skin sensitization and several in chemico and in vitro assays have been validated to be used in testing strategies. However, these OECD assays concern neat chemicals and use mainly cell lines cultivated in 2D which are not easily adapted to medical devices extracts where potential leached sensitizers are diluted in polar and nonpolar solvents. The recent validation of in vitro skin irritation methods for medical device extracts demonstrated the added value of reconstructed human models (RhE). For skin sensitization, assays with RhE alone (Petry 2018, Andres 2017, Cotterez 2018, Coleman 2015) or in co-culture with 2D cells (Schellenberger et al. 2019) showed promising results for complex test systems. Skin sensitization is under discussion by the experts of the ISOTC194/WG8 to evaluate how it would be possible to adapt and validate the testing strategies proposed for pure chemicals to the specific context of MD. Unlike the situation of skin irritation, the existence of quantitative data for skin sensitization generated from human (NOAEL) or animal data (EC3) will facilitate production of reference test samples to robustly and comprehensively evaluate adaptation of OECD methods to the context of medical devices products. This presentation will give an overview of different in vitro approaches for skin sensitization and will present the last results in medical devices context with methods based onto 3D models as test system.

References

Keywords
skin sensitization; ; medical devices; ; reconstructed human epidermis; ; RhE; co-culture
For more than twenty years EPISKIN has been an active player in the development of alternative methods, whether by providing experimental systems based on reconstructed human 3D tissues, by developing non-animal methods (NAMs) or by supporting the implementation of these methods in industrial, academic and CRO laboratories. Validation of alternative methods and regulatory evolutions in many countries result in an increasing demand for support and training. In line with our commitment for dissemination of non-animal methods (NAMs), we created EPISKIN Academy in 2012 to (a) facilitate the deployment and acceptance of validated alternative methods and (b) prepare new generations of scientists and toxicologists to use these methods and to participate in the development of future ones.

In order to respond to the diversity of needs we have developed a modular program ranging from a short awareness and demonstration of these methods to full theoretical and practical laboratory training leading to certification. Our presence on 3 continents has enabled us to build long-term partnerships with various public, academic and governmental actors in several countries. These collaborative approaches in education are best suited to reach the right audience and provide holistic solutions in which trainees can not only receive hands-on training in methods but also acquire the scientific and regulatory knowledge essential to the success of these approaches. In 8 years, EPISKIN Academy has trained several hundred students, scientists and toxicologists from public and private organizations. 280 have been certified on OECD methods of corrosion and skin and eye irritation (TG431, TG439, TG 492). EPISKIN Academy is committed to accompany today challenges and to prepare the future of alternative methods in toxicology by engaging, whenever possible, long-term partnerships with institutional partners.

References

Keywords
alternative to animal methods; training; Toxicology; public-private partnerships; certification
Besides ethics and science, attitude and behaviour are key components in 3R development. Researchers, as main users of research animals and animal free models, have opportunities and responsibilities to contribute to 3R development. Students is an important target group for 3R education as next generation of scientists and specialists in academia, authorities and private industry.

We have been following students in the Global Master’s Programme in Toxicology at Karolinska Institutet, in which the 3Rs are integrated into all courses. Students’ attitudes were compared with researchers at Karolinska Institutet in a 3R-survey using the response alternatives Strongly disagree, Disagree, Neither/Nor, Agree, Strongly Agree. Respondents were divided into three groups; students, junior researchers and senior researchers (N=44, 63, and 44, respectively).

When asked about usefulness of the 3Rs when defining research question and reporting data, the majority agreed or strongly agreed (69-71%, no differences between the groups). All three groups agreed to a higher extent (79-100%) to usefulness related to animal handling, housing and euthanasia, students even more than the researchers (95 to 100%, p< 0.05), indicating high engagement in Refinement. The vast majority in all groups agreed that stressed animals yield less valid results (93, 95, 91%). Students agreed that 3R results in increased scientific quality more than junior and senior researchers, respectively (Replacement 55, 23, and 28%; Reduction 52, 24, and 28%; Refinement 93, 79, and 72%, p<0.05).

In general, all respondents showed an equal positive attitude towards the 3Rs, especially Refinement. Observed differences indicate that students in some cases are more positive. This might be due to differences in responsibilities/experience, and that younger generations have more recent and updated knowledge in animal ethics and behaviour and higher confidence in computer and cell models. This will be further explored e.g. by filming the students learning in a documentary during 2020.

References

Keywords
3R survey; 3R attitudes; 3R awareness; 3R education; documentary film
680 TOWARDS A REGULATORY APPLICATION OF CACO-2 ADVANCED INTESTINAL BARRIER MODEL

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It is a matter of fact that advanced in vitro models should be complex as required to reflect the in vivo situation but simple and defined enough to be reproducible and transferable. This implies that a careful definition of model parameters and/or experimental conditions is critical for their applicability to toxicological/pharmacological studies.

Tri-culture model of intestinal barrier of Caco-2 cells co-cultured with different intestinal cells types as mucosecretory cells (HT29-MTX) and lymphoblastoid cells (Raji B) was extensively utilized in evaluation of chemicals and nanomaterials (NMs) absorption. However, it still shows quite variability, which invalidate its wider use as in vitro model of intestinal barrier.

A pilot study to standardize and optimize Caco-2 tri-culture model is running at ISS, in the perspective of its application to NMs translocation studies. The study is finalized to develop an OECD Guidance Document on the definition of an in vitro approach for gastrointestinal fate of ingested NMs.

Several model parameters were considered as insert pore dimension, microfold cell (M cell) induction and characterization, mucus production. Different endpoints of M cell phenotype induction on Caco-2/Raji B co-culture, such as transepithelial resistance (TEER) decrease, ZO-1 protein expression, and barrier permeability to FITC-dextran and fluorescent silica nanoparticles were evaluated. Mucus influence on silica nanoparticles absorption was also investigated.

Furthermore, to determine if a direct contact between the two cell types can better stimulate M cell induction, an inverted co-culture model was developed in which Caco-2 cells facing the basolateral compartment and Raji B cells were added to the apical compartment. Both models, normal and inverted, were able to induce M cells transformation in Caco-2, but the latter seems to be more performant.

As a whole, study results furnish a relevant contribute to move this three-culture model versus regulatory context.

Supported by Italian Ministry of Health, section 4145 REACH

References

Keywords
Intestinal barrier; Advanced in vitro model; Regulatory application; Nanoparticles absorption
A variety of human skin models have been developed for applications in in vitro studies. Typically, these reconstructed skin models employ protein-based scaffolds along with human skin cells: fibroblasts and keratinocytes. A key limitation of these models is that they still fail in recapitulating the cellular and microenvironmental complexity, such as the presence of vasculature, multiple cell types (e.g., melanocytes, neural and immune skin cell) and adnexal structures (e.g., hair follicles, sebaceous and sweat glands), that are representative of human physiology. In parallel, 3D bioprinting technology has been gaining attention as a platform for tissue engineering given the possibility for precise cell positioning, flexibility, reproducibility, and high-throughput production. We have explored these advantages for the development of two models of the human hair follicle. For the reconstructed skin model, a bioink containing dermal papilla cell (DPCs) and human umbilical vein cells (HUVECs) was precisely printed within the gelled dermis. These cells formed spheroids inside the dermis which were enveloped by keratinocytes and melanocytes migrating from the epidermis through the vertical opening left by the nozzle. The resulting model contained a hair follicle-like structures whose morphology and biomarker pattern mimics that of the native tissue. Additionally, we have developed a hair follicle spheroid model formed by a core of DPCs and HUVECs (step 1) enveloped by a sheath of epithelial cells (step 2). The resulting spheroid, generated in an automated, precise and reproducible manner by 3D bioprinting, also resembled the structure of the native hair follicle and could potentially be used for high-throughput screening of substances. The development of reconstructed skin models with increased complexity that better mimic the native tissue can have an important impact on the diversification of in vitro models available for safety and efficacy assessment of chemical compounds.

References
A DYNAMIC PERFUSION BASED BLOOD- BRAIN BARRIER MODEL FOR CYTOTOXICITY TESTING AND DRUG PERMEATION

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The blood-brain barrier (BBB) serves to protect and regulate the CNS microenvironment. The development of an in-vitro mimic of the BBB requires recapitulating the correct phenotype of the in-vivo BBB, particularly for drug permeation studies. However, the majority of widely used BBB models demonstrate low transendothelial electrical resistance (TEER) and poor BBB phenotype. The application of shear stress is known to enhance tight junction formation and hence improve the barrier function. We utilised a high TEER primary porcine brain microvascular endothelial cell (pBMEC) culture to assess the impact of shear stress on barrier formation using the Kirkstall QuasiVivo 600 (QV600) multi-chamber perfusion system. The application of shear stress resulted in a reorientation and enhancement of tight junction formation on both coverslip and permeable inserts, in addition to enhancing and maintaining TEER for longer when compared to static conditions. Furthermore, the functional consequences of this was demonstrated with the reduction in flux of mitoxantrone across PBMEC monolayers. The QV600 perfusion system may serve as a viable tool to enhance and maintain the high TEER pBMEC system for use in in-vitro BBB models.

References

Keywords
blood brain barrier; shear stress; Primary porcine endothelial cells; perfusion
A considerable portion of the biomedical industry is focused on materials for wound healing. The requirements these materials need to meet are not trivial: biocompatibility, anti-bacterial activity, anti-oxidant effects, anti-inflammatory efficacy. All of these are required during the in vitro characterizations and during the subsequent analysis.

Some of these qualities are present in naturally available protein-based materials (Pignatelli et al., 2018) (silk fibroin, wool keratin, zein from corn), and polysaccharides (i.e. alginate from brown algae, hyaluronic acid) (Contardi et al. 2019, Setti et al., 2018). To these matrices we added natural active compounds (i.e. essential oils, fruit extracts) (Contardi et al., 2019) during the design of biocomposite materials as advanced patches for an efficient wound closure. In addition, we recently developed an innovative allmycelium-based substrate, a promising and natural platform for patches design.

Primarily human dermal fibroblast, human epidermal keratinocytes and human red blood cell were used during the following in vitro test: biocompatibility essays (MTT, CellTiterGlo), analysis of the cellular anti-oxidant and inflammatory processes, and assessment of the emolysis action. Particular attention was devoted to the evaluation of cell growth and substrate adhesion through confocal imaging (actin, nuclei, focal adhesion) and SEM analysis. These morphological studies constitute useful criterion for the performance of specific material-architecture combination.

In parallel, anti-bacterial properties of our materials were tested with inhibition area test and bacterial colonies counting; gram-negative E.Coli cultures were used to this end. Finally, we are currently setting up an in vitro wound healing model based on scratch test to evaluate the efficacy our materials extracts to promote cell migration toward the lesion margin and accelerate its closure.

Taken together we believe this series of tests can constitutes a complete and all rounded protocol for the assessment of the upcoming new generation of advanced materials for the wound caring management.

References

Keywords
biocompatibility; anti-bacterial activity ; anti-oxidant effects; anti-inflammatory efficacy; emolysis
701 USE OF THE FULL THICKNESS MODEL, T-SKIN™, TO INVESTIGATE THE EFFECTS OF UVA AND UVB ON SKIN AND PHOTOPROTECTIVE EFFECTS OF VITAMIN C

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The skin represents a major barrier to penetration of chemicals into the systemic circulation. It is also subject to damaging effects from environmental factors such as UV radiations. The safety of topically applied pharmaceuticals and cosmetics, as well as topically exposed environmental chemicals, requires a robust and predictive skin model. T-Skin™ is a reconstructed full-thickness model with a well stratified, differentiated and self-renewing epidermis and a dermal compartment composed of functional fibroblasts embedded in a matrix of collagen I (1). We have used this 3D model as a reproducible, invivo-like, and predictive human skin model to (a) characterize the effects of UVA1 and Solar Sun radiation UVA1+UVB (UVSSR) exposure on skin tissue and (b) to investigate the protective effects of vitamin C against UVA1 radiation damage. Endpoints measured were tissue viability, histology, fibroblast number and cytokine and chemokine release. Classical photoageing responses of human skin due to UVA1 exposure (Biological Efficient Dose) were observed i.e., a viability decrease mainly located in the dermis associated with a loss of a dermal fibroblasts disappearance, a metalloproteinase-1 proinflammatory mediators releases. UVSSR caused damages to both epidermis and dermis parts, and a more extensive release of inflammatory mediators than UVA1 exposure. Vitamin C protected against UVA1 induced damages, evident at level of dermal damage (preventing a loss of viability and the number of fibroblasts in the dermis and the attenuation of metalloproteinase-1 release), as well reducing the release of IL-1α and IL-8. This study shows that the T-Skin™ model mimics the main responses to UV radiation and supports its use as screening tool to develop new UVA1-protective ingredients.

References

Keywords
full thickness; skin model; UV effects; dermis; cytokine
One of the great challenges of periodontal and peri-implant surgery is the need to gain the volume of attached gingiva and connective tissue. The success depends on an adequate supply of connective tissue, and the presence of active cells on the replacement and recovery of injured or lost tissue. Platelet-rich fibrin (PRF) is a fibrin matrix obtained by centrifuging autologous blood (Miron, 2017) and this is rich in cytokines, growth factors (Saluja et al, 2011; Duregger et al, 2018), in addition to clustered cells to be released, serving as an absorbable membrane in procedures of Guided Bone Regeneration (GBR). It also becomes a tissue healing stimulator, once it promotes angiogenesis and cell proliferation on Guided Tissue Regeneration (GTR) methods (Fan et al, 2020). In this work, we aim to use PRF as a healing agent in connective tissue grafts while serving as scaffold for the transport of gingival fibroblasts cultivated by humanized technique. Autologous oral fragment is removed to obtain the desired cells (fibroblasts) using explant technique. The humanized culture medium consists of: DMEM, 5% human platelet lysate, 1% antibiotic/antimycotic and 1% L-glutamine. PRF preparation: autologous venous blood taken in a dry tube is centrifuged at 2700 rpm for 12 minutes. After centrifugation whole blood became separated to red blood cells and supernatant. PRF is formed from the supernatant due to natural clotting. The serum is removed and the PRF membrane is pulled off by sterile forceps from the inner side of the tube. The PRF membrane was used immediately after preparations to seed fibroblasts and cultivation. Our results showed us the most effective method of using PRF membranes to obtain a greater quantity of viable cells with proliferation capacity. Thus, we can state that PRF membranes can be used as scaffold for tissue regeneration in periodontal and peri-implant surgery.

References

Keywords
PRF (Platelet-Rich Fibrin); Fibroblasts; Humanized Culture; Tissue regeneration; Scaffold
708 EXTENSIONS OF THE TOXTRACKER ASSAY FOR MECHANISTIC INSIGHT INTO THE MODE OF ACTION OF GENOTOXIC COMPOUNDS

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ToxTracker is a mammalian stem cell-based reporter assay that detects activation of specific cellular signalling pathways upon chemical exposure. ToxTracker contains six different GFP-tagged reporter cell lines that together can detect the induction of DNA damage, oxidative stress and/or protein damage in a single test. Genotoxic agents activated the Bscl2-GFP reporter, activated by promutagenic DNA lesions and subsequent DNA replication stress, and/or the Rtkn-GFP reporter, which is activated in response to DNA double strand breaks.

High levels of oxidative damage caused by the production of reactive oxygen species (ROS) can indirectly lead to genotoxicity. To assess whether the genotoxicity observed in ToxTracker is direct or indirect, ToxTracker was performed in the presence of two ROS scavengers. If the observed genotoxicity is indirect, genotoxicity reporter activation should be abrogated in the presence of the ROS scavengers.

Both directly DNA reactive clastogenic agents and indirectly genotoxic aneugenic agents both activate the Rtkn-GFP and/or the Bscl2-GFP reporter in ToxTracker. To better discriminate these types of agents, we included cell cycle and aneuploidy analysis in the ToxTracker assay. Aneugenic agents arrested the cells in G2/M phase after 4h of exposure and caused an increase in the number of aneuploid (>4n DNA content) cells after 24h of exposure, while clastogenic and non-genotoxic agents did not.

Exposure to aneugenic agents gives rise to an abnormal number of chromosomes. Broadly, aneugenic agents can be divided into two main categories: microtubule disrupting agents and kinase inhibitors that affect mitotic progression. To gain more insight into the mode of action of microtubule disrupting agents, we created a GFPtubulin assay. In this assay, microtubule stability as well as cell cycle progression are assessed. Both microtubule stabilising and destabilising agents could be detected.

References

Keywords
ToxTracker; Genotoxicity; Aneugens; Oxidative stress; Mode of action
General batch safety tests for veterinary vaccines as the Laboratory Animal Batch Safety Test (LABST) or the Target Animal Batch Safety Test (TABST) are supposed to demonstrate that a vaccine does not cause abnormal local or systemic reactions. They have been introduced decades ago during the development of the first veterinary vaccines. However, over the last 25 years, the relevance of the TABST and LABST was questioned due to the introduction of more specific safety tests, strict control of starting material and the introduction of Good Manufacturing Practice. Retrospective analysis of LABST and TABST data revealed that the two tests are no longer relevant and not able to detect problematic batches. The LABST (or abnormal toxicity test) has been removed from European Pharmacopoeia monographs for veterinary vaccines in 1997, and the TABST in a stepwise approach until its complete deletion in 2013.

In 2008, Europe proposed to The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) to aim at harmonisation of general batch safety tests across the VICH regions (USA, Japan, Europe) in order to minimise the need to perform separate studies for regulatory authorities of different countries. However, due to the great divergence in requirements between the regions it was concluded to adopt a phased approach with the first step to harmonise the criteria on data requirements for waiving of the TABST for inactivated vaccines in regions where it is required, and the respective VICH GL50 came into force in 2014. A comparable guideline for live vaccines (GL55) is available since 2018 and VICH GL59 on waiving criteria for the LABST is under public consultation (end in April 2020).

References

Keywords
Veterinary vaccines; general batch safety test; international harmonisation
712 THE HET-MN (HEN’S EGG TEST FOR MICRONUCLEUS INDUCTION): AN IN VITRO ASSAY REFLECTING SYSTEMIC AVAILABILITY OF TEST COMPOUNDS.

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Genotoxicity hazard identification across different sector industries starts with in vitro testing. These assays are based on two-dimensional cell cultures, which are limited in reflecting routes of exposure and intrinsic metabolic capacity. To address these aspects, 3D test systems were introduced into in vitro genotoxicity testing and combined with established read-out parameters to apply them as follow up solutions to positive findings from standard testing under regulations that prohibit in vivo testing as detailed in the Cosmetics Europe Genotoxicity Program.

Here, we report on the HET-MN, which reflects the systemic availability of compounds and allows the analysis of micronuclei in erythrocytes of the developing chicken eggs. It considers important toxicokinetic aspects: compounds are applied through a small hole in the eggshell onto the egg membrane. After compounds passed the egg membrane, they are taken up by the vascularized chorioallantoic membrane prior to their distribution via the blood vessel system. The metabolism of compounds occurs via enzymes present in the yolk sac membrane and the developing liver before metabolites are excreted into the allantois, a bladder equivalent.

A validation exercise in which > 30 compounds were tested double-blinded in three laboratories showed a good specificity of > 90% and sensitivity of > 80%. The chemicals covered a balanced data set of true positives (e.g., 2-aminoanthracene, taxol), true negatives (e.g., cyclohexanone) and misleading positives (e.g., resorcinol), representing different chemical classes and modes of actions.

In summary, the HET-MN combines the advantages of in vitro approaches with the ability to mirror the systemic availability normally intrinsic to in vivo experiments, while being in accordance with animal protection regulations and ethical aspects, which makes the assays an excellent tool to supplement existing in vitro genotoxicity toolboxes.

The work was funded by the German Ministry for Research and Education as well as by Cosmetics Europe.

References

Keywords
genotoxicity; systemic availability; ADME; micronucleus
In 2016 the Netherlands National Committee for the protection of animals used for scientific purposes published their recommendation "Transition to non-animal research - on opportunities for the phasing out of animal procedures and the stimulation of innovation without laboratory animals". One of the ideas to accelerate the transition to animal-free research is to create target images on animal-free research. These describe clear transition objectives for each research domain aimed at reducing the use of laboratory animals with equal or better research quality.

Target images are drawn up from a dialogue with and between the various social groups, including patient and animal interest organizations, and the scientific field, including transition / innovation experts. This must result in transition goals for the next 10 years that are both ambitious and realistic. It requires a well-considered approach that starts with an analysis of the most important tasks in the relevant research domain, and the opportunities and possibilities for non-animal innovation. In addition, knowledge from other (research) disciplines is used. A description of the requirements for achieving these transition goals forms part of the objective.

A target image for the complex domain of fundamental scientific brain research has been delivered already and the creation of target images for the domain of cardio-vascular research and for the broad field of education is ongoing. Lessons learned along the way are taken into account when developing new target images. The professional group concerned has to be owner of the target image. The Netherlands National Committee stimulates, supports and monitors the creation and implementation of these target images outlines.

References

Keywords
target images; transition; animal-free research
Artificial organs for circulatory support are lifesaving systems that support or replace the function of failing organs in critically ill patients. While these devices enable complex lifesaving interventions, increasing evidence implicates sub-optimal system design is likely to cause blood damage (not identified in earlier pre-clinical tests). Development processes of artificial organs are limited by international standards that recommend the use of bovine blood for predicting success of human life-support systems. Thus, we aimed to compare the suitability of human and bovine blood for artificial organ haemocompatibility testing.

Human blood was sourced, tested, and compared to data obtained from previous bovine blood studies for haematological and rheological parameters, including specific assessment for mechanical sensitivity of blood cells and high-shear tolerance.

Haematological assessment identified that when compared with bovine blood, human blood contains: ~25% more blood cells·µL⁻¹; erythrocytes with ~25% larger diameter, ~50% larger volume and surface area, and 10-15% less cytosolic haemoglobin (implicating substantially decreased cytosolic viscosity). Rheological profiles were also identified to be drastically different; human plasma is approximately half the viscosity of bovine plasma; however, as bovine erythrocytes do not aggregate, low-shear whole blood viscosity is markedly increased in human blood. Further, while human erythrocytes are substantially more deformable than bovine, human erythrocytes exhibit far greater susceptibility to shear-induced damage (i.e., half the strength of bovine blood).

Due to inherent biological differences that exist between human and bovine blood, it is likely that current bovine recommendations have resulted in the development of nonrepresentative models of blood-device compatibility. To improve the outcomes and quality of life of patients receiving artificial organ therapies, future devices must be designed, tested, and optimised for humans; bovine blood is a poor model of human tissue and should not be used as a surrogate.

References

Keywords
artificial organ development; haemorheology; haematology; haemocompatibility ; comparative biology
In contrast to animal models, human brain organoids replicate the architecture and neuronal composition of the human brain, which is useful for the study of human brain development and disease. However, current methods for human brain organoid culture are low-throughput and not suitable for long-term high-resolution imaging, which is required to study developmental processes and disease progression within physiologically relevant time frames (i.e. days, weeks, months). Here we demonstrate the utility of 3D-printed microplate inserts, which enable the scaling up of brain organoid culture and the growth of brain organoids in pre-defined XYZ coordinates. Together, these innovations facilitate high-resolution and high-throughput imaging of brain organoids over long periods of time (up to 2 months). We show that brain organoids grown using 3D-printed microplate inserts do not significantly differ in terms of gene expression, tissue architecture, and growth rates from brain organoids obtained using standard protocols. Finally, we applied this technology to visualize the growth of patient-derived glioblastoma stem cells as tumours within healthy brain organoids. Overall, this new bioengineering platform constitutes a significant advance that permits high-throughput studies of several brain diseases using organoids and high-content phenotypic imaging, thereby replacing the use of animal models in brain cancer research.

FUNDING: This work was supported by grants from the National Health and Medical Research Council of Australia (NHMRC); the Cure Brain Cancer Foundation; the University of South Australia; the Neurosurgical Research Foundation; the Cancer Council SA Beat Cancer Project; the Beat Cancer Project and Health Services Charitable Gifts Board, the Australian Research Council (ARC), a Premier’s Research and Industry Fund grant provided by the South Australian Government Department for Innovation and Skills and the Medical Advances Without Animals trust (MAWA).

References

Keywords
Brain organoids; high-throughput; screening; live-imaging; high-content microscopy
There is a growing number of applications for various engineered nanoparticles (ENP), however, their impact on human health and various organs is poorly understood. In this study we utilized a 3D reconstructed human intestinal microtissues, EpiIntestinal tissue model, to develop an in vitro system for assessment of toxicological profiles of ingested nanomaterials. The tissues were exposed to various concentrations of three types of nanoparticles: copper (II) oxide (CuO) (50 nm in size), zinc oxide (ZnO, 35-45 nm in size), and titanium oxide (TiO2, 40 nm in size). Sonicated nanoparticles were resuspended in 40 μl of buffer and applied apically onto the tissues for 4 hours. Following application, the tissues were washed and incubated for additional 24 hours in standard medium. Subsequently the barrier integrity (TEER) and viability (MTT) were determined for each tissue. In addition, medium was collected to determine levels of selected proinflammatory cytokines released following the nanoparticle exposure. Using IC15 (concentration of nanoparticles that reduces barrier function or tissue viability by 15%) as a cut off, we observed a dose response reduction of barrier integrity and tissue viability for CuO and ZnO. On the other hand, the titanium oxide did not induce toxic effects even at the highest concentration. Similar observation was detected through the cytokine production — we have seen a dose-dependent increase of interleukin 8 (IL-8) in tissues exposed to CuO and ZnO. Overall, we have shown that the TEER measurement is very reproducible and more sensitive endpoint than MTT. In conclusion, these results suggest that reconstructed small intestine tissues might become a sensitive tool not only for determining the toxicity of ingested nanoparticles, but also for further studying interactions of nanoparticles with host gastrointestinal system.

References

Keywords
EpiIntestinal tissue model; Nanoparticles; Barrier integrity ; Tissue viability; gastrointestinal system
THERMOPLASTIC POLYURETHANE FILMS AS BIOMATERIAL – PHYSICAL CHEMICAL AND BIOLOGICAL STUDY OF THE MODIFICATION CAUSED BY IONIZING RADIATION

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New materials are being studied and widely applied in the health area, highlighting biocompatible polymers as the most versatile. Among these polymers, we developed the methodology for the manufacture of thermoplastic polyurethane films for application as biomaterials, replacing possible devices from material of animal origin. The sterilization proposed by ionizing radiation requires the study and characterization of the material to assess possible losses or modifications, due to the influence that radiation can cause on the polymer chains, losing the characteristics for the purpose used (Abuj, 1996). Therefore, the present work evaluates, by chemical and physical-chemical characterization, the possible extension of the alterations caused by the ionizing radiation in the polyurethane film. The material is produced in an environment with controlled temperature and humidity and subject to increasing doses of gamma radiation (15, 25 and 50 kGy), ethylene oxide and plasma as comparative techniques. The techniques DSC (Differential Scanning Calorimetry), TGA (Thermogravimetry), traction test, RDX (X-Ray Diffraction) and VEM (Scanning Electron Microscopy), proved that the material, after application of sterilization techniques, maintains its physical – chemical characteristics and does not change after treatment. While the techniques used to verify the Biocompatibility of the material such as Cytotoxicity, Adhesion and Proliferation define the best method for sterilizing the polymeric film.

References

Keywords
Biomaterials; Thermoplastic; Ionizing Radiation; Biocompatibility; Caracterization
Mineral (glass and stone) wool is one of the most used insulation material, due to its outstanding effectiveness, but also because of extensive and robust studies supporting the fact that they are safe to use when standard safe work practices are followed. Specific protocols to characterize fibre biopersistence have been defined by European authorities [Bernstein et al., 1999]. Tested according to one of these protocols, the fibres have to demonstrate a low biopersistence to not be classified as carcinogenic under the EU CLP Regulation (Note Q, Regulation (EC) n°1272/2008). Mineral wool manufacturers, such as Saint-Gobain, need to reduce as much as possible the number of these in-vivo tests, as they raise ethical issues and are costly and time-consuming. Thus, the development of in-vitro tests reliable, quicker, cheaper and predictive of in-vivo biopersistence is required.

EURIMA (EUropean Insulation Manufacturers Association) [Sebastian et al., 2002] aims to develop an acellular in-vitro test, in which fibres dissolution in a flow-through system is followed by chemical analysis of the solutions. Saint-Gobain has a long experience on dissolution tests [de Meringo et al., 1994] [Thelohan et al., 1994] [Guldberg et al., 2000] and has decided to develop an acellular in-vitro test in a different way, with a closed system in which the dissolution fluid is agitated but not circulating.

In this work, the Saint-Gobain in-vitro test is presented; the parameters impacting glass wool and stone wool dissolution, such as the fluid composition and pH, are studied; and differences between “circulating” and “agitated” in-vitro dissolution tests are discussed. The “agitated” fluid allows to reduce the test duration and to maintain a constant pH during experiment, which increases the reproducibility. This test is still under development as it also displays limits. Further efforts will be needed to obtain a robust predictive tool.

References

Keywords
mineral wool; dissolution; bio-persistence; fiber; in-vitro test
INCREASING THE RELIABILITY OF PRECLINICAL DATA: ENABLING APPROACHES

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Data generated in nonclinical research studies drive decisions with long-term consequences. Concerns about the extent to which these data can be relied upon, initially raised in evidence-based scientific articles, have become part of a worldwide conversation on what is known as the ‘reproducibility crisis’. In neuroscience research, for example, systematic reviews, meta-analyses and multicenter studies continue to demonstrate that multiple sources of bias and differences in practices can affect data and conclusions, and ultimately decision-making. While the debate continues to unfold, initiatives aiming to reduce the risks of bias and to increase the reliability of preclinical data are being developed. Reporting guidelines and best practice recommendations have made important contributions, but there is still a need for enabling approaches. A common framework, with comprehensive evidence-based guidelines and practical tools would help scientists ask a scientific question, appropriately design experiments to answer the question, and generate and share data that they and others will be able to rely upon.

References

Keywords
National Platforms for alternative methods were created about twenty years ago with the aim to promote and inform about alternative methods to speed up their regulatory acceptance. To achieve these goals effectively and incisively, collaboration between the following four areas was considered as a priority: Research - Industry - Government Institutions - Associations for animal protection.

The Italian Platform on Alternative Methods (IPAM), in full compliance with this approach, pursues at: i) promoting research and information on alternative methods in animal experimentation in Italy, ii) building synergies to accelerate development and acceptance of alternatives methods in basic, applied, and regulatory research. Within this framework, IPAM regularly organises national and international events and meetings (Rovida et al., 2013; Nagy et al., 2016; Caloni et al., 2018) aimed at different stakeholders (students, researchers, general public). Among the most recent, the exhibition “Science & Consciousness, a journey inside the 3Rs” a didactic itinerary on alternatives in animal experimentation which was hosted by several Italian universities and research institutes, and the IPAM-ecopa symposium 2019 on “Non Animal Methodologies (NAMs): research, testing, assessment and applications”, recently coorganised with ecopa (European consensus-platform for alternatives) (Lorenzetti et al., 2020).

Moreover, since 2007, the IPAM-Farmindustria award is assigned every two years to a young researcher author of a paper and/or a thesis degree relevant to the application of 3Rs Principle in pharmacological research. IPAM also actively dialogue with national and international regulatory bodies and its members frequently share their expertise in training events organized by university, industries, and public entities.

Finally, the IPAM’s website (www.ipamitalia.org) and Facebook social (www.facebook.com/IPAMITALIA) represent an important point of reference of information, updates, and discussion for anyone who is interested on the 3R Principle, alternative methods and their applications in science.

References


Keywords
3R Principle; National Platform; Alternative Methods; Training and education
770 A HUMAN HEPATOCYTE-LIKE CELL BASED IN VITRO MODEL FOR HEPATIC INSULIN-DRIVEN DE NOVO LIPOGENESIS

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Steatosis, marked by increased intra hepatic triglyceride accumulation, is a hallmark of non-alcoholic fatty liver disease (NAFLD) and precedes the progression to non-alcoholic steatohepatitis (NASH) and liver fibrosis. Hepatic de novo lipogenesis (DNL), activated by glucose and insulin, is a major pathway in the development of steatosis and contributes to 38% of the intrahepatic triglyceride-palmitate content in NAFLD patients (Smith et al., 2020). Recent studies in both animal models and NAFLD patients demonstrated that a reduction in steatosis is associated with an improvement of NASH and hepatic fibrosis, indicating the therapeutic potential of drugs acting on hepatic steatosis (Harrison et al., 2019; Gapp et al., 2020). Currently there is a lack of human in vitro hepatocyte models that can support the identification of novel drugs inhibiting hepatic DNL. None of the existing models are described to be sensitive for insulin driven DNL, while the available rodent hepatocyte models (ex vivo or precision-cut liver slices) have insufficient throughput for effective drug discovery. In collaboration with the lab of In Vitro Toxicology and Dermato-Cosmetology of the Vrije Universiteit Brussel (VUB), we identified that the human hepatocyte-like cells (HLCs) (Natale et al., 2018, Boeckmans et al., 2019), derived from skin precursor cells (hSKP), are uniquely sensitive to insulin driven DNL, shown by both gene expression and lipid accumulation readouts. We demonstrated that the sensitive HLCs showed an increased SREBP-1C expression, a key transcription factor for DNL, upon insulin stimulation. Moreover, inhibition and activation of the DNL pathway could be demonstrated using reference inhibitors (ACCi and AKTi) and activators (LXRa). After miniaturization of the lipid accumulation assay to a 384-well plate format, a library of publicly available mode-of-action chemical substances was screened to validate the relevance of the model and to identify novel targets involved in the DNL.

References

Keywords
Non-alcoholic fatty liver disease (NAFLD); De novo lipogenesis (DNL); High throughput assay; Human hepatocyte-like cells (hSKP-HPC); Drug discovery
The number of non-human animals used in research has increased in line with advances in medical technology, although it has previously been shown that these experiments demonstrate poor human utility (Knight A., 2007). This study aimed to determine the effectiveness of animal studies on rats that were performed as part of medical doctors’ residency master’s theses prepared in Turkey between January 2006 and December 2015. The number of thesis-derived published papers from each year, as well as the subsequent citation rate of these papers, was determined. Results from 34% of the 656 analysed studies (226/656) were published as papers in PubMed-indexed journals. These 226 studies got 1803 subsequent citations in total. Citation counts were statistically significantly different in 2009 and 2010, as compared to 2011, 2013, 2014 and 2015. Previous studies showed that the usual main objective for carrying out animal studies in Turkey was the preparation of a thesis or the furthering of an academic career (i.e. personal self-interest) (Mayir B. et al., 2016) In the current study, the publication rate and the number of subsequent citations of these thesis-derived papers were both low, and thus, the contribution of these animal studies to scientific progress is doubtful. It is recommended that institutional research ethics committees should be much more highly selective in approving the use of animals for the purposes of student thesis preparation.

References

Keywords
publication; citation; analysis; theses; residency
EVALUATION OF THE SINGLE-CELL LEVEL IMMUNO-EFFICACY OF RECOMBINANT PROTEIN FOR AVIAN INFLUENZA SUBUNIT VACCINES WITH AN NOVEL AAT INTEGRATION PLATFORM

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Avian influenza virus (AIV) has extensively circulated in migratory waterfowl and poultry. The potential of AIV infecting humans might be a significant threat. Therefore, the development of a new vaccine against AIV is urgent. Subunit vaccines is a new type of vaccine technology. The AIV subunit vaccine do not contain whole virus particles or live components of the influenza virus. It differs from inactivated whole virus vaccines of influenza from chicken embryo eggs, it contain only the antigenic parts of the major surface structure proteins. Even it is only a sequence that would been recognized by a neutralizing antibody (He et al., 2014). Just for this reason, it is advantageous in business and production. Global efforts to promote development of next-generation influenza subunit vaccine, universal and long-lasting protective vaccine. However, the development of next-generation vaccines often requires a large number of experimental animals, and the promotion of alternatives to animal testing (AAT) has become a global goal in recent years. The author’s past researches, including "Pathogenesis and molecular modeling of avian influenza virus (He et al., 2013)", "A novel secretory bicistronic baculovirus protein expression platform (Hsieh et al., 2018)", "Modular microfluidic chip control system (He et al., 2015)" and "Peripheral blood mononuclear cell (PBMC) isolation microfluidic chip" were integrated into a AAT project. We developed a molecular modeling process for structural regions of key protein for avian influenza subunit vaccines. In addition, we prepared influenza virus segmented expression proteins with the baculovirus protein expression platform based on the prediction of molecular modeling. Finally, we tried to use the PBMC microfluidic chip to screen immunodominant, universal and long-lasting protective epitopes of Taiwan H6N1 avian influenza virus. These efforts accelerated the AAT of the avian influenza subunit vaccine development.

References

Keywords
Avian influenza virus (AIV); Subunit vaccine; Molecular modeling; Microfluidic chip; Alternatives to animal testing (AAT)
In 2019, Brazil joined the growing list of countries banning animal experiments. In 2014, the National Council for Animal Experimentation (ConceA) Normative Resolution No 18 (RN18) made the use of 17 Alternative Methods mandatory, establishing five years deadline for the public and private sector to adjust to this new reality. Among the endpoints, skin irritation and corrosion tests required full availability, as this endpoint is broadly demanded several industries and different types of products. Since 2012, The National Institute of Metrology, Quality and Technology (Inmetro) has been one of three Central Laboratories of the National Network of Alternative Methods (Renama), established to promote the implementation, dissemination and validation of Alternative Methods to Animal Experimentation.

The PReMASUR (MERCOSUR's Regional Platform for Alternative Methods for Animal Experimentation) National initiative had organized by far 19 training courses focusing on in vitro methodologies leading the training approximately 200 professionals from Brazil and Mercosur member countries. In this context, training and dissemination efforts are crucial to prepare and anticipate the needs in terms of demand, but also to create an efficient network to widely deploy these methods throughout the country. It’s worth mentioning that the production in Brazil of the in vitro reconstituted human epidermis by Episkin’s subsidiary in Brazil was a milestone, making available an emblematic model of replacement alternative for research, training and regulatory purposes.

In 2014 there was no GLP laboratory in Brazil for RN18 CONCEA in vitro methodologies. Nowadays, there is currently at least one test facility for the OECD TG 431, 439, 437, 428, 487 methodologies required by RN18 CONCEA, allowing Brazil to adhere to 3Rs (reduction, refinement and replacement) principles and the sequential access to markets abroad. The partnership between the public and private sectors was crucial to achieving this success.

References

Keywords
Brazilian Regulation; Alternative Methods to Animal experimentation ; hands-on training ; PReMASUR
Safety evaluation of medical devices is a complex process and evaluation of skin irritation potential is an indispensable part of this process. The rabbit skin irritation test developed by Draize has been successfully replaced by reconstructed human epidermis protocol (RhE) (OECD TG 439). However, this protocol is optimized for neat chemicals and medical device (MD) extracts are dilute solutions with low irritation potential. To reflect the requirements of ISO 10993 directive, an optimized protocol using known irritants and spiked polymers was developed in 2013 (1). After successful transfer and standardization, a validation scheme was prepared. All 17 laboratories that trained in the protocol worldwide produced results with almost 100% agreement with predictions for selected references (2).

In follow up approach, several medical devices benchmark materials (5 irritants and 2 vehicles) were evaluated in the controlled human patch testing (4 h and 18 h) and in EpiDerm in vitro skin irritation protocol. Results were then compared to existing rabbit skin irritation test data.

Based on the preliminary studies, an international round robin validation study was conducted in 2016 to confirm the ability of the RhE models to correctly predict the intracutaneous irritation of extracts from medical devices (4 irritants and 3 non-irritant materials). Blinded polymer samples were extracted with sesame oil and saline according to ISO 10993-12 (3).

The protocol employing EpiDerm tissues was able to correctly predict virtually all of the irritant polymers in the saline, sesame oil as well as in both solvent extracts. Our results confirmed the ability of in vitro approach using RhE tissue models to detect the presence of skin irritants at low concentrations in dilute medical device polymer extracts (3). The use of the reconstructed tissue models, as replacements for the rabbit intracutaneous test is currently being implemented into the ISO 10993 standards used to evaluate medical device biocompatibility.

References

Keywords
EpiDerm; in vitro skin irritation protocol; medical devices extracts; validation; ISO 10993:23
Pain can be challenging to recognize and treat in laboratory rodents and pain management remains an important ethical consideration for those working with rodents in science. Additionally, animals in pain may not have normal physiologic function or demonstrate normal behaviors, decreasing the reliability of studies in which these animals are used. The International Association for the Study of Pain (IASP) has designated 2020 as the Global Year of Pain Prevention providing an opportunity to focus on pain detection and mitigation strategies for rats and mice. Newer pain assessment techniques, such as facial grimace scoring, burrowing, and nest-building evaluate changes in spontaneous behaviors or animals in their home environments and may prove more useful than traditional evoked response reflex testing. Similarly, an evidence-based review of clinical pain management in rodents suggests that updated recommendations are needed for effective pain mitigation. Careful planning based on study design, knowledge of pharmacokinetics and mechanisms of action of analgesic agents, regular observation of animals for individual differences, and ensuring an institutional culture that recognizes the sentience of laboratory mice and rats is needed for effective clinical management of pain.

References

Keywords
ethics; animal welfare; pain recognition; pain management; rodents
822 A COMPARISON OF TRAINING STANDARDS AMONGST INTERNATIONAL COLLEGES OF LABORATORY ANIMAL MEDICINE

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To assist new colleges of laboratory animal medicine to develop and define quality standards for training and examination of candidates and to help promote intercollege recognition of credentials of Diplomates between the American, European, Japanese and Korean Colleges of Laboratory Animal Medicine (ACLAM, ECLAM, JCLAM, and KCLAM), the International Association of Colleges of Laboratory Animal Medicine (IACLAM) conducted an in-depth review and comparison of oversight, training, credential, and examination standards for each college. Specifically, this included a review of: national or regional college support structures, the college Constitution and Bylaws, a detailed description of qualifying mechanisms for candidates wishing to sit the certification examination, training program criteria, the process for credentialing candidates for examination, the mechanism for examination development, evaluation, and quality assurance, as well as comparing the detailed role delineation documents (RDD) or task analyses for newly qualified Diplomates. While a number of differences were found in processes, there was good general harmonization in approaches to training program duration, qualifications of candidates and credentialing processes, RDDs between the colleges. Areas requiring more detailed review include harmonization of didactic training and certification examination preparation, review, and quality assurance.

References

Keywords
education; laboratory animal veterinarians; quality assurance
The tools and approaches used within veterinary education and training to gain knowledge and skills are undergoing changes as new technologies are being developed and as ethical and animal welfare concerns are being given greater prominence. This 45-minute beta version documentary film investigates the veterinary curriculum and follows the disciplines, courses and practical classes that veterinary students and trainees will face. The film presents innovative, humane learning tools and approaches that have been developed and implemented by educators to better meet teaching objectives. Interviews with educators, teaching assistants and students illustrate their experience of the alternatives and how they support effective education and training. Demonstrations and practical classes show a range of tools and approaches in use. These include virtual laboratories and virtual reality software for anatomy and physiology education, mannekins and advanced synthetic cadavers for surgery training, client donation programs to provide ethically sourced cadavers, and clinical learning opportunities with animal patients. The film demonstrates that veterinary education and training can be achieved in a fully humane way without harmful animal use, to the benefit of the students, the educators, the animals, and the profession itself.

References

Keywords
education; training; veterinary; animal experiment; replacement alternative
828 THREE-DIMENSIONAL CULTURE IMPROVES THE MORPHOLOGY AND FUNCTION OF CARDIOMYOCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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So far, we have been conducting animal tests or simple in vitro assays for preclinical studies in drug development. However, the animal models often cannot recapitulate an entire disorder or disease and the human system and in vitro assays are too simple to reproduce the detailed physiological features observed in vivo. The cells derived from a human pluripotent stem cell (hiPSC) offer important advantages for drug toxicity screening against relevant human organ cells and may facilitate drug development. Especially, the hiPSC-derived cardiomyocyte (hiPSC-CMs) represents a potentially powerful tool to model aspects of heart physiology relevant to disease and adverse drug effects. However, two-dimensional (2D) monolayers of hPSC-CMs are limited in their ability to mimic native cardiac tissue structurally and functionally. To elucidate the physiological changes according to culture conditions, we compared the morphological features with microscopy imaging analysis and electrophysiological functions with cardiac action potential and the relative gene profiling in 2D- or 3D-cultured hiPSC-CMs. In this study, we found that 3D culture induced the intracellular morphological changes of hiPSC-CMs with the increased population of organized myofilaments and highly quantified mitochondria. The 3D culture also enhances the action potential parameters in electrophysiological analysis, which is consistent with the result of the increase in cardiac ion channel expression important to cardiac function. These results demonstrate that the 3D culture improves the phenotypical maturity and electric functionality of hiPSC-CMs. [Acknowledgement] This work was supported by the Technology Innovation Program funded by the Basic Science Research Program funded by the Ministry of Education (NRF2019M3A9H1103719).

References

Keywords
Three-dimensional culture; Human pluripotent stem cell-derived cardiomyocytes; Cardiac maturation; Electrophysiological function; Morphological change
Microfluidics systems linked with biosensors cell-monitoring is one of the major advances in the field of healthcare. These systems reflect functional advances that mimic relevant cell and tissues properties. As a proof-of-concept this work is intended to assess how graphene oxide (GO) is suitable to build better biosensors for skin-cell-monitoring systems and how that the exchange of skin metabolites can be monitored in a human tissue-engineered skin substitute. Functional properties of a 3-D fullthickness skin substitute has been previously demonstrated by our group (Mathor et al, 2014) and GO nanocomposites were synthesized, characterized and bio-functionalized as a electrochemical sensing (Sakata et al, 2018). In this way in Brazil three technological centers of Nuclear and Energy Research Institute (IPEN/CNEN-SP) an agency of the Ministry of Science, Technology, Innovations and Communications (MCTIC) of the Federal Government are collaborating to develop the Skin-On-A-Chip Project. It is a groundbreaking example of the partnership to bring innovative technologies into the drug discovery and safety testing paradigm. Integrating biosensors technology has a huge potential over conventional methods, including high-throughput screening, low limit of detection, real-time analysis and less sample volume requirement to be analyzed by our future microfluidic system. We believe this work will provide a predictive model, future prospects and challenges ahead for the next generation of testing will use appropriate extracellular matrices to better approximate the human cellular microenvironment.

References

Keywords
Microfluidics Systems; Skin-On-A-Chip; Graphene-based Biosensors; Drug Discovery; Cell Metabolites Monitoring
Diphtheria antitoxin (DAT) is a life-saving drug, but the way it and other antitoxins are manufactured hasn’t changed in more than 100 years. As with most therapeutic antitoxins, DAT is produced from the serum of equines who have been hyperimmunised by repeated toxin injections. In addition to documented animal welfare problems at facilities where hyperimmunised equine serum is produced, equine antitoxins can cause adverse health effects in humans who receive them. In recent years, public health authorities have noted a global shortage of equine DAT and called for the development of alternative products. As a first step toward developing a non-animal replacement for equine DAT, the PETA International Science Consortium Ltd. funded the development of human monoclonal antibodies against diphtheria toxin that can be produced in cell culture. In 2017, we introduced this project and presented early results at Word Congress 10. Here, we report the successful results of the completed project, which has led to the development of a set of fully human diphtheria toxin-neutralising antibodies that are ready for clinical development (1). The process used in this project serves as a template for other groups interested in replacing animal-derived therapeutic and diagnostic antibodies with human recombinant antibodies.

References

Keywords
recombinant; antibody; nonanimal; diphtheria; antitoxin
Current medicine, which is predominantly based on small molecule drugs, has only taken us so far in beating disease and tissue degeneration. Extracellular vesicles (EVs), which are highly specialised yet ubiquitous nanoscale messengers secreted by cells, have emerged as a new generation in medicine. The problem is their power cannot be harnessed because they are heterogeneous, and little is known about them. We developed a therapeutic formulation of EVs derived from mesenchymal stem cell EVs and determined the correlation between the molecular composition of EVs and their therapeutic efficacy using human physiology mimicking models.

To characterize molecular composition of individual vesicles we used atomic force microscopy nano-infrared spectroscopy (NanoIR). Our study showed that EVs isolated from cells exposed to different level of oxidative stress have major differences in protein, RNA/DNA and lipid content. To confirm the composition and establish subpopulations with the desired composition suited for lung repair we conducted high-resolution nano flow cytometry analysis (NanoFCM). Our assessment of EVs on lung repair using single cell culture and human lung-mimicking models (MucilAir) demonstrated efficacy of EVs to promote tissue repair.

Taken together, we showed that single vesicle characterisation tools, AFM-IR coupled with NanoFCM was critical to select therapeutic subpopulations of EVs while human physiology-mimicking models, that replace animal models, enabled assessment of the therapeutic potential of our EV-medicines.

FUNDING: This work is supported by grants from The Medical Advances Without Animals Trust and Australian Ethical.

References

Keywords
Extracellular vesicles; human physiology-mimicking models; AFM-IR; nano flow cytometry; nanocharacterisation
The EU Cosmetics Directive prohibits the use of in vivo genotoxicity models and, while the in vitro 2-test battery has a high sensitivity for prediction of in vivo genotoxic/carcinogenic agents, it tends to over-predict the genotoxicity hazard, resulting in misleading positive results. To address this, the Cosmetics Europe Genotoxicity Task Force has established two in vitro skin genotoxicity models as follow up assays to the 2-test battery for substances with dermal exposure: the reconstructed skin (RS) Comet assay and the RS micronucleus (RSMN) test. Here, we report on the completed validation of these assays. Both assays exhibited good sensitivity and specificity: 77% and 88% for 3D Skin Comet (32 compounds) and 80% and 87% for the RSMN (47 compounds). A combination of these assays enables detection of DNA damage leading to all 3 types of genotoxic damage (mutation, clastogenicity and aneugenicity). In the validation dataset, most of the true positive chemicals were positive in both assays of the 2-test battery i.e. the Ames and micronucleus assays; therefore, the results of both the RSMN and the 3D Skin Comet assay were considered to make a final call on the chemical’s genotoxic potential. By applying this endpoint-triggered strategy, the sensitivity increased to 89%. In conclusion, the high predictivity of the expected genotoxicity in vivo observed for these higher tier in vitro assays supports their use as follow-up tests to the standard 2-test battery. For topically applied chemicals, the RSMN assay is recommended for in vitro micronucleus positive chemicals; whereas, Ames positives should be followed-up with a RS Comet assay. This tiered strategy shows great promise as an in vitro-only approach for genotoxicity testing of cosmetic ingredients. Both assays were accepted into the OECD guideline development program in April 2019. Funded by Cosmetics Europe and the German Ministry for Research and Education.

References

Keywords

genotoxicity testing; human reconstructed skin models; validation; comet assay; micronucleus test
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Gene therapy is an emerging therapeutic field with promise of slowing down disease progression or even curing inherited diseases. uniQure is developing a number of gene therapy programs for treatment of neurodegenerative diseases like Huntington’s disease, spinocerebellar ataxia’s, amyotrophic lateral sclerosis and others. One of the main advantages of gene therapy is that will be injected once in the brain of patients and provide life-long therapeutic benefit. The translatability of gene therapy from preclinical studies to the clinic is challenging because of the lack of harmonization of the use of in vitro and in vivo models. Typically, the development of a gene therapy product would require extensive toxicology-safety package in animals, often non-human primates. At uniQure, we have started developing and testing induced pluripotent stem cell (iPS) cells and iPS-derived 3D organoids as a model for efficacy testing of AAV-based gene therapies for neurodegenerative diseases. The advantage of the iPS and organoid system is that we can evaluate the efficacy and safety of the treatment in a system closer to the human situation. In organoids, transduction with AAV-based gene therapies led to high levels of vector DNA, transgenic mRNA and protein expression that translated in efficacy read-outs. Furthermore, AAV5 mediated delivery of a human sequence specific engineered microRNA to cerebral organoids led to a lower expression of its target ataxin3. Future implementing of iPS and organoid testing during gene therapy development could reduce the usage of animal models and improve translation to the clinic.

References

Keywords
Pyrrolizidine alkaloids (PAs) are common plant constituents relevant for safety assessment because some have been shown to be hepatocarcinogens in rodent studies following metabolic bioactivation and DNA-adduct formation. Similarly, at high doses in humans they have been associated with hepatic sinusoidal obstruction syndrome, following protein-adduct formation. Hundreds of PAs exist in nature, and we and others have shown that their potency differs due to structural differences (Merz and Schrenk 2017, Allemang et al, 2018; Lester et al, 2019; Louisse et al 2019). This allows for a risk assessment approach that relies on dose addition, scaled for individual potency, relative to a reference PA by way of a Relative Potency Factor (RPF). As toxicokinetic, metabolism and genotoxicity are key contributors to the differences, we examined different PAs and their N-Oxides representing a variety of structural classes using in vitro technologies. A number of PAs have been examined in human and rat liver cell models. Micronuclei were examined via flow cytometry in HepaRG cells and bridged to DNA adduct and kinetic measurements in rat sandwich culture hepatocytes. Each PA could be ranked according to its potency relative to the reference PA riddelliine, with the diesters being more potent than the monoesters and the N-Oxides having negligible potency. The differences in gut absorption between the PAs and their N-Oxides was investigated in a Caco-2 cell model, and due to the importance of the microbial reduction of N-Oxides to their free bases, integrated with biotransformation rates obtained from a faecal polymicrobial assay. PBPK modelling was performed to integrate all in vitro data, the calculated RPFs closely reflecting those first proposed by Merz and Schrenk.

References

Keywords
Integration of specific readout compatible with Blood-Brain Barrier (BBB) Biochip will lead to “Next Generation Organ-on-Chip”. To meet this challenge, we have developed a platform, which combines an in-house developed biochip and a perfusion system, compatible with several real-time readouts. The biochip consist in a dual compartment with a large exchange surface, which can be connected with any pump systems using Luer-lock fluidic connectors. Moreover, this biochip is compatible with any microscope for standard slide format. In addition, this platform can be installed inside an incubator and remotely controlled by Bluetooth® with a user-friendly graphical user interface (GUI). We used two Human BBB cell types hCMEC/D3 [2] and CD34+ derived cells [3] and the commercial ibidi® pump system to set up and validate our in-house perfusion system. Immunolabelling within our in-house biochip confirms an increase of tight-junction markers with both cellular types. In co-culture, both compartments can be visualized to study cell transport. Cell viability is improved with the use of continuous perfusion. Our in-house perfusion system is made of four peristatic pumps and four valves which enables to perform up to four biochips experiments simultaneously. In injection mode, our perfusion system enables to perform drug delivery or drug exposure experiments but also real time analysis in sampling mode. Long-term exposure can be programmed using our GUI to simulate chronic exposure or to exchange medium. In perfusion mode, permeability coefficient (Papp) can be measured in real time to monitor the barrier integrity in order to detect quick events. Our system can also induce shear stress, which results in an improvement in the tightness properties of the BBB. To conclude, we have developed a biochip suitable for in vitro BBB modelling, which can be also used in any other biological cellular barriers and with any perfusion systems.

References

Keywords
Blood-brain Barrier; Shear-stress; Biochip; Pump; in vitro model
Establishment of an In Vitro Rabbit Neurosphere Model to Evaluate Neurodevelopmental Adverse Effects Induced by Intra-Uterine Growth Restriction (IUGR) and to Test Future Therapies

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Intrauterine growth restriction (IUGR) is defined as a significant reduction of fetal growth rate leading to a birth weight below the 10th percentile for the corresponding gestational age. The prevalence accounts for 5-10% of all pregnancies, approximately 600,000 cases in Europe. Placental insufficiency reduces placental blood flow leading to fetal development under chronic hypoxia which is associated to neurodevelopmental damage, cognitive dysfunctions and cardiovascular adverse outcomes (Eixarch et al., 2012). The characterization of neurostructural changes in fetus with IUGR is essential to design therapeutic strategies directed to limit its deleterious effects.

We have established for the very first time an in vitro model based on primary rabbit neuronal progenitor cells (NPCs) cultured as three-dimensional cell aggregates called neurospheres. Neurospheres are able to mimic basic processes of fetal brain development like proliferation, migration and differentiation into neurons, oligodendrocytes and astrocytes (Baumann et al., 2014; Kühne et al., 2019). We successfully developed new endpoints like neurite outgrowth, branching and synaptogenesis. By comparing the functionality of control and IUGR neurospheres we identified that rabbit NPCs from IUGR individuals have a significantly reduced ability to form oligodendrocytes.

To find a neuroprotective therapy preventing/reversing adverse effects of IUGR we tested six different compounds at increasing concentrations (Docosahexaenoic acid (DHA), choline, lactoferrin, melatonin, zinc, and 3,3′,5-Triiodo-L-thyronine (T3)). Basic processes of neurogenesis and cell viability were assessed to determine the maximum tolerated concentration (MTC) and effective concentration (EC). DHA (MTC=10µM; EC=1μM), melatonin (MTC=3μM; EC=1μM) and T3 (MTC=30nM; EC=0,1nM) have been selected as the most promising therapies due to their promoting effects on oligodendrogenesis.

The in vitro model allows us to evaluate different processes of neurogenesis in a fast, economic and ethic way and contributes to a better understanding of IUGR induced neurodevelopmental damage and to the selection of new neuroprotective therapies.

References

Keywords
Intrauterine growth restriction; fetal brain development; in vitro; neurosphere model; therapy testing
Flame retardants are chemicals or mixtures widely used in commercial and consumer products to reduce flammability. Following the ban of long-used polybrominated diphenyl ethers, a wide range of novel flame retardants (nFRs) used as a replacement are consistently found at relatively high levels in the environment and human matrices. Some evidence indicates the toxic effects of individual nFRs in mammals, but generally, toxicity data remains insufficient. Considering the widespread presence of these compounds, this is a crucial knowledge gap, and more toxicological studies on nFRs, individually and as mixtures are critically needed (Bajard et al., 2019). In line with the 3Rs (reduction, refinement, and replacement) principle of regulatory toxicology, in vitro approaches combined with the Adverse Outcome Pathways (AOPs; an OECD endorsed framework) allow to link mechanistic studies to apical endpoints.

The present study aims to better understand the mechanisms driving the liver toxicity of prioritized nFRs, focusing on hepatic steatosis, and to contribute to the development of quantitative AOP (qAOP). For steatosis, AOPs and AOP network linking several molecular initiating events (MIEs) have been suggested. Evidence indicates that the prioritized nFRs affect some relevant MIEs. Experimental research is being conducted to quantify the effects of the nFRs on different Key Events of the AOPs proposed for hepatic steatosis, using in vitro toxicological studies in 2D and 3D cell culture. Our first results indicate that several nFRs may induce lipid accumulation in the hepatocytes at subcytotoxic concentrations. Additional in vitro test battery, such as nuclear receptor activation, expression of associated downstream proteins, are currently being examined to understand the molecular mechanism and to develop quantitative data for follow-up development of qAOP using the computer simulations through Artificial Intelligence-based tools. Potential mixture effects of nFRs on the studied endpoints are being assessed as well, and relevant AOP networks will be discussed.

References

Keywords
Hepatic steatosis; Quantitative Adverse Outcome Pathways; Flame Retardants; 3Rs; Mixtures
There is a great need for in vitro models to predict developmental toxicity to reduce the number of laboratory animals and to make test methods for chemicals more relevant for humans (Seidel 2018). Human stem cells are an excellent tool to model human embryonic development as they can form embryoid bodies (EBs). These EBs mirror the early embryo in many structural and functional aspects and therefore are a promising model for predicting developmental toxicity (Brickman and Serup 2016).

Here we describe the setup of an innovative assay to study developmental toxicity using human induced pluripotent stem cells (iPSCs). iPSCs do not have ethical constraints such as the previously used embryonic stem cells, they are made by converting somatic cells from donors back to a pluripotent state and they can undergo EB formation and differentiation (Yamanaka and Takahashi 2006). In our assay, we guided three iPSC lines in an 8-day protocol to become cardiomyocytes-containing EBs that exhibit autonomous and clearly visible contractions as in the heart. These contractions serve as initial readout together with EB diameter, however, we are also evaluating genetic modified iPSCs to develop more robust, sensitive and reproducible test methods.

Importantly, we showed that the 8 days of differentiation mimic human embryonic development up until the first heartbeat at day 21, by measuring expression of marker genes for mesoderm, cardiac progenitors and cardiomyocytes. Because many developmental pathways are conserved, we hypothesize that our model can identify general developmental toxicants. We have validated this by identifying thalidomide and the rodent toxicant epoxiconazole as positives in our assay, while valproic acid, as a mild developmental toxicant, did not reduce the portion of beating EBs significantly. Lastly, we addressed the molecular basics of our assay by measuring changes in gene expression, which might help to reveal novel markers for developmental toxicity.

References

Keywords
induced pluripotent stem cell; developmental toxicity; embryonic development; cardiomyocyte
919 USING HUMAN TO BENCH TO HUMAN CIRCULAR APPROACHES FOR HEALTH AND MEDICINES RESEARCH

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Last decade sea of science/technology progress is deeply changing society functioning, including health/disease management, medicines development. Animal-based research has been gold-standard on disease/medicines research (eg disease models, genetically modified animals) also for regulatory purposes. But animal’s to human prediction are limited and alternative/complementary approaches are needed. Deeply interconnected, science and technology are providing human-based innovative tools based as those using human iPSC technology, for disease research, human associated-targets, or investigational molecules as potential therapies. Human cells are increasingly used for (dysfunctional) target identification in health/disease conditions. Information is used by computational chemistry and biology to conceive an develop corrective molecules/approaches. Patient’s variability on disease expression, progression, response to therapies is identified from big data captured by health systems/clinical research and provide basis for patient and disease stratification. Specific patient-derived samples are used to identify molecular/genetic attributes behind variabilities, providing the basis for conceiving more adjusted, personalised treatments. Therefore, medicines research can increasingly include 1. patient’s samples selected based on specific attributes, 2. identifying dysfunctional targets 3. computational conception of target molecules screened in vitro, in silico to select the most promising/safe candidates 4. testing those in human-based cell/organ systems, anticipating efficacy and potential safety hurdles, replacing animal models, hopefully towards their elimination. Those should enable progression into carefully designed human studies, incorporating safety/efficacy biomarkers and digital tools for events reporting and patient monitoring. Identification of responders/non-responders/adverse reactions can be achieved using those tools, providing foundation for another wave of research using patient’s samples to enable again identification of targets responsible for those variabilities and search for solutions. Successive waves of circular, continuous research, from patient to bench to patient will hopefully result in tailored treatments towards personalised medicine.

References

Keywords

circular research; in vitro models; personalised medicine; animal reduction
920 INVESTIGATING EPILEPSY USING A COMBINATION OF MATHEMATICAL MODELLING AND VOLUNTARY HUMAN DATA AS A VIABLE REPLACEMENT FOR ANIMAL MODELS.

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Epilepsy is characterised by abnormal electrical brain activity (seizures) that vary considerably between individuals. Both diagnosis and treatment of epilepsy are highly patient specific. The aim here is to understand patient specificity by studying individual brain structure-function relationships. We investigate the relationship between a patient’s brain structure (connectivity) and dysfunction (seizures), which is a fundamental problem of 21st century epilepsy research.

The overwhelming majority of neuroscience research is based on animal experiments. Animal models (typically mice & rats) are used to test hypotheses by artificially inducing epilepsy by administration of drugs, electrical stimulation, and by breeding genetically modified animals that produce seizures. Whole brain or brain slices of animals are used to study the relationship between brain connectivity and seizures. Since brain connectivity is unique for each individual animal, experiments are limited specifically to each animal. We propose an alternative to animal models as a combination of voluntary human data and a mathematical model of brain connectivity and epileptic behaviour. Although a computational model is not as detailed as an actual physical brain, the model parameters are rigorously understood and can be manipulated at will, unlike a physical experiment. Within this framework we can modify many aspects of brain connectivity with ease and study its effect on seizure dynamics. This work moves away from traditional neuroscience paradigms and replaces animal models with computational models based on human data. We are interested in how the brain connectivity of individual patients’ influences the transition from a normal state into an abnormal seizure state. This novel multidisciplinary approach in combination with voluntary human data, will serve as a replacement for animal models and will contribute to the paradigm shift away from conventional neuroscience research.

Supported by Medical Advances Without Animals Trust (MAWA).

References

Keywords
Epilepsy; Neuroscience; mathematical modelling; computational modelling; animal replacement
921 EVALUATING DRUG-INDUCED LIVER TOXICITY OF ACETAMINOPHEN, TROVAFLOXACIN AND LEVOFLOXACIN IN A TRIPLE-CELL MICROPHYSIOLOGICAL LIVER SINUSOIDAL MODEL

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Introduction:
Drug withdrawal from commercial markets due to the occurrence of hepatotoxic adverse events is frequently reported (Watkins 2011). The antibiotic compound Trovafloxacin was withdrawn due to idiosyncratic drug-induced liver injury (DILI) (EMEA/17438/99, 1999). Levofloxacin, as a structurally related non-DILI drug, is still marketed with only rare cases of hepatic failure (Karim, Ahmed et al. 2001; Schloss, Becak et al. 2018). Recently developed microphysiological systems (MPS) of the human liver are a promising approach for the assessment of DILI and designed to offset the lack of predictability of preclinical animal models (Baudy, Otieno et al. 2020). This study investigates the toxicity of acetaminophen and the two antibiotic drugs in a triple-cell MPS of the human liver.

Methods:
Human MPS of the liver were composed of a vascular, non-parenchymal compartment and a hepatic parenchymal compartment by using liver sinusoidal endothelial cells, primary monocyte-derived macrophages and the hepatic HepaRG® cell line, respectively. The liver MPS was cultured for seven days under continuous vascular perfusion. All drugs were applied relating to their respective toxic human plasma concentrations and with redosing every 24 hours. Supernatants were collected and analysed for liver injury markers (LDH, ASAT). Expression of specific cell markers in DILI were surveyed by immunofluorescence (FcγR2b, CD206, ASGPR-1).

Results:
Acetaminophen and trovafloxacin at high dose concentrations resulted in loss of tissue integrity and elevation of specific liver-injury markers in the liver sinusoidal model. The treatment with levofloxacin did not result in commensurable DILI.

Conclusions:
In this study, we could demonstrate that DILI was induced by acetaminophen and trovafloxacin in a triple-cell human liver model, but not for the structural-related antibiotic levofloxacin. This work highlights the urgency to develop human-relevant MPS to ameliorate drug safety and to minimize prediction failure of preclinical animal models.

References

Keywords
microphysiological systems; organ-on-chip; liver; drug safety; pharmacy
922 REDUCTION THROUGH REFINEMENT (FOR RABBITS)

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At GSK we research, develop and manufacture innovative medicines, vaccines and consumer healthcare products. Animal studies, conducted with high standards of humane care and treatment, represent a small but vital part of our procedures in the release and development of vaccines. The Rabbit Pyrogen Test (RPT) is a vaccine release test that identifies pyrogenic substances in our vaccines. We currently use an animal-free alternative called the Monocyte Activation Test (MAT - uses human blood, in an ELISA), though for certain countries, we still must perform the animal test. Where this test is performed, the rabbits go through a specialised acclimatisation process (including training/habituation sessions) and standardized handling techniques.

The caging, used during the testing, has been redesigned and improved, both ergonomically and with increased space. With these refinements we have shown a reduction of animal numbers in 4 places:
Pre-test animal selection was reduced from (on average) 7 to 2 rabbits per month.
Batches passing the test first time increased 58% to 73%
Batches re-tested went from 50% to 28%
Rabbits injured in caging reduced by 100% (to 0)

During this time the capacity of the RPT was also doubled (from 30 to 60), with no effect to results of the refinements, in fact the numbers continued to reduce after this change.

While GSK continues to work toward an era of non-animal based research and development, we remain committed to acting ethically and practicing good animal welfare when animal use is still required.

References

Keywords
Rabbits; Acclimatisation; Handling; Pyrogen; Reduction
Rebuilding epidermis has been of interest to replace animal models for toxicological studies, and six reconstructed human epidermis (RHE) were successfully validated to investigate in vitro potential skin irritants (OECD Test Guideline N°439). However, the use of these RHE models has a high degree of dependability in the commercial strategies of the suppliers, and they have limited accessibility for companies of countries with customs barriers. Thus, this study reports the development and characterization of a novel epidermal equivalent meant to be used for in vitro skin irritant testing. For developing the in-house RHE, we have modified published protocols (Poumay et al., 2004; Pedrosa et al., 2017). The in-house RhE, constructed with neonatal KCs (nKCs) plated on collagen IV-coated inserts, presented a well-differentiated epidermis (~ six layers of differentiated viable cells, mature stratum corneum, 64.5 μm thickness), and resembles human epidermis (Hematoxylin staining). This model also demonstrated similarities to native human epidermis in terms of marker expression (Keratin-10, Keratin14, Filaggrin, Involucrin). Parameters of cell viability (optical density average at 570 nm of 1.6) and barrier function integrity (IC50 of 3.23 mg/mL - sodium dodecyl sulfate) match the quality control criteria adopted by OECD 439. The performance of in-house RhE as skin irritation model was evaluated by the SkinEthic™ test method with 19 reference substances (OECD 439); and results shown that the in-house RHE was able to discriminate between irritating and non-irritating substances. Moreover, its performances as a skin irritation test were similar to the ones described in the OECD 439 (Alépée et al 2010; Kojima et al. 2013; OECD 439). Taken together, these results demonstrate the potential use of a novel RHE (in-house RHE) as skin irritation model especially for those countries in which validated RHEs have limited accessibility.

References

Keywords
OECD 439; Non-animal method; Skin irritation; Toxicology
933 USE OF FIBROBLAST PRIMARY CELL CULTURE TO STUDY PHOTOBIOMODULATION IN HORSES

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The photobiomodulation is a non-invasive therapy, with low cost, using laser or led light, under the infrared near or red spectrum in low potency densities, in order to promote the activation and modulation of some cellular mechanisms. Laser therapy is commonly used to induce analgesia, reduce inflammatory process and edema, and, to hasten the regeneration of injured tissues as, skin wounds, tendon and bones lesions. Data from in vitro experiments with cell cultures can be informative, especially at biochemical, functional and molecular levels, as well as in studies to analyze pharmacological targets and biological products production. The present study was designed to investigate the effects of the photobiomodulation at equine fibroblast primary culture in vitro as a model of equine skin fibroblast laser response. The fibroblast culture cells were divided in 5 groups (10000 cells/each): control, and irradiated groups with different energy densities 0.5J/cm², 2J/cm², 5J/cm² and 10J/cm², the groups received one or two laser irradiation 24 hours apart. Samples were obtained before (control) and after 24h of each irradiation. The cell concentration was obtained by direct counting (NeuBauer Chamber), Trypan Blue Stain was applied to access the cellular viability, DNA Integrity tested using the Comet Assay and, for nuclear alterations were made the Micronucleus test. Data were analyzed with ANOVA or chi-square test, both with 5% of significance level. Was concluded that the laser had shown some anti-mutagenic effect, and, according the energy density, can interfere at the cellular viability and proliferation. In this way, the laser act as a citoprotector agent, by the reduced DNA damage observed at the laser irradiated equine fibroblast cultured cells, effect that could be also expected in the equine skin fibroblast exposed to different laser energy density. The in vitro results aim to safely guide the default parameters for forthcoming in vivo research.

References

Keywords
biostimulation; cell model; low-intensity laser therapy
Photodynamic therapy (PDT) is a treatment modality with selective tumoricidal effects and has been used in feline skin alterations as Cutaneous Squamous Cell Carcinoma (SCC), SCC in situ (Bowen’s disease) and Actinic Keratoses (AK). PDT involves the administration of a photosensitizing agent that is preferentially retained by neoplastic tissue. After its administration, neoplastic localized photosensitizing agent is activated when it absorbs light of a specific wavelength, then interacts with oxygen leading to a subsequent formation of cytotoxic free radicals. The use of a LED source allows the operator to control the activation of the photosensitizing agent at regions of interest. The cat skin tumors share some similarity with human tumors, especially in biology, clinical evolution, response to treatment and prognosis. Due its high incidence in cats, SCC cat model is more suitable to modeling the human tumors than the largely used rat induced tumor model. Clinical response to PDT therapy is dependent on the type of photosensitizing agent, tumor/normal tissue photosensitizer ratio, and energy dose delivered to the target tissues. The treatment for SCC is the removal of the lesion, with a safety margin, which is traumatic and lead to mutilation and/or aesthetic alterations that could influence the relation owner/animal. The objective of the present study is to evaluate the response to PDT of different cat’s tumors. Cats with different types of lesions were biopsied to determine tumor and cell alteration, all had total or partial remission and reported less tumor recurrence than expected. The observed recurrences could be related to the depth of the neoplasm cells, due the lack of effectiveness in deep tissue. The PDT had shown to be an efficient and safe to treat superficial skin neoplasm, less invasive, with less recurrences and with no mutilation or other aesthetic major issue when compared to the conventional approach.

References

Keywords
Photomodulation; Photosensitizing Agent; Cutaneous Squamous Cell Carcinoma; LED
Microfluidic microphysiological systems (MPS) have proven to be a powerful tool for recreating human tissue-and organ-like functions at research level. This provides the basis for the establishment of qualified preclinical assays with improved predictive power. Reduction and replacement of laboratory animals and healthy volunteers are envisioned once human MPS-based models and assays translate into valid preclinical platforms. However, industrial adoption of MPS-based assays is progressing slowly due to their complexity. The presentation first highlights rational and state of the art of MPS developments (Marx, U. et al., 2020). Subsequently, examples of human single and multiorgan models, such as human bone marrow (Sieber, S. et al., 2017) and human liver-thyroid co-culture will be introduced. The underlying universal microfluidic HUMIMIC® platform of a size of a microscopic slide integrating an on-chip micro-pump and capable to maintain various organ model combinations or single organ equivalents of 16 different human tissues will be described. Challenges of industrial adoption of the platform, with the focus on models supporting repeated dose toxicity testing and simultaneous evaluation of safety and efficacy aspects (Huebner, J. et al., 2018) will be discussed. Furthermore, the creation of more complex physiology-based autologous multi-organ arrangements (Ramme, A. et al., 2019), mimicking adsorption, distribution, metabolism, excretion and crucial organismal feedback loops will be pointed out. We finally, introduce the concept of universal physiological templates (Dehne, E. -M., 2020), which might pave the way towards on-chip patient models. Design criteria, aspects of long-term performance, their impact on the drug development paradigm and ethical issues will be elaborated.

References

Keywords
Microphysiological system; Organ-on-Chip; Scientific state-of-the-art; Ethics; Industrial adoption