Société de Toxicologie
Cellulaire et Moléculaire

CONGRÈS FONDATEUR

LA TOXICOLOGIE VERS LA 3ème DIMENSION

13 et 14 juin 2019

FIAP Centre Jean Monnet - Paris

AU PROGRAMME

6 SESSIONS SUR LES MODELES 3D EN TOXICOLOGIE

Avec le soutien de :

CHANEL

Jeudi 13 juin 2019

8h45 **Accueil des participants**

9h15 **Ouverture du congrès par Saadia Kerdine-Römer,** *Présidente de la STCM*

9h30 *Les avatars biologiques (organoïdes, organes sur puce, bioimpression 3D) en recherche biomédicale*
Xavier Gidrol, Institut de Biosciences et Biotechnologies de Grenoble (BIG)

10h15 *Nouvelles approches d'ingénieurie tissulaire par bioimpression 4D*
Marine Salducci, POIETIS

10h45 **Pause-café & session poster**

Session 1 : Modèles 3D dans la peau (Modérateurs : S. Kerdine-Römer & C. Laperdrix)

11h15 *Développement par génie tissulaire d'un modèle de peau humaine innervée, vascularisée et immunocompétente pour l'étude des réactions inflammatoires cutanées*
Vincent Flacher, CNRS UPR 3572 I2CT

11h45 Tests toxicologiques in vitro : validations des modèles 3D
Christelle Videau, Episkin

12h05 Activation des kératinocytes en réponse à une exposition répétée d'une molécule allergisante cosmétique dans un épiderme reconstruit
Romain Vallion, INSERM UMR 996

12h20 *Enhanced Micronucleus genotoxicity assay using 3D reconstructed human epidermis*
Eric Andres, OROXCELL

12h35 *« Come to see my poster »*

12h45 **Déjeuner & session poster**

Session 2 : Modèles 3D dans le foie (Modérateurs : K. Andréau & A. Guillouzo)

14h15 *Bioingénierie du foie : promesses et limites actuelles des modèles 3D*
Cécile Legallais, BMBI, CNRS/UTC

14h45 *HepatoPearls: New generation of liver-mimicking spheroids in toxicology*
Noushin Dianat*,* CYPRIO

15h05 *In vitro* 3D culture systems for the study of human liver diseases
Sharma Ashwani, Biopredic

15h20 *Culture en 3D d'hépatocytes : nombreux avantages et quelques limites*
Camille Savary**,** INSERM U1066

15h35 **Pause-café & session poster**

Session 3 : Modèles 3D dans l'intestin (Modérateurs : B. Salles & P-J Ferret)

16h00 *Utilisation d'organoïdes humains pour étudier la biologie intestinale*
Maxime Mahé, Inserm UMR 1235, Nantes

16h30 *High Content Screening sur les organoïdes intestinaux*
Audrey Ferrand, IRSD

16h50 *The potential of 3D reconstructed Human intestinal models for biowaiver studies and finished products testing*
Christophe DINI, OROXCELL

17h05 *La STCM se présente*

18h00 **Cocktail**

Vendredi 14 juin 2019

Session 4 : Modèles 3D dans le poumon (Modérateurs : A. Baeza & S. Billet)

9h00 *A human breathing alveolus-on-chip: applications in toxicology and clinically-relevant in vitro testing*
Nuria Roldan, AlveoliX AG

9h30 *Exposition en interface air/liquide de cellules pulmonaires à des nanomatériaux*
Ghislaine Lacroix, INERIS

9h50 *Transcriptomic alterations induced by air pollution-derived PM2.5 reflect the shift from healthy to COPD-diseased human bronchial epithelium*
Sébastien Anthérieu, EA4483

10h05 *Modulation of the biological response of human reconstituted airway epithelium exposed to fine particles differing by their emission sources (traffic vs industry)*
Sophie Achard, INSERM UMR 1153

10h20 *Etude de la toxicité d'effluents gazeux issus du traitement catalytique du toluène lors d'expositions répétées en Interface Air-Liquide*
Clémence Méausonne, UCEIV EA 4492

10h35 Pause-café & session poster

Session 5 : Modèles 3D dans les autres organes (Modérateurs : Fanny Boislève & M. Pallardy)

11h00 Une approche supramoléculaire pour la culture de cellules neuronales en 3D
Juliette Fitremann, UMR5623 CNRS-UPS

11h30 Dispositifs microfluidiques pour les essais de toxicité oculaire : un modèle de culture de compartimentation pour l'étude des effets toxiques du chlorure de benzalkonium sur le trijumeau
Michael-Adrien Vitoux, Institut de la Vision

11h45 AG de la STCM

12h15 Déjeuner & session poster

13h30 *Le placenta perfusé et ses perspectives en toxicologie*
Sophie Gil, INSERM UMR-S1139

14h00 *Conséquences de l'exposition de la barrière placentaire humaine à des polluants de type nanoparticules de CeO2 et benzo(a)pyrène*
Margaux Nedder, INSERM UMR-S 1139

14h15 *« Mon projet de recherche en 180 sec »*

Session 6 : Les outils des 3D (Modérateurs : S. Kerdine-Römer & K. Andréau)

14h30 *Organoids: new tool for toxicology?*
Jessica Feteira, STEMCELL Technologies

14h40 *xCELLigence Real-Time Cell Analyzer: Advancing Discovery Through Innovation*
Pascale Daou, ACEA Biosciences

14h50 *Properties and use of a unique physiological hyaluronic acid-based hydroscaffold for scalable 3D cell culture*
Zied Souguir, HCS Pharma

15h00 Remise des deux prix & Clôture du congrès

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Résumés des présentations orales

Nouvelles approches d'ingénierie tissulaire par bioimpression 4D

Marine Salducci, Poietis Bioparc Bordeaux Metropole, 27 Allée Charles Darwin, 33600 Pessac

Poietis développe des solutions de biofabrication basées sur les technologies de bioimpression pour la conception et la production de tissus, comme le modèle de peau totale Poieskin®. L'automatisation des processus de production devrait être essentielle pour obtenir une biofabrication contrôlée, reproductible et de qualité.

Le processus de fabrication suit trois étapes principales : (1) la conception du tissu où les motifs 3D de cellules et de biomatériaux sont définis par un logiciel de CAO de tissu interne ; (2) la biofabrication du tissu consistant en un dépôt couche par couche de collagène, de fibroblastes et de kératinocytes; (3) la maturation tissulaire au cours de laquelle les motifs cellulaires évoluent vers l'architecture tissulaire fonctionnelle finale. La biofabrication est réalisée à l'aide de notre plate-forme qui combine la bioimpression assistée par laser à la bioimpression microvalve (Next-Generation Bioprinting platform). Les contrôles unitaires sont effectués à l'aide d'outils d'analyse d'images intégrés pour évaluer la qualité de la production.

Nous démontrons un contrôle de l'organisation 3D des cellules et une relation entre le motif de cellule 3D initial et la dynamique de réorganisation cellulaire. Les tissus ainsi fabriqués sont utilisés pour des tests de principes actifs et pourraient également l'être pour des études de toxicologie.

La stratégie de biofabrication décrite ici est générique et peut être appliquée à la conception et à la production de n'importe quel tissu. La production en routine de Poieskin® pour des applications de recherche in vitro démontre la maturité industrielle de la bio-impression.

An innervated, vascularized and immunocompetent human skin model to evaluate the properties of chemical substances.

Quentin Muller[1,2], Marie-Josée Beaudet[2], Sabrina Bellenfant[2], Rémy Pépin[2], Adrien Brulefert[1], Christopher G. Mueller[1], François Berthod[2]* and **Vincent Flacher[1]*** (*equal contribution)

[1] Laboratoire CNRS UPR3572 Immunology, Immunopathology and Therapeutic Chemistry (I(2)CT), Institut de Biologie Moléculaire et Cellulaire, Université de Strasbourg, Strasbourg, France, [2] Centre LOEX de l'Université Laval, Centre de recherche du CHU de Québec-UL, and Département de Chirurgie, Faculté de Médecine, Université Laval, Québec (Québec), Canada.

Pathogens, sensitizing chemicals and autoimmune diseases trigger T cell-driven inflammation in the skin under control of cutaneous Dendritic Cells (DCs). No in vitro human model recapitulates the features of cutaneous immune responses. Indeed, such models frequently lack an epidermal barrier, necessary to investigate topically applied compounds, and fail to acknowledge the influence of non-immune cells which modulate the activation of DCs and T cells. This represents a major shortcoming and prevents accurate pre-clinical evaluation of anti-inflammatory drugs, vaccine adjuvants or potentially allergenic chemicals (sensitizers). To resolve this, we developed a novel Innervated and Vascularized immunocompetent Tissue-Engineered Skin Tissue-Engineered Skin (IV-iTES) combining all structural and functional elements of the healthy skin.

The IV-iTES model integrates human keratinocytes, fibroblasts, dendritic cells and pseudo-capillaries, as well as a sensory nerve network derived from either murine embryos or human induced pluripotent stem (iPS) cells. Our culture conditions were adjusted to guarantee a stable phenotype for all cell populations, thereby mimicking steady-state human skin. The structure of the resulting co-culture and cell-specific markers were thoroughly characterized. Sensory nerves could be triggered in situ by agonists of their specific receptors and subsequently released neuropeptides substance P and CGRP. When exposed to known sensitizers and control molecules, cytokines were released in the supernatant with a pattern that reflected the properties of the chemicals. Altogether, the IV-iTES should allow in-depth investigations and predictions on cutaneous toxicity, angiogenesis and inflammation.

Liver on chip and other 3D hepatic models: Relevance and limits for predictive toxicology

Cécile Legallais, Université de Technologie de Compiègne, UMR CNRS 7338 Biomécanique et Bioingénierie, Compiègne, France

Organ on chip or organoids are promising platform for preclinical studies of new drugs or in predictive toxicology for chemicals. Such alternative methods are promoted to follow the 3R recommendations: reduce, refine, and replace animal trials.

The culture of cells in biochips or microstructured devices, in an adequate environment, demonstrated better and prolonged maintenance of cells' functions or differentiation. However, such devices are not easy to handle, which might limit their use in classical laboratories. To overcome this limit, we have developed at UTC a specific platform where 24 biochips can be positioned in series or in parallel. ADME processes can thus be mimicked in such configuration.

The biotransformation of xenobiotics achieved in the liver represents usually a key element to assess their toxicity either in the same organ or in others located downstream. Coupled to omics approaches, it may lead to the improved knowledge on the effects of substances alone or in mixture on different intracellular pathways.

In this presentation, we will illustrate the benefit of developing such tools in the framework of a hospital-university research project "Innovation in Liver Tissue Engineering" and for toxicology studies regarding pesticides promoted by ANSES agency. We will also comment the potential limits associated with these systems and with data analysis.

"HEPATOPEARL®: NEW GENERATION OF LIVER-MIMICKING SPHEROIDS"

Dr Noushin DIANAT, Cyprio

Despite extensive screening in preclinical stages, Drug-Induced Liver Injury (DILI) remains the main cause for attrition of drugs from late-stage clinical trials and from market, highlighting failure of current *in vitro* models to efficiently predict toxicity. Contrary to monolayer culture of Primary Human Hepatocytes (PHH) traditionally used in hepatotoxicity screening, three-dimensional (3D) liver models seem more promising due to their vivo-resembling functions and extended life span, more fitting to chronic toxicity studies.

To circumvent this drawback, we have developed a novel technology named “BioPearl”, to fabricate miniaturized 3D spheroids from primary human hepatocytes with a high production rate adapted to high throughput screening. Using this technology, micrometric core-shell capsules composed of a thin layer of alginate and a liquid core of cells are generated. "HepatoPearl®" display vivo-mimicking characteristics such as: a) polarized epithelial morphology with the presence of bile canaliculis, b) functional detoxification transporters, c) lifespan of more than 45 days d) high and stable metabolic activity of phase I and II metabolizing enzymes e) albumin secretion f) urea synthesis and g) CYP inducibility over 6 weeks.

During this talk, Cyrpio’s HepatoPearl® model, it’s characterization and application in acute and chronic hepatotoxicity screening will be presented.

Etude de la biologie intestinale par l'utilisation d'organoïdes humains.

Mahe MM[1,2]

1) Inserm UMR 1235 - TENS, University of Nantes, Inserm, 1 Rue Gaston Veil, 44035 Nantes Cedex 1, France

2) Division of Pediatric General and Thoracic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

L'étude des mécanismes associés au maintien, renouvellement et à la différenciation des cellules souches a permis à la communauté scientifique d'avancer dans la compréhension du développement intestinal et des maladies gastro-intestinales. Ces connaissances nous ont également permis de générer de nouveaux modèles physiologiques de «haute fidélité» ou organoïdes. Au cours de cette présentation, je décrirai nos travaux précédents, qui portent sur la production d'organoïdes intestinaux humains à partir de cellules souches adultes et à pluripotence induite. Sur la base de ces modèles, je soulignerais la complexité supplémentaire que nous pouvons ajouter afin de mieux comprendre l'effet du microenvironnement intestinal en physiologie et physiopathologie (exemple du système nerveux entérique). Enfin, je soulignerais comment nous pouvons utiliser de tels modèles pour incorporer un environnement luminal avec des nutriments et des microbes.

A human breathing alveolus-on-chip: applications in toxicology and clinically-relevant in vitro testing

Noria Roldan, AlveoliX AG, Bern, Suisse

The lungs represent one of the largest surfaces exposed in the human body. Their branched and alveolarized structure is essential to ensure oxygen retrieval from the air through gas exchange, as it is the cyclic compression of the diaphragm that allows air to flow in and out. However, this large surface can make lungs vulnerable when air pollutants, nanoparticles and other air insults come into play and can be compromised during proinflammatory processes leading to severe respiratory disorders. When it comes to in vitro studies, models of the alveolus that recapitulate the mechanical constraints and complex subcellular structure of the organ have been so far very limited. This emerging need has led to the development of a breathing lung-on-chip that operates by a nature-inspired microdiaphragm. This breathing-like mechanism allows for the 3D cyclic deflection of an elastic 3 µm-thin porous PDMS membrane onto which human primary alveolar epithelial cells (hAEpC) can be cultured, or co-cultured with other lung cell types, such as endothelial cells, to mimic the air-blood barrier. We have demonstrated that our system allows for the long-term culture of hAEpC that establish a functional and tight barrier after some days in culture. In this context, we have also shown that physiological mechanical stress is important for barrier functions, such as molecule transport across the epithelial layer.

To get insights into the utility of such a system from a toxicological perspective, we have challenged our model employing proinflammatory molecules such as bacterial lipopolysaccharide (LPS) or aerosolized zinc oxide (ZnO) nanoparticles. Preliminary data indicates that stretched hAEpC are more susceptible to ZnO induced nanotoxicity. LPS, on the other hand, although is not sufficient to alter hAEpC barrier integrity in either static or dynamic conditions, requires the integration of endothelial and immune cells which establish a close interplay in the lung-on-chip. These results reveal the relevance of using the lung-on-chip system which not only recapitulates the breathing motion, but can also be tuned to resemble more closely the cell complexity in vivo.

Évaluation de la pertinence de modèles in vitro pour prédire les effets pulmonaires aigus chez le rat après exposition à des nanoparticules de TiO2 et de CeO2

G. Lacroix

Les modèles animaux restent à ce jour l'outil de référence pour caractériser les effets pulmonaires des nanoparticules (NPs) chez l'homme. Cependant, pour des raisons éthiques et économiques, le recours à des méthodes de substitution est nécessaire. Les modèles *in vitro*, utilisant des cellules pulmonaires, représentent une alternative intéressante, néanmoins, leur pertinence doit être démontrée.

Les méthodes *in vitro* utilisent classiquement des cultures constituées d'un seul type de cellules, exposées par contact avec la substance d'intérêt via le milieu de culture (exposition en mode submergé). Des modèles plus élaborés font appel à des co-cultures (comprenant deux types cellulaires ou plus), cultivées à l'interface air-liquide (ALI) pour recréer les conditions physiologiques observées au niveau des poumons. Ces cultures sont par la suite exposées directement à des aérosols de NPs pour mimer au mieux une exposition par inhalation.

L'hypothèse testée dans le cadre de ce travail est que les modèles cultivés en ALI et exposés à des aérosols de NPs permettent de prédire, de manière plus fiable que les modèles exposés en submergé, les réponses biologiques observées *in vivo*.

Les réponses obtenues à l'aide de différents modèles *in vitro* ont été comparées aux réponses pulmonaires de rats exposés par voie respiratoire par instillation intra-trachéale aux mêmes NPs. *In vitro*, une co-culture de cellules épithéliales alvéolaires (lignée A549) et de macrophages (lignée THP-1 différenciée) a été exposée à des NPs peu solubles (TiO_2 ou CeO_2) en mode submergé ou à l'interface air-liquide (ALI). Après 24h d'exposition, les réponses pro-inflammatoires cellulaires ont été évaluées et des comparaisons quantitatives ont été effectuées avec les réponses inflammatoires obtenues *in vivo*, en utilisant des métriques identiques. Quelque soit la métrique utilisée (masse/surface alvéolaire ou masse/nb de macrophages), la méthode *in vivo* s'est révélée la plus sensible. Les réponses biologiques observées étaient similaires entre l'*in vivo* et l'*in vitro* mais la méthode ALI, censée le mieux mimer l'environnement alvéolaire, s'est révélée la plus prédictive des effets *in vivo*, en termes de doses d'effet. Les différences entre l'*in vivo* et l'*in vitro* étaient moins marquées quand la masse était normalisée par le nombre de macrophages par rapport à la surface alvéolaire. En revanche, il n'a pas été possible de conclure clairement sur le classement relatif (« ranking ») des NPs en raison de la faible toxicité des NPs étudiées.

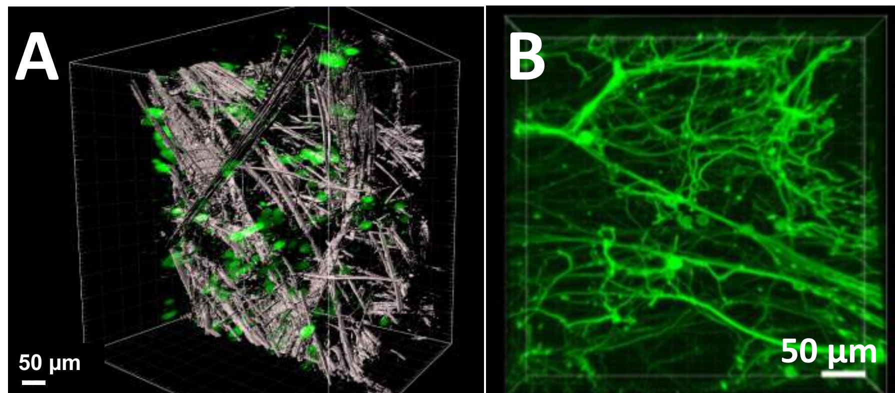
En conclusion, l'utilisation de modèles cellulaires *in vitro,* plus représentatifs de l'organisation cellulaire au niveau des poumons et de méthodes d'exposition *in vitro* plus réalistes, permet d'obtenir des informations plus fiables et pertinentes sur la toxicité à court-terme des NPs de TiO_2 et CeO_2 (en termes de doses d'effet). Des études complémentaires doivent être menées pour évaluer si le classement relatif des NPs est modifié par la méthodologie mise en œuvre.

Une approche supramoléculaire pour la culture de cellules neuronales en 3D

Laurence Vaysse[b], Anaïs Chalard[a,b,c], Pierre Joseph[c], Laurent Malaquin[c], Sandrine Souleille[c], Barbara Lonetti[a], Jean-Christophe Sol[b,d], Isabelle Loubinoux[b] , **Juliette Fitremann[a]**

[a]IMRCP, Université de Toulouse, CNRS, Bat 2R1, 118 Route de Narbonne, 31062 Toulouse Cedex 9, France. [b] TONIC, Toulouse NeuroImaging Center, Université de Toulouse, Inserm, UPS, France. [c]LAAS-CNRS, Université de Toulouse, CNRS, UPS, Toulouse, France, [d] Centre Hospitalier Universitaire de Toulouse; Pôle Neurosciences; CHU Toulouse, France

Différentes approches sont développées actuellement pour obtenir des modèles de tissu neuronal en 3D. Après avoir discuté quelques exemples issus de la littérature, nous présenterons comment nous avons développé une matrice synthétique à base de molécules auto-assemblées, non polymère. L'assemblage spontané de ces petites molécules fournit un enchevêtrement de fibres hétérogènes et un hydrogel très peu rigide et permissif aux cellules (Fig. A). La croissance de cellules souches neurales humaines adultes sur cette matrice conduit à un réseau mixte et hétérogène de cellules gliales et de cellules neuronales. D'autre part les fibres, structurées à l'échelle microscopique, guident la croissance de neurites de formes variées sur de très longues distances (Fig. B).



Références:

(1) Chalard, A.; Vaysse, L.; Joseph, P.; Malaquin, L.; Souleille, S.; Lonetti, B.; Sol, J.-C.; Loubinoux, I.; Fitremann, J. Simple Synthetic Molecular Hydrogels from Self-Assembling Alkylgalactonamides as Scaffold for 3D Neuronal Cell Growth. *ACS Applied Materials & Interfaces* **2018**, *10* (20), 17004–17017. https://doi.org/10.1021/acsami.8b01365.

(2) Zhuang, P.; Sun, A. X.; An, J.; Chua, C. K.; Chew, S. Y. 3D Neural Tissue Models: From Spheroids to Bioprinting. *Biomaterials* **2018**, *154* (Supplement C), 113–133. https://doi.org/10.1016/j.biomaterials.2017.10.002.

Le placenta perfusé et ses perspectives en toxicologie

Sophie Gil, Faculté de Pharmacie de Paris, Université Paris Descartes

UMR-S 1139 « Physiopathologie et pharmacotoxicologie placentaire humaine »

L'un des axes développés au laboratoire est l'étude du passage transplacentaire et des effets sur les fonctions placentaires de médicaments et de polluants environnementaux. L'exposition des femmes enceintes à des agents toxiques est inévitable. De fait, pendant la grossesse, ces toxiques peuvent se retrouver dans la circulation maternelle et être en contact avec le placenta.

Dès lors que nous parlons de toxicité pendant la grossesse, l'intérêt premier a toujours porté sur la connaissance du passage ou du non passage d'une molécule dans la circulation fœtale. Ces études de passage ont toujours suscité un vif intérêt pour appréhender le risque encouru pour le développement du fœtus. Pour répondre à ces questions, le modèle du cotylédon perfusé isolé a été développé durant les années 1970. Il est le seul modèle à reproduire la double circulation fœto-maternelle tout en respectant la particularité anatomique du placenta humain à terme. Techniquement, cette approche permet d'étudier le transport de molécules, de varier des paramètres maternels et/ou fœtaux, d'utiliser des placentas pathologiques ou de mimer certaines pathologies. De plus, depuis quelques années, avec les avancées de la biologie placentaire, nous savons qu'il ne faut plus ignorer les répercussions des xénobiotiques sur le placenta. En effet, le placenta représente une cible privilégiée des toxiques circulants compte tenu d'une perfusion élevée (> 600 mL/min à terme). Il va les capter, il peut les métaboliser, les stocker, les relarguer dans la circulation maternelle ou les laisser passer dans la circulation fœtale. Le placenta joue alors un vrai rôle de rempart mais cette exposition peut engendrer un dysfonctionnement placentaire ayant des répercussions sur le déroulement normal de la grossesse.

Après avoir décrit les particularités spécifiques du placenta tout au long de la grossesse, une revue de la technique de perfusion sera faite avec l'application de diverses conditions de perfusion, mettant en évidence les avantages de cette technique. Les limites de ce modèle ainsi que les apports récents seront détaillés au cours de la présentation, tout particulièrement pour les études en début de grossesse.

Résumés des posters

POSTER N°1

Modulation of the biological response of human reconstituted airway epithelium exposed to fine particles differing by their emission sources (traffic vs industry)

<u>Achard Sophie</u>[1], Seurat Emeline[1], Chang A-L[1], Courcot D[2], Momas I[1], Seta N[1], Verdin A[2], Cazier F[3].

[1] Inserm UMR 1153 - CRESS - HERA team (Health Environment & Risk Assessment), Faculty of Pharmacy of Paris, Paris Descartes University, Paris, France.
[2] Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV)-EA 4492, SFR Condorcet FR CNRS 3417, University of Littoral Côte d'Opale, Dunkerque, France.
[3] Centre Commun de Mesures, Maison de la Recherche en Environnement Industriel, University of Littoral Côte d'Opale, Dunkerque.

Many epidemiological studies have shown associations between respiratory and cardiovascular diseases and airborne pollutants, including particulate matter (PM).
For a better understanding of the PM's health impact, the present study aims to evaluate the impact of two samples of fine particles ($PM_{2.5}$) differing by their emission sources (traffic vs industry) and thus by their chemical composition (heavy metals, paraffin, polycyclic aromatic hydrocarbons) on the human airway epithelium.
To mimic the real human exposure to PM a 3D *in vitro* model was used: a human airway epithelium reconstituted co-cultured with human airway fibroblast (MucilAir™). The epithelia were exposed to PM twice a week for 2 consecutive weeks. 48h after each exposure, the culture medium on basal side was collected and inflammatory response was assesed. At the end of each week the membrane integrity was evaluated using the TEER (trans-epithelial electrical resistance) measurement and part of the epithelia was sacrificed for gene expression assessment. RNA extraction was conducted in order to analyze the PM's influence on inflammatory, oxidative stress and metabolic gene expressions by RTqPCR.
Without loss of membrane integrity, a significant increase of the inflammatory response appears. IL8, IL6 and GM-CSF secretions were higher after PM-Traf exposure compared to PM-Ind. In addition, some gene expression modulation appears: increase of inflammatory biomarkers (IL8, IL6 and GM-CSF) and decrease of oxidative stress biomarkers (NOS, Hmox1, SOD). Finally, a significantly induction of metabolic gene expression (Cyp1A1, Cyp1B1) was observed after exposure to PM-Ind and to a lesser extent to PM-Traf.
The highlights of this study are: 1/ the importance of considering of the chemical composition of the particulate matter in the risk assessment, and 2/ the importance to use an *in vitro* model that approximate the real human exposure to evaluate the impact of repeated exposures to environmental pollutants.

POSTER N°2

HEALS: transcriptomic study following exposure of hepatic HepaRG cells to a mixture of phthalates and heavy metals

Martine Aggerbeck, Emilie Distel, Spyros Karakitsios, Eléonore A Attignon, Béatrice Le-Grand, Etienne Blanc, Milena Horvat, Robert Barouki, Denis Sarigiannis

UMR-S 1124 INSERM Université de Paris

Throughout their lives, human are exposed to xenobiotic mixtures and other stressors, defined as the exposome. The European project HEALS (Health and Environment-wide Associations based on Large population Surveys) aims at the comprehension of the role of the global environment in the outcome of pathologies.
Phthalates metabolites and metals were measured in biological samples of 2 european cohorts (REPRO PL and PHIME). The levels in the human liver for the 3 most abundant phthalates and 4 metals (methylmercury, mercury, cadmium and lead) were estimated by a PBBK (Physiologically Based BioKinetic) model. We treated the human hepatic cell line HepaRG with the combination of the phthalates and metals at 2 concentrations: Mix 1 (estimated concentrations in the liver) and Mix 2 (10 times Mix 1) for 3 weeks. We verified that the treatments did not affect the cell viability, compared to the control (1.7% DMSO and 1% HNO_3) condition.
After 3 weeks, mRNA, proteins and cellular extracts were prepared for multiple OMICs analyses. We present the preliminary transcriptomic results. Although the concentrations of phthalates and metals used were low, using the Ingenuity (IPA) software, a number of networks was deregulated as well as Tox Lists describing toxicity-relevant gene sets. The ten most up-regulated genes in both Mix1 and Mix2 conditions were different except for one gene (RNA variant U1 small nuclear 14) and for the ten most down-regulated ones, 4 common genes were found (inhibin subunit BE, SPINK1, complement C8 beta chain, retinol dehydrogenase 16). These genes are involved in endoplasmic reticulum stress, pancreatitis, acute phase response, retinoic acid metabolism.
PCR experiments will be performed. The proteome and the metabolome of the cells after the treatments compared to the control samples are currently analyzed and the comparison of the 3 OMICs data should provide information on the metabolisms affected by the pollutants tested.

POSTER N°3

Enhanced Micronucleus genotoxicity assay using 3D reconstructed Human epidermises

Andres Eric

OROXCELL, Romainville

The Genotoxic potential of dermato-cosmetic substances is generally determined using *in vitro* methods. Among them, the micronucleus assay is recommended by regulatory agencies. Up to now, this assay has been performed using cells in suspension and required a testing of substances in the absence and in the presence of non-human relevant metabolic activation. However, despite its regular use, this process has some limitations, mostly when tested substances are non-water soluble and to also consider the risk associated with topical exposure and not only related to a systemic exposure. In addition, the applied metabolic activation is of rat origin and is non-human relevant.
In order to circumvent these drawbacks, reconstituted human epidermis models, which are now extensively used for safety assessment of novel topical products, is proposed to substitute cells in suspension. Due to their physiological properties, some commercially available RHE models are strongly mimicking Human natural skin, moreover, the use of these models ensures an entire exposure of the test substance to the test system, independently to the lipophilicity displayed by the evaluated substance. Furthermore, the metabolic capacity of the reconstructed epidermises is close to that observed in native human skin, thus making the identification of the genotoxic potential of resulting metabolites more predictive.
In this work, we describe our results on the evaluation of the reconstructed epidermis model suitability for the performance of the cytokinesis-block micronucleus assay. We established appropriate culture conditions, by determining the basal level of micronuclei and evaluated the possible effect of generally used solvents on micronuclei scoring.
We investigated the performance of cytokinesis-block micronucleus assays in reconstructed epidermis to predict in vivo genotoxins by topical administration of known clastogens, aneugens, progenotoxics and non-genotoxics, and the performances of the assay were determined.

POSTER N°4

Chemical characterization and toxicological effects of emissions generated from new tobacco- and smoking-related products: comparison with conventional cigarette smoke

Romain DUSAUTOIR, Gianni ZARCONE, Marie VERRIELE, Guillaume GARÇON, Isabelle FRONVAL, Nicolas BEAUVAL, Delphine ALLORGE, Véronique RIFFAULT, Nadine LOCOGE, Jean-Marc LO GUIDICE , **Sébastien ANTHERIEU**

EA4483, Université de Lille

Cigarette smoke exposure is responsible for almost 30% of cancer deaths and the cause of nearly 90% of lung cancer. Smoking cessation is, at present, the only effective way to slow down the progression of cancer. Electronic cigarettes (e-cig) and more recently heated tobacco products (HTP) provide an alternative for smokers as they are generally perceived to be less harmful than conventional cigarettes. These new devices can quickly gain popularity, even before there is sufficient scientific evidence to determine their effects on the user.

This work was performed (i) to characterize the chemical composition (carbonyl compounds and polycyclic aromatic hydrocarbons) of these tobacco-related product emissions, (ii) to investigate their potential toxicity in human bronchial epithelial cells (BEAS-2B) cultured at the air-liquid interface, and (iii) to compare their emissions and their respective toxicities to those of cigarette smoke. Aerosols were generated by a smoking machine from HTP, different models of e-cig (low or high power) or conventional cigarette (3R4F). The numbers and concentrations of carbonyl compounds and PAHs emitted by HTP and e-cig are lower than with cigarette smoke. At the cellular level, HTP exposures induce a cytotoxicity higher than the different e-cig models tested, but lower than conventional cigarettes. Significant increases in IL-6, IL-8, IFNg and GM-CSF secretion are observed in BEAS-2B cells after HTP or 3R4F exposures, while only IL-6 is increased for e-cig.

Overall, these data evidence lower emissions of harmful chemicals and lesser *in vitro* toxicity of HTP compared to cigarette smoke. By contrast, HTP generate higher levels of hazardous compounds and result in greater cytotoxicity than e-cig. Moreover, effects observed with e-cig are more marked when they are used at high rather than low power. These first conclusions shall be further comforted by additional experiments conducted in animal models after long-term exposures.

POSTER N°5

Impact of the inflammatory mediators produced by the ocular surface exposed to environmental pollutants on the respiratory tract

Ashrin Aya$**, **Sawaya Rami$, Bossou Marc, Pratama Septa, Chang Anne-Laure, Momas Isabelle, Seta Nathalie, Achard Sophie.

$ Equal contribution
[1]Inserm UMR 1153 - CRESS - HERA team (Health Environment & Risk Assessment), Faculty of Pharmacy of Paris, Paris Descartes University, Paris, France.

Air pollution consists of different pollutants: chemicals like pesticides, particulate matter (PM) emitted by combustion sources for example, and biological contaminant such as mycotoxins. Regardless of the type of pollutant, pollution is an important issue associated with various health impacts and concerning whole population: occupational and general population. Besides respiratory and cardiovascular problems, air pollution can affect the eye, causing eye redness, irritation, blurring of vision. However, the link to the environment is still too often overlooked. A repeated exposure by ocular contact may cause the release of inflammation mediators cytokines. These biological substances will be eliminated through the nasolacrimal duct, and will enter into contact with the nasal mucosa, increasing the sensitivity of the respiratory system and leading to a mucosa hyper-reactivity reaction.
The main purpose of this study was to evaluate the inflammatory response of Human Corneal Epithelial (HCE) cells under various environmental exposure situations: pesticides (Captan, Folpet and Chlorpyrifos), fine particles (PM2.5 from industry and traffic influence) and, mycotoxins (secondary metabolites produced by fungi, such as *Aspergillus* and *Fusarium*). The secondary goal was to assess the role of inflammation markers produced by the HCE cells after exposure on the inflammatory response of human airway epithelial cells (hAEC).
Regardless of the pollutants tested, our results indicate an increase in the biological activity in terms of inflammatory response, with a modulation of gene expression (inflammatory and oxidative stress) after pollutant exposures. The outcomes of the inflammatory mediators produced by ocular surface modulate the biological response of the nasal epithelial cells.
Our results indicate that the biomarkers produced by the ocular surface could contribute in the development or the exacerbation of the respiratory diseases.

POSTER N°6

Amorphous silica nanoparticles trigger human dendritic cell maturation *in vitro* and provoke CD4+ T Cell proliferation

Alexia Feray, Marie Hullo, Natacha Szély, François-Xavier Legrand, Emilie Brun, Eléonore Guillet, Sabrina Barillet, Marc Pallardy and Armelle **Biola-Vidamment**

INSERM UMR996, Université Paris-Sud, Châtenay-Malabry

Amorphous silica nanoparticles (aSNPs) are widely used in dietary supplements, biomedical applications, cosmetics or construction materials. General population is probably more exposed than initially anticipated and occupational exposure, particularly via the inhalation route, should be better evaluated. Indeed, despite considered as highly biocompatible as compared to their crystalline counterparts, aSNPs could contribute to or exacerbate the onset of allergic airway disease. Dendritic cells (DCs) could sense these nanomaterials and undergo a maturation process enabling them to migrate to regional lymph nodes and to activate naive T-lymphocytes. The aim of this work was to evaluate the effects of aSNPs on human DCs *in vitro*. Human monocyte-derived DCs were exposed for 16 hours to final concentrations of 12,5 and 25 µg/ml of fumed silica nanoparticles. Endotoxin levels were unlikely to have any effect on DCs since no activity was found in the media. Results showed that the aSNP significantly upregulated the CD86 costimulatory molecule, as well as the CD83 maturation marker and the CXCR4 chemokine receptor surface expressions. Secretions of inflammatory cytokines were significantly enhanced in a dose-dependent manner in the DC culture supernatants. To evaluate whether aSNPs could induce DC to become functionally mature, we assessed their capacity to activate allogeneic T-cells. Results showed that the increase in T-lymphocytes proliferation in presence of aSNP-treated moDCs was statistically significant for all tested DC/T ratios compared to unloaded DCs. Moreover, analysis of the co-culture supernatants for the production of T cell-derived cytokines showed a significant increase of IL-9 and IL-17A and F, and an upregulation of IL-5, consistent with the pro-inflammatory phenotype of DCs described above. Taken together, these results suggest that aSNPs are able to induce functional DCs maturation and could act as adjuvants of the immune system.

POSTER N°7

Impact on the human HepG2 cell metabolome of exposure to a mixture of 2 persistent organic pollutants.

Cédric Caradeuc[1], Béatrice Le-Grand[2], Aude Catalayud[1,2], Adama Ka[1,2], Martine Aggerbeck[2], Gildas Bertho[1,$], **Etienne Blanc**[2,$,].

[$] Equal contribution
[1] Paris Descartes University, USPC, CNRS UMR 8601, 45 rue des Saints Peres, 75006 PARIS, France
[2] Paris Descartes University, USPC, INSERM UMR-S 1124, 45 rue des Saints Peres, 75006 PARIS, France

Over the past decades, human exposure to environmental pollutants has become a chronic insult. The general population is impacted by the increasing amounts and types of xenobiotics which are associated with long-term pathologies such as chronic liver diseases and metabolic diseases. The mechanisms leading to these effects are multi-factorial and remain largely unknown. Our work focuses on the Persistent Organic Pollutants (POPs) which are resistant to biological and chemical degradation and accumulate along the trophic chain. This study uses metabolomic analyses to detect changes in specific metabolite levels to identify modifications of signaling or metabolic pathways. We aimed to find modifications in hepatic metabolism after exposure to two environmental pollutants (TCDD, a dioxin and ✔-endosulfan, an organochloride pesticide), alone or in combination. To obtain metabolomic profiles, we performed untargeted NMR-based metabolomics on the *in vitro* liver model, HepG2. Proliferating HepG2 cells were exposed for 48h to TCDD (25nM), ✔-endosulfan (10µM), or the mixture. For NMR analyses (500 MHz), extra-cellular media were collected whereas cell pellets underwent chloroform-methanol extraction to obtain the polar phase. The analysis of the exo- and endo- metabolomes revealed several changes in the amounts of metabolites after exposure (phospho-choline, glutathione, lactate, redox co-factors, some amino-acids). Some of these metabolites might represent biomarkers for the hepatotoxicity of POPs and shed light on the disturbed pathways *in vitro* (sphingolipid metabolism, ROS regulation, energetic metabolism) which could contribute to hepatotoxicity. Our work validates the use of untargeted NMR profiling of metabolites from different fractions of *in vitro* cultured HepG2 cells to study the cocktail effect of pollutants on the cell metabolome. We also plan to use our workflow on other models (such as HepaRG cells) exposed to various pollutants.
European Heals Project n°603946.

POSTER N°8

Cryogel integrated microchip: towards a human 3D model of liver cancer

Lilandra Boulais[1], Rachid Jellali[1], Ulysse Pereira[1], Patrick Paullier[1], Eric Leclerc[1], Sidi A. Bencherif[1,2,3], Cécile Legallais[1]

[1] UMR CNRS 7338, Laboratoire de Biomécanique et Bioingénierie, Université de Technologie de Compiègne, Compiègne, France
[2] Laboratory for Advanced and Multifunctional Polymer Biomaterials, Department of Chemical Engineering, Northeastern University, Boston,USA
[3] Harvard John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, USA

Recently, a number of studies have highlighted that the cell microenvironment plays a critical role in various biological processes. In this context, 3D cell culture systems have been the focus of much attention to mimic more closely the natural environment found in vivo. To this end, we have developed an innovative technology combining biomaterials and microfluidics. A polydimethylsiloxane (PDMS) microchip containing a macroporous alginate hydrogel was designed for hepatocyte culture to create a liver tissue.
The microfluidic device was successfully engineered to ensure a homogeneous perfusion while providing a micro-sized cell culture chamber in 3D. Inside this microchip device, alginate was covalently crosslinked at subzero temperatures to create a macroporous scaffold which was then coated with collagen before cell seeding. HepG2C3A, a human liver cancer cell line, were cultured in this device in dynamic conditions (10µL/min) for 7 days.
The physical properties of the integrated cryogels were characterized: the SEM observations showed a macroporous structure with an average pore size of 100µm (Fig.1). The cryogel presented a high degree of pore connectivity and swelling ratio allowing cell culture medium circulation through its 3D structure.
The HepG2C3A cells adhered on alginate cryogel and spread inside this macroporous scaffold. The SEM observations showed that after 7 days of culture, these proliferative cells formed 3D structures which remained inside the microchip after the removal of the alginate cryogel by alginate lyase (Fig.2).
Looking into the future, this innovative microchip may be a promising device to improve predictive toxicology and to develop personalized medicine.

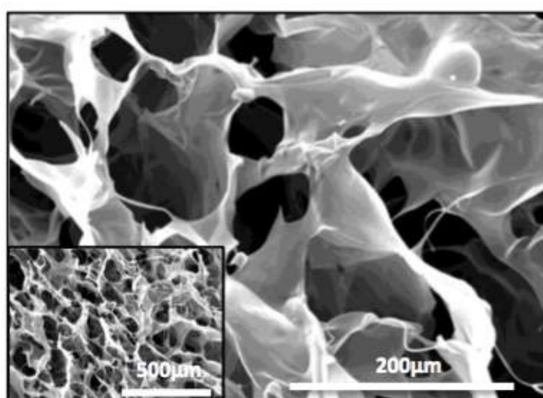


Fig.1 SEM image of alginate cryogel

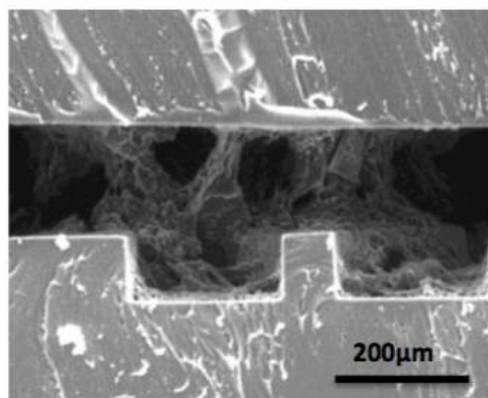


Fig.2 SEM image of HepG2C3A at day 7 in dynamic conditions (10µL/min) after removal of alginate cryogel

POSTER N°9

Using Impedance-Based Approaches for Measuring Cell-Mediated Cytotoxicity and Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

Leyna Zhao

ACEA Biosciences Inc

The most common method for measuring cell mediated cytotoxicity is the release assay based on the loss of target cell membrane integrity. Within up to 4 hours following effector cell addition resulting in target cell lysis, either the radioactive release from target cells pre-labeled with Chromium-51 or Indium-111 is measured, or the release of naturally occurring substances, such as lactate dehydrogenase (LDH), into the culture medium is assayed. Release of these substances thus serves as an indirect measure of the extent of cell damage due to effector cell-mediated target cell lysis. Alternative endpoint methods also include flow cytometry, enzyme-linked immunosorbent assay-based granzyme measurement, and morphometric analyses by microscopy.
Here we describe an impedance-based real-time label-free method that can automated capture the kinetics of the cell mediated or antibody dependent cell mediated cytolysis (ADCC) of the target cancer cells. To determine if cell-mediated cytotoxicity, and specifically ADCC can be investigated using an impedance-based xCELLigence system, the response of tumor cells as target cells) to natural killer (NK) cell activity (as effector cells), in the presence or absence of immunoglobulin G isotype-specific antibody, was measured. Importantly, it is shown that the addition of NK cells in suspension, over a monolayer of adherent tumor cells, does not produce impedance changes, because the NK cells do not come in contact with the electronic sensor. However, the secretion of perforins and granzymes by these non-adherent NK cells does activate caspases inducing tumor cell apoptosis. Dysfunctional and dying tumor cells detach from the sensor electrode, reducing the number of viable and adhering cells on the electrode surface.
Overall, our results show that the impedance-base technology is a label free alternative to the traditional end-point assays. The automated readouts provide direct, sensitive and specific measurement of target cell changes both short-term (hrs) and long-term (days). It allows the easy quantification of the cell-mediated cytotoxicity and evaluation of the potencies for specific antibodies.

POSTER N°10

The potential of 3D reconstructed Human intestinal models for biowaiver studies and finished products testing

<u>Christophe DINI</u>

OROXCELL, Romainville

Biowaiver approaches consists in waiving in vivo bioavailability and /or bioequivalence requirements using regulatory non-clinical approaches. These studies are considered as non-clinical toxicological studies and are to help the spreading of certain API generic forms, while avoiding non necessary exposure of healthy volunteers to APIs in pivotal clinicals studies.
In the context of *in vitro* studies, Caco-2 cell lines have become the most frequently used models to perform such studies. Recently, we have explored novel organotypic 3D reconstructed Small Intestine models based on Human cells to at least conduct biowaiver studies, but also using formulated API to estimate the impact of formulation in promotion of absorption and to make comparison of an adult versus a pediatric form which are currently in development, at doses corresponding to those employed in clinics.
The object of this presentation is to give an insight into the added value that might bring these novel models over Caco-2 cells, to evaluate finished products.

Développement d'un modèle in vitro pour l'évaluation de l'hépatotoxicité à dose répétée

Sylvie EMERY, Anne RIU, Renaud Barbier, Hélène Riesterer, Reine Note, Silvia Teissier, Valérie Michaut

LOREAL, Aulnay sous Bois

Le développement de méthodes d'évaluation, capables de mimer les fonctions physiologiques d'organes humains cibles de toxicité à dose répétée, est devenu un enjeu clé, afin de faire face aux challenges actuels en évaluation de la sécurité, notamment depuis l'interdiction de l'évaluation sur l'animal pour les ingrédients cosmétiques.
Vinkent et al (2012) ont montré que le foie était l'organe cible dans des études d'exposition par voie orale chronique *in vivo* chez le rat, du fait de son rôle dans la transformation et l'élimination des xénobiotiques.
Nous avons développé un test 3D hépatique de sphéroïdes HepG2, simple et rapide sur 10 jours. Les sphéroïdes sont formés en 6 jours, puis traités quotidiennement pendant 96h, avec renouvellement intégral des solutions. Les mesures réalisées sont la viabilité cellulaire, ainsi que la quantification du biomarqueur hépatique précoce : l'albumine, le dernier jour de traitement.
La calibration et la caractérisation de ce modèle ont été validées avec un set de molécules de références connues. Les résultats ont montré une nette amélioration des fonctions hépatiques de la lignée hépatocytaire HepG2 cultivée en sphéroïde 3D par rapport à la culture 2D ; aussi bien en activité enzymatique, qu'en fonction de biosynthèse, et expression génique. Le modèle 3D Sphéroïde a été ensuite évalué avec un set de composés hépatotoxiques et non hépatotoxiques. Un profil de transcriptomique ciblée a été réalisé pour un sous set de composés permettant d'identifier des modes d'action spécifiques.

POSTER N°12

PREVITOX, un réseau national pour l'évaluation préclinique et pharmacogénétique de la toxicité médicamenteuse

Pierre-Jean FERRON*, Bruno Clément, les membres du réseau PREVITOX

NuMeCaN, INSERM, Rennes

L'administration de produits de santé est susceptible d'induire des effets toxiques pouvant causer des effets indésirables graves (EIG) associés à une morbidité et à une mortalité importante. Certains facteurs peuvent expliquer la forte croissance des EIG dont le nombre a doublé au cours des 15 dernières années, notamment l'augmentation de l'incidence des pathologies chroniques et du nombre de patients polymédicamentés, les troubles du métabolisme ainsi que les susceptibilités individuelles. Dans ce contexte, le projet PREVITOX a pour objectif d'établir un réseau d'experts, de laboratoires et de plateformes afin de proposer des solutions alternatives aux modèles animaux pour l'évaluation de la toxicité des produits de santé. Le réseau est composé de 32 laboratoires, 4 infrastructures nationales et 1 accélérateur de technologie (ART-Bioprint), qui sont organisés en 4 groupes de travail (GT) :le GT1 pour la toxicologie in vitro sur des modèles cellulaires (2D, 3D, organoïdes) de lignées cellulaires et de cellules différenciées issues d'iPS humaines ; le GT2 pour l'identification de biomarqueurs dans les modèles in vitro par des approches protéomiques et métabolomiques ; le GT3 pour la collection des données biologiques issues de la pharmacovigilance et de pharmacogénétique pour valider des paramètres de susceptibilités individuelles ; le GT4 pour la modélisation des données obtenues d'une part in vitro et chez les patients, et d'autre part in silico, à partir des structures moléculaires des médicaments. L'objectif du réseau et de proposer un outil polyvalent pour l'évaluation de la toxicité de produits de santé, notamment dans les phases pré-AMM. Le réseau PREVITOX pourra également intervenir lors de l'apparition de EIG chez des individus isolés ou dans des sous-groupes des populations cibles, dans le but d'analyser les mécanismes cellulaires et moléculaires impliqués ; d'identifier des biomarqueurs spécifiques à ces toxicités inattendues ; et d'évaluer le rôle des susceptibilités individuelles de réponse à ces traitements.

POSTER N°13

Transcriptomic modifications induced by air pollution-derived $PM_{2.5}$ reflect the shift from healthy to COPD-diseased human bronchial epithelium.

Sébastien ANTHERIEU, Bérénice LECLERCQ, Laurent Y ALLEMAN, Esperanza PERDRIX, Patrice CODDEVILLE, Jean-Marc LO GUIDICE, **Guillaume GARÇON**

EA4483, Université de Lille

The chronic exposure to air pollution-derived fine particulate matter ($PM_{2.5}$) is suspected of exacerbating and even initiating chronic inflammatory lung diseases. However, the knowledge of the underlying mechanisms by which PM exerts its harmful health effects is still incomplete and detailed *in vitro* studies using novel tissue engineering tools, helping in recapturing the native lung environment *ex vivo*, are highly needed. The goal of this study was to assess transcriptomic modifications in human bronchial epithelial cells exposed to $PM_{2.5}$ and better understand how this exposure may lead to a shift from healthy to COPD phenotype.
Normal human bronchial epithelial (NHBE) or COPD cells were differentiated at the air-liquid interface and repeatedly exposed to $PM_{2.5}$ for 72 h. Their transcriptomes were analyzed using human pangenomic microarrays. Basal transcriptomes of both the control cell phenotypes were first compared and 1280 transcripts were significantly deregulated in COPD vs NHBE cells. The transcription of 1168 genes was significantly altered by $PM_{2.5}$ exposure in NHBE cells. Interestingly, among these deregulated mRNA, a large number (679) were in common with those found in shift from NHBE to COPD phenotype. Indeed, within this set of genes, we observed increased expression of genes involved in inflammation (e.g. TGF-b, IL-17, IL-33, CXCL1, CXCL3, CXCL6, CXCL8, CXCL14, CCL20), as well as genes associated with extracellular matrix remodelling (e.g. EGF, FGF1, KRT4, MMP9, MMP 13, TIMP-1). By contrast, only 107 transcripts were modulated by $PM_{2.5}$ in COPD cells, evidencing an exacerbation of COPD. All these alterations were thereafter validated by RT-qPCR and at functional protein level.
Overall these results showed that the transcriptomic modifications induced by $PM_{2.5}$ deregulated key pathways involved in COPD pathogenesis. These first conclusions shall be further comforted by additional experiments conducted in animal models after longer-term exposures.

POSTER N°14

Differential miRNA expressions related to air pollution-derived $PM_{2.5}$ in repeatedly exposed healthy and diseased 3D organo-typic mucocilary-phenotype models

Sébastien ANTHERIEU, Céline GRARE, Djamal ACHOUR, Bérénice LECLERCQ, Laurent Y ALLEMAN, Esperanza PERDRIX, Patrice CODDEVILLE, Jean-Marc LO GUIDICE, **Guillaume GARÇON**

EA4483, Université de Lille

The chronic exposure to air pollution-derived fine particulate matter ($PM_{2.5}$) has been associated to severe lung diseases, including lung cancer. Although genome-wide approaches have been sometimes used to assess the effect of $PM_{2.5}$ on human health, miRNA expression remains largely under-investigated. To better unravel the cellular and molecular mechanisms activated by $PM_{2.5}$ in human bronchial epithelial cells, in this work, attention was carefully focused on the differential miRNA expressions in repeatedly exposed healthy and diseased human bronchial epithelial cells. RNA-seq analyses were used to study the miRNA expression of normal human bronchial epithelial (NHBE) and COPD cells, differentiated at the air-liquid interface, and repeatedly exposed to $PM_{2.5}$ for 72 h. A combination of different bioinformatics tools facilitated the analyses of miRNA expression patterns, their interactions with gene expression, and the gene networks related to cellular and molecular responses to $PM_{2.5}$. The miRNomes of both the control cell phenotypes were first compared and 32 miRNA, 23 up- and 9 down-regulated, were reported in COPD vs NHBE cells. There were 38 miRNA found as significantly up- (31) or down (7)-regulated in NHBE cells repeatedly exposed. Interestingly, among them, 29 miRNA, involved in inflammation, autophagy, as well as β-cathenin/wnt or pro-fibrogenic/TGF-β pathways, were also significantly differentially expressed in basal COPD versus NHBE phenotypes. Only 6 miRNA were down-regulated in repeatedly exposed COPD cells, supporting thereby only a slight exacerbation of COPD. Identification of the critical gene targets of the more relevant candidate miRNA is actually under study.
Taken together, these data highlighted the differential miRNA expressions induced by $PM_{2.5}$ in repeatedly exposed healthy and diseased 3D organo-typic mucocilary-phenotype models and shed new lights on possible relevant mechanisms by means of which $PM_{2.5}$ acts as harmful factor for human health.

POSTER N°15

Culture of primary pulmonary cells at air-liquid interface: a suitable 3D model to study exposure to particles from pyrotechnic smokes?

V. Martin de Lagarde, C. Corbière, C. Monteil

Université de Rouen Normandie, Laboratoire ABTE EA 465, équipe Toxicologie de l'Environnement : Milieux Aériens et Cancers, Rouen, France

Pyrotechnic smokes are widely used for civilian and military applications: protection, security, festivity, obscuring or signaling. Smokes combustion induce severe particulate matters (PM) pollution episodes and because of their size, PM penetrate deep into the respiratory system and impair cellular defenses. There is a lack of data on the toxicity associated with smoke particles. Consequently, the aim of this study is the toxicological assessment of particles generated from combustion of two different smokes, a red signaling smoke (F1) and an obscuring one (F4), on a 3D model of primary human pulmonary cells. Normal Human Bronchial Epithelial cells (NHBE) were grown at an Air Liquid Interface where they differentiated and formed a pseudo-stratified epithelium. Cytotoxicity (MTT) and genes expressions (RT-qPCR) of inflammation and anti-oxidant responses were explored after 24h exposure and 24h recovery. Results show that both particles did not induce cytotoxicity and only F1 significantly altered genes expression. Particles significantly induced superoxide dismutase 1 (SOD1) and 2 (SOD2), NADPH quinone oxidoreductase-1 (NQO1) and heme oxygenase-1 (HO1) expressions. After 24h recovery, the expressions of SOD1, SOD2 and HO-1 returned to normal while the expression of NQO1 stayed significantly increased. Catalase was not modified but its expression was significantly increased after 24h recovery. Concerning IL8 gene expression, particles induced its expression and it remained increased after 24h recovery. These results showed that F1 particles induce a reversible anti-oxidant response. Inflammation will be more investigated by quantification of other cytokines. Furthermore, the different composition of particles between F1 and F4 may explain the lack of effects observed for F4. In conclusion, the 3D culture model used in this study seems to be relevant to assess the toxics effects of smokes and could be useful to evaluate human health risk.

Etude de la toxicité d'effluents gazeux issus du traitement catalytique du toluène lors d'expositions répétées en Interface Air-Liquide

Clémence MÉAUSOONE, Rebecca EL-KHAWAJA, Gauthier TREMOLET, Stéphane SIFFERT, Renaud COUSIN, Fabrice CAZIER, Yann LANDKOCZ, Dominique COURCOT, Sylvain BILLET

Unité de Chimie Environnementale et Interactions sur le Vivant, UCEIV EA 4492, SFR Condorcet FR CNRS 3417, Université du Littoral-Côte d'Opale, Dunkerque, France

Le toluène est un des solvants les plus utilisés en industrie. Ce composé organique volatil (COV) a pourtant été classé parmi les substances cancérogènes, mutagènes ou reprotoxiques par l'Union Européenne. Afin de diminuer l'exposition humaine, son élimination à la source est de plus en plus recherchée. Ainsi, dans le cadre du projet de recherche DepollutAir, financé par le programme européen Interreg V, une solution de dégradation catalytique a été développée. L'oxydation par catalyse hétérogène permet d'atteindre des bons taux de conversion, mais peut produire dans certaines conditions de fonctionnement des sous-produits dont nous nous proposons de tester la toxicité lors d'expositions répétées de cellules pulmonaires humaines BEAS-2B.

Dans un premier temps, nous avons testé la possibilité d'exposer en interface air/liquide les cellules BEAS-2B de manière répétée à un flux gazeux. Les cultures cellulaires ont ensuite été exposées durant 1 heure par jour pendant 1, 3 et 5 jours au toluène non traité (100 et 1 000 ppm) ainsi qu'à deux concentrations des émissions catalytiques (10 et 100%). L'étude toxicologique a ensuite été menée par l'évaluation de la cytotoxicité, de la réponse inflammatoire et de l'expression génique des enzymes de métabolisation des xénobiotiques.

La mesure de la cytotoxicité a montré un effet global modéré. Toutefois, l'exposition a entraîné une sécrétion dose-dépendante non significative des cytokines pro-inflammatoires IL-6 et IL-8. En outre, l'induction significative de plusieurs gènes impliqués dans le métabolisme des xénobiotiques organiques aromatiques, tels que *CYP2E1* et *CYP2F1*, est cohérente avec la présence de toluène non converti et de benzène identifié par analyse chimique comme un sous-produit. Enfin, seules les expositions répétées ont permis de montrer l'expression tardive de *CYP1A1, CYP1B1 et CYP2S1*, probablement liée à la formation d'hydrocarbures aromatiques polycycliques non détectés par les méthodes analytiques classiques utilisées pour le développement de catalyseurs.

En conclusion, les résultats obtenus dans ce projet montrent l'intérêt de mener des travaux *In Vitro* d'exposition répétée à l'aide d'un dispositif en interface air/liquide. L'application de ce modèle permet de souligner la pertinence de la validation toxicologique des systèmes catalytiques avant leur formulation en semi-pilote et pilote industriel pour le traitement des mélanges gazeux contenant des COV.

Conséquences de l'exposition de la barrière placentaire humaine à des polluants de type nanoparticules de CeO2 et benzo(a)pyrene

Margaux Nedder[1], Sonja Boland[2], Stéphanie Devineau[2], Amal Zerrad-Saadi[1], René Lai-Kuen[3], Jasmina Rogozarski[1], Jean Guibourdenche[1], Sophie Gil[1], Xavier Coumoul[4], Thierry Fournier[1] et Ioana Ferecatu[1]

[1] INSERM UMR-S 1139, Université Paris Descartes, Paris, France
[2] CNRS UMR 8251, Université Paris Diderot, Paris, France
[3] INSERM UMS 025 – CNRS UMS 3612, Université Paris Descartes, Paris, France
[4] INSERM UMR-S 1124, Université Paris Descartes, Paris, France

La pollution atmosphérique est une préoccupation majeure et l'exposition de la femme enceinte peut entrainer des effets néfastes sur le développement et la croissance du fœtus. Le placenta est un organe transitoire, dont les échanges entre le sang maternel et fœtal et les fonctions endocrines jouent un rôle physiologique crucial dans le bon déroulement de la grossesse. Le placenta agit également comme une barrière sélective contre des substances environnementales se retrouvant dans la circulation sanguine maternelle. La barrière placentaire est constituée d'un épithélium trophoblastique, composé de cytotrophoblastes villeux bordés par un syncytium en contact avec le sang maternel. Les polluants tels que les hydrocarbures aromatiques polycycliques (HAP) et les nanoparticules (NP) sont des contaminants environnementaux majeurs et susceptibles d'impacter les fonctions placentaires. Le Benzo(a)pyrène (BaP), un prototype de HAP, est formé durant la combustion incomplète de sources organiques et retrouvé dans l'air (fumée de cigarette, gaz d'échappement) et l'alimentation. Les NP de CeO_2 sont nouvellement utilisées comme additif de cigarette, agents pharmaceutiques antioxydants potentiels et ajoutées systématiquement dans le carburant, donc émises dans l'atmosphère. Nous étudions l'impact des NP de CeO_2 et du BaP sur l'intégrité et les fonctions de la barrière placentaire humaine en utilisant des cytotrophoblastes primaires qui se différencient spontanément en syncytiotrophoblaste. Les concentrations de polluants utilisées vont de très faible, correspondant aux concentrations trouvées dans l'organisme, jusqu'au niveau le plus élevé mesuré dans l'air. Nous avons évalué l'internalisation dans les trophoblastes et la cytotoxicité des NP et du BaP. A dose sublétale, nous avons déterminé l'impact des ces polluants sur la capacité des trophoblastes à former un syncytium et sur leurs fonctions endocrines. A notre connaissance, il s'agit de la première étude portant sur la toxicité conjointe des NP de CeO_2 et du BaP sur l'intégrité et les fonctions de la barrière placentaire.

POSTER N°18

PLACENTAL CELLS IN TOXICOLOGY STUDIES: A PROMISING TOOL TO REVEAL PREGNANCY DISORDERS

Elodie OLIVIER[a], Sophie FOUYET[a,b], Anaïs WAKX[a], Mélody DUTOT[a,c], Patrice RAT[a]

a) Chimie Toxicologie Analytique et Cellulaire (CNRS UMR 8038), Université Paris Descartes, Sorbonne Paris Cité, Faculté de Pharmacie, Paris, France
b) Léa Nature, 3 Rue Pierre de Fermat, 17180 Perigny
c) Yslab, 2 rue Félix Le Dantec, 29000 Quimper, France

Placenta supports the normal growth and development of the fetus by coordinating exchanges of nutrients and wastes between maternal and fetal circulatory systems and by secreting numerous hormones including polypeptide and steroid hormones. Placental dysfunctions are therefore commonly associated to pregnancy disorders that can be consecutive to chemicals exposure. It is therefore vital to conceive placenta as a target organ for toxic agents and not just as a barrier between mother and fetus. Our objective was to develop a human in vitro cell model for evaluating toxicity for placenta. We selected the human placenta JEG-3 cell line and first optimized culture conditions to highlight chemicals cytotoxicity using sodium lauryl sulfate as a death inducer. We then checked the expression of the established trophoblast marker CK7 and the release of both polypeptide (hPl, HCG) and steroid (oestradiol, progesterone) hormones under the modified culture conditions.

The JEG-3 cell line, under specific culture conditions, represents a promising model to study chemicals toxicity like pollutants, drugs, biocides, food contaminants and then better understand some pregnancy disorders. Since the cell model we developed releases hormones, it could be also used in endocrine disruption studies.

POSTER N°19

Primary Human Hepatoctye 3D Spheroids for Studying Hepatic Function and Drug Toxicity

David Kuninger, Jennifer C. Hoover, Michael P. Connolly, Debra L. Johnson, Mark Kennedy, Deborah K. Tieberg, Mark J. Powers and Sujoy Lahiri

Division of Cell Biology, Thermo Fisher Scientific Inc

Primary Human Hepatocyte (PHH) culture provides the closest in vitro model to human liver that can produce a metabolic profile of a given drug very similar to that found in vivo. Hence, PHH culture is the gold standard for studying the in vitro hepatic biology, liver function, and drug induced hepatotoxicity. The conventional 2-dimensional (2D) PHH culture is limited by de-differentiation and rapidly loss of hepatic specific functions. Therefore, there is a need for more robust in vitro models that reflects in vivo liver biology with better culture longevity. Recently, 3-dimensional (3D) in vitro models for hepatocytes have gained a lot of attention for their ability to recapitulate the hepatic function and greater longevity. Recently we have developed an easy-to-assemble in vitro PHH 3D-spheroid model. Our initial work shows that PHH can assemble into spheroids using NuncTM SpheraTM super low attachment 96-well U- bottom plates and standard centrifugation method within 5 days of seeding. Interestingly, we have also found that not every lot of PHH can assemble into 3D-spheroids. We have shown that seeding 1,500 PHH/well resulted in spheroid formation with homogenous morphology and consistent size (~200 µm diameter). The PHH spheroids can live up to 28 days in culture and can retain hepatocyte-specific functions. To assess whether hepatocyte-specific functions were maintained in the PHH spheroids during prolonged culture, albumin secretion, CYP3A4 activity and levels of ATP synthesized were analyzed. These parameters were found to remain stable during prolonged culture period. Also, gene expression profiles at 5, 7, 14 and 21 days showed a relatively higher expression of hepatocyte specific genes, such as albumin and CYP3A4, compared to that of the 2D-culture. Finally, we have performed cytotoxicity assay using compounds causing drug induced liver injury (DILI), such as Chlorpromazine and Diclofenac, and found comparable IC50 values between the 2D and 3D cultures using PHH. These results indicate that the PHH 3D-spheroid system developed by us constitutes a promising in vitro tool to evaluate hepatic function. As part of our future work, we are investigating the possibility of introducing nonparenchymal liver cells like Kupffer and Stellate cells to the spheroid system to assess feasibility of creating various liver disease models.

Co-culture model Caco-2/HT29-MTX: a promising tool for toxicity investigation of phycotoxins on the intestinal barrier

Reale Océane, Antoine Huguet, Valerie Fessard

Unité de Toxicologie des Contaminants, ANSES, Fougères

Lipophilic phycotoxins produced by marine microalgae can accumulate in edible shellfish. Some of them are documented to affect the gastrointestinal tract provoking acute intoxications in humans. However, for some toxins, the absence of proven human intoxications makes it difficult by public health authorities to estimate the risk for humans following acute exposure. Investigation of toxins toxicity through both in vitro and in vivo studies can provide key information. In fact, several phycotoxins have been shown in vivo to induce toxic effects on the intestinal epithelium such as cell detachment, fluid accumulation and villous erosion. Nevertheless, most of the toxicity data have been obtained in vitro on intestinal epithelial cell monolayers with a single cell type. Recently, co-culture models have been developed to mimic more closely the human intestinal barrier and are expected to improve evaluation of the toxicity of ingested compounds. Using such relevant co-culture model with enterocytic Caco-2 cells and HT29-MTX goblet cells, we investigated the effects of four phycotoxins (okadaic acid (OA), yessotoxin (YTX), pectenotoxin-2 (PTX2) and azaspiracid-1 (AZA1)). Cell viability, permeability, production of mucus and inflammation were evaluated using various approaches such as TEER, ELISA, histology and High Content Analysis. Our results showed that OA and PTX2 affected the monolayer permeability and that YTX and AZA1 increased the mucus layer through histological analysis. Only OA seems to induce inflammation through IL8 cytokine release. Additional results using RT-PCRq will highlight the pathways and genes affected by these toxins on the investigated processes. This co-culture model appears to be a promising tool to evaluate and compare the effects of phycotoxins on the human intestinal barrier.

POSTER N°21

Development of a 3D bronchial epithelial model for chronic exposure to nanomaterials

Daniel Sanchez-Guzman, Sonja Boland, Stéphanie Devineau, Armelle Baeza

Unité de Biologie Fonctionnelle & Adaptative (BFA), Université Paris Diderot

The production and use of nanomaterials has increased exponentially and nanomaterials have become a potential hazard for human health. To investigate chronic exposure and long term effects of nanomaterials on the lung, we have developed a 3D cell culture model of human bronchial epithelium. The model is composed of Calu-3 cells which grow at the air-liquid interface (ALI) on Transwell® inserts.
To monitor the epithelial barrier integrity over time, we measured the transepithelial electrical resistance (TEER) and permeability to the fluorescent dye Lucifer Yellow (LY) from the apical to the basal compartment. The TEER remained higher than 300Ω and we observed less than 1% of LY diffusion after 30 days at ALI. The formation of tight junctions was confirmed by immunolabelling of zonula occludens-1. The morphology of the epithelium was characterized by scanning electron microscopy and confocal microscopy showing mucus production. The identification of mucins in the apical secretome was confirmed by mass spectrometry. Furthermore, we observed that the concentration of protein in the secretome increases with the percentage of fetal bovine serum (FBS) in the basal compartment. Interestingly, Calu-3 cells maintain a tight barrier without FBS in the culture medium, but this is associated with a decrease in the protein production. The composition of the secreted proteins was compared to one clinical sample of Broncho-Alveolar Lavage (BAL). We identified 413 extracellular proteins in the BAL and 411 in the apical secretome. The polymeric gel-forming MUC5AC and MUC5B, and the cell-tethered MUC1 mucins were common to BAL and Calu-3 secretome. The model was exposed to fluorescently labelled silica nanoparticles (20nm and 200nm), which were found to be internalized by Calu-3 cells after 48h. To conclude, our results show that Calu-3 cells form a functional epithelium which can be used to investigate chronic effects of nanomaterials at the bronchial level. Now, we are investigating the effect of chronic exposure to silica and silver nanoparticles on the epithelial barrier.

POSTER N°22

Properties and use of a unique physiological hyaluronic acid-based hydroscaffold for scalable 3D cell culture

Zied Souguir, Elodie Vandenhaute, Marie Lesaffre, Véronique De Conto, Nathalie Maubon

HCS Pharma, Loos

3D cell culture systems have recently emerged as tools for reproducing the cellular environment and organization as tissues/organs, where cells are connected to each other and to the surrounding extracellular matrix (ECM). Such systems could be of great benefit in toxicity and efficiency studies in the early stages of the drug discovery process, where predictivity of in vitro models is crucial for success in later stages. Given the key role of the ECM in the behavior of cells in health and disease, scientists try to mimic this component of the microenvironment by using coatings, and more recently by using matrix-like or matrix-based 3D culture systems, e.g. decellularized organs, hydrogels based on synthetic or natural polymers, or solid scaffolds - all of which with pros and cons. By analyzing the composition and physicochemical properties of the ECM in vivo, we considered the presence of both (i) insoluble structural macromolecules (collagens), and (ii) hydrophilic biopolymers (glycosaminoglycans or GAGs) that can trap water and bind growth factors. Therefore the ECM has a dual physicochemical behavior, being at the same time a hydrogel composed of GAGs and a solid scaffold made of insoluble macromolecules, that we called a “hydroscaffold”. To mimic the ECM as a physiological hydroscaffold, HCS pharma has invented a 3D cell culture system based on Hyaluronic Acid – a major GAG currently underrepresented in cell culture systems – that can be biofunctionalized with other ECM components, according to the organ/tissue of interest. The resulting hydroscaffold shows a very good stability for long-term culture (2 months at least), allowing chronic/repeated-dose studies. It is scalable from healthy to pathological tissues and is provided in a ready-to-use multi-well format (up to 384-well plate). We will present the use of this hydroscaffold on an automated platform, showing examples of toxicity/efficiency studies performed on liver cells, neurons and cancer cells.

POSTER N°23

Le potentiel sensibilisant cutané : utilisation d'une co-culture 3D kératinocytes / THP-1 pour l'évaluation concomitante de différents évènements clés de l'AOP

Amélie Thélu, Laetitia Beaudequin, Adeline Josseaume, Florent Dumont, Claudie Deloménie, Angela Patatian, Maxime Floreani, Sabrina Soum, Bastien Page, Hervé Ficheux, Sophie Catoire, Saadia Kerdine-Römer

Département de toxicologie in vitro, THOR Personal Care, Compiègne
UMR-S 996, Université Paris Sud / Paris Saclay, Chatenay-Malabry

Dans le cadre de l'évaluation du potentiel sensibilisant cutané, une co-culture composée d'un épiderme humain reconstruit (RhE), appelé VitroDerm, associé à des cellules THP-1 a été mise en place. Cette approche reproduit en parallèle plusieurs événements du mécanisme de la sensibilisation retranscrit dans le concept de l'Adverse Outcome Pathway (AOP) : la pénétration et le métabolisme cutanés, l'activation des kératinocytes et des cellules dendritiques. Ce type de co-culture maintient la communication intercellulaire entre les kératinocytes et les cellules dendritiques telle qu'elle a lieu *in vivo* tout en facilitant l'analyse indépendante des types cellulaires après traitement.
La co-culture RhE VitroDerm / THP-1 a été caractérisée (viabilité cellulaire, histologie et expression basale de CD54 et CD86 par cytométrie en flux). Ensuite, le traitement topique avec différents produits sensibilisants et non sensibilisants de référence a été réalisé afin de rechercher les marqueurs d'intérêt de la sensibilisation cutanée. Pour cela, la cinétique de perméation à travers le RhE VitroDerm a été mesurée et la modulation des marqueurs CD54 et CD86 situés à la surface des cellules THP-1 a été quantifiée par cytométrie en flux. Les résultats obtenus ont corrélés avec ceux précédemment obtenus lors du test h-CLAT (monoculture THP-1). Une signature génique spécifique aux molécules allergisantes a été identifiée grâce à l'analyse par puce à ADN. Enfin, pour étudier les interactions cellulaires de type paracrine entre les cellules de la co-culture, nous avons mesuré les cytokines et avons mis en évidence que CCL3 et CXCL8 sont produites en réponse aux produits sensibilisants.
Cette co-culture préserve l'interaction cellulaire et permet de mimer la biodisponibilité *in vivo* en plus de l'activation des kératinocytes et des cellules dendritiques. Elle apparait être un modèle d'étude approprié aux composés ou mélanges peu solubles, ne pouvant pas être étudiés avec les méthodes classiques.

POSTER N°24

In vitro 3D culture systems for the study of human liver diseases

Loïse Valentin, Solenne Chardonnet, Karine Rondel, Chriss'Even Mouanga, Agnès Jamin, Flyuza Roshchina, Pierre Morice, Mallaury Bordessoulles, Basile Gurchenkov, Bouchra Lekbaby, Aurélie Schnuriger, Mohamed-Oussama Mouri, Renaud Piarroux, Patrick Soussan, Ashwani Sharma, Christophe Chesné, Ruoya Li, and Valérie Soulard

Biopredic international, Saint-Grégoire

Presently, the most common culture system used to address *in vitro* human hepatic disorders and/or infections is based on 2D or 2D+ (collagen-matrigel sandwich) monoculture systems of primary human hepatocytes (PHH). Although these models have proven to be very useful in the past, they reproduce neither the complete physiology of the human hepatocytes, nor the cellular complexity of the liver. With the aim of establishing *in vitro* culture models closer to the *in vivo* situation, we set up 3D mono- and multi-cellular spheroids, respectively composed of PHH, or PHH combined with non parenchymal cells (NPCs). Here, we describe the process of spheroid generation and the viability of the formed spheroids. Then, we show that 3D monocellular spheroids exhibit enhanced liver-specific functions, such as increased albumin secretion and higher phase I substrate metabolism, compared to the same batches of PHH cultivated as 2D+. With the aim of better characterizing these models, we also present preliminary data obtained from microproteomic analyses. Finally, we show that these models can be used for the study of hepatic stage of malaria, the deadliest parasitic liver infection worldwide, and HBV infection.

Activation of keratinocytes in response to multi-exposure of a cosmetic sensitizer in a reconstructed epidermis

Romain Vallion, Chloé Raffalli, Agnieszka Jaracz-Ros, Carmen Gallego, Pierre-Jacques Ferret, Géraldine Schlecht-Louf, Marc Pallardy, Françoise Bachelerie, Saadia Kerdine-Römer

Pierre Fabre Dermo-Cosmétiques / UMR-S 996 Université Paris Sud

Keratinocytes (KCs) are the main component of the epidermis, an epithelium in continuous self-renewal. The four distinct layers are characterized by the differentiation status of keratinocytes and during their maturation process, KCs move from the basal to the upper layer and orchestrate immune responses if microbes and molecules enter the stratum corneum due to mechanical or pathological skin barrier defects.
In allergic contact dermatitis (ACD), KCs play a key role since they are the first cells to encounter the contact sensitizer (CS) in the skin. KCs contain enzymes that have metabolic activity to transform prohaptenes into biologically active haptenes, facilitating protein binding to form the antigenic complex. In addition, by expressing chemotactic factors and inflammatory cytokines when exposed to CS, KCs could initiate the immune response.
In this study, we investigate how repeated exposure to CS influences the process of epidermal differentiation. To answer this question, a 3D skin model composed of KCs (NIKS cell line) grown on a matrix of collagen and primary human fibroblasts was used and exposed to cinnamaldehyde (CinA), a well-known electrophilic compound. At the end of the differentiation, the 3D skin model was analyzed by immunohistochemistry, western blot and RT-qPCR. A biochip was also carried out to highlight new genes of interest.
Our results show that repeated exposure to CinA induces a slight increase in skin thickness and a lower percentage of apoptotic cells. An induction of filaggrin expression is measured in response to a chronic exposure to CinA. In addition, the transcription factor Nrf2 is activated and antioxidant genes are induced. Preliminary results from the microarray show a high degree of segregation between groups.
This work shows that a low concentration of CS can modify the epidermis and seems relevant for cosmetic products often used with low doses of sensitizing molecules.

POSTER N°26

Microfluidic devices for ocular toxicity assays: a compartmentalization culture model as a valuable tool to study toxic effects of benzalkonium chloride on trigeminal neurons.

Vitoux MA[1,4], Peyrin JM[5,6], Baudouin C[1,2,3], Melik Parsadaniantz S[1], Réaux-Le Goazigo A[1], Brignole-Baudouin F[1,2]

[1] Vision Institute, INSERM / CNRS / Sorbonne Université, Paris, France
[2] Quinze-Vingts National Ophthalmology Hospital, Paris, France
[3] Versailles-Saint-Quentin-en-Yvelines University, Versailles, France
[4] HORUS PHARMA, Saint-Laurent-du-Var, France
[5] CNRS UMR 8246, Neurosciences, Paris, France
[6] Sorbonne Universités, UPMC, Institut de Biologie Paris-Seine, Paris, France

Introduction: Cornea is the most innervated tissue of the body and ocular irritation stimulates corneal trigeminal nerve endings. Benzalkonium chloride (BAK) is a quaternary ammonium widely used as preservative in eyedrops and as disinfecting agent in house-cleaning sprays. Here, we aimed to develop a compartmented culture model of trigeminal ganglion (TG) neurons in order to evaluate in vitro the neuronal toxic effects of BAK.
Methods: We performed a primary culture of TG cells from adult male mice, using microfluidic devices allowing the physical separation of soma from axons thanks to microchannels. After 10 days of culture, axons that have reached the distal compartment were exposed to 5.10-2 % or 5.10-3 % BAK for 15min, followed by a 2h- or 4day-recovery. Morphological alterations in the somal and distal compartments were analyzed using CFSE staining in microscopy. RT-qPCR was performed in the somal compartment for ATF3 (neuronal injury), cFos (neuronal activation), proinflammatory cytokines (CCL2, IL-6), GFAP (glial activation) and microRNA-9 (repression of axonal regeneration).
Results: After the 2h-recovery, 15min of 5.10-2% BAK exposure altered distal axons and increased mRNA levels of ATF3, cFos, CCL2, IL-6 and GFAP. Cells were also altered in the somal compartment after 4day-recovery. Whereas 5.10-3% BAK exposure showed alteration in the distal compartment, any gene expression was detected. Interestingly, we observed a microRNA-9 decreased expression after 2h. Any somal alteration was also observed after 4 days.
Conclusions: This preliminary study presents a new compartmented culture model as an interesting tool to study the impact of any xenobiotic on sensory neurons. This model could also represent a first step on the way of a 3D-reconstructured corneal epithelium after combining corneal epithelial cells and TG endings in order to better understand relationships between these two main ocular surface cell structures directly exposed to toxic stresses.

Financial Support: CIFRE HORUS PHARMA, INSERM, CNRS, UPMC