



Session V-3: Cell culture and tissue engineering

Session V-3: Oral presentations

V-3-121

Human organs-on-chip: 3D human tissue engineering as a technological innovation, an intelligent replacement alternative to animal testing

H. Hosseinkhani¹, M. Hosseinkhani² and A. Tampieri³

¹National Yang Ming University, Taipei, Taiwan; ²Mount Sinai School of Medicine, New York, USA; ³National Research Council of Italy, Faenza, Italy

hosseinkhani@ym.edu.tw

Three-dimensional (3D) *in vitro* living organs that can mimic organ and tissue structure and function *in vivo* will be of great benefit for a variety of biological applications from basic biology to toxicity testing and drug discovery. There have been several attempts to generate 3D tissue models but most of these models require costly equipment, and the most serious disadvantage in them is that they are too far from the human organs *in vivo*. Because of these problems research and development in drug discovery, toxicity testing and biotech industries are highly expensive, involve sacrifice of countless animals and it takes several years to bring a single drug/product to the market or to find the toxicity or otherwise of chemical entities. Our group has been actively working on several alternative models by merg-

ing biomaterials science, nanotechnology and biological principles to generate 3D *in vitro* living organs, to be called "Human Organs-on-Chip," to mimic natural organ/tissues in order to reduce animal testing and clinical trials. We have fabricated a novel type of mechanically and biologically bio-mimicking collagen-based hydrogel that would provide for interconnected mini-wells in which 3D cell/organ culture of human samples in a manner similar to human organs with extracellular matrix (ECM) molecules would be possible. These products mimic the physical, chemical, and biological properties of natural organs and tissues at different scales. The presentation will review the outcome of our experiments so far in this direction and the future perspectives.



V-3-269

Temporal transcriptomic alterations during renal epithelial monolayer formation

L. Aschauer, L. N. Gruber, A. Limonciel, M. O. Leonard, W. Pfaller, A. Wilmes and P. Jennings

Innsbruck Medical University, Innsbruck, Austria

Lydia.Aschauer@i-med.ac.at

The ability of the kidney to concentrate, metabolise and secrete compounds underlies its susceptibility to xenobiotics. The proximal tubule epithelium is one of the most susceptible regions of the nephron. Even minor disturbances in proximal tubule function can have serious consequences for homeostasis. Injury to the epithelial cells releases cell to cell contacts and promotes proliferation and tissue repair. In order to better understand these processes, we analysed temporal transcriptomic alterations of human renal proximal tubule cultures during monolayer formation.

Primary human proximal tubule cells and the recently developed human RPTEC/TERT1 cell line were seeded at ~30% confluence. Cell cultures were maintained in hormonally defined DMEM/F12 and fed every day for 16 days. At day 1 and then every third day, cultures were harvested for RNA isolation and cell cycle analysis. In addition, supernatants were collected to

analyse glycolysis rates. Using Illumina HT-12 whole-genome expression arrays 1390 temporally differently expressed probes were identified.

The time of culture had a large impact on the gene expression, stabilising around day 13 after seeding, concomitant with retardation in G1/0 cell cycle phase. Temporal increases in cell adhesion (CDH-1 and 16) and tight junction proteins (ZO-3, CLDN2, CLDN3, CLDN10) were observed. Additionally, subunits of Na,K-ATPase were up-regulated (ATP1A1, ATP1B1, FXYD2), whereas FXYD5, TGFbeta 1, PCNA and cyclin D1 were down-regulated during monolayer formation. Downgraded glycolysis was accompanied by alterations in energy metabolism genes (e.g. LDHB, HKDC1, PFKFB3/4, PDHA1, NDUFB5, PRKAB1, ACACB, FASN, ACOT7, ACSM3, ACSS1, ACAD10). The generated data set will be useful to identify mechanistically linked injury biomarkers of epithelial cell injury processes.

V-3-419

Direct oxygen supply to liver-derived cells using oxygen permeable membranes

Y. Sakai, F. Evenou, M. Hamon, K. Komori and T. Fujii

University of Tokyo, Tokyo, Japan

sakaiyas@iis.u-tokyo.ac.jp

A study in the 1960s pointed out the fact that a confluent adult hepatocyte monolayer cultured in conventional tissue-culture-treated polystyrene (TCPS) is usually in an extremely anaerobic condition. To completely overcome this problem in static cultures using microplates, we prepared a special plate that has bottom surfaces made from polydimethylsiloxane (PDMS), an oxygen-permeable material. This plate enables direct oxygen supply to the cell layers cultured beneath the culture medium layers, thus meeting the cellular oxygen consumption at well-controlled exposure oxygen concentrations determined by its atmospheric concentrations. Using this plate, various liver-derived cells, such as human hepatocarcinoma Hep G2 cells and adult/fetal rat hepatocytes, could be cultured in thick pseudo-

3D tissues comprised of several cell layers. In addition, glucose consumption and lactate production revealed that these cells utilized mainly their aerobic energy production as opposed to when they are in conventional polystyrene plates, leading to remarkably enhanced protein synthesis. Control of actual oxygen concentrations at the cells was also important in growing fetal hepatocytes; initially low (5%) but later high (21%) oxygen concentrations showed remarkable self-organization of fetal rat hepatocytes into heterogenic thick liver tissues. These results demonstrate the high future feasibility of this simple plate system in the culture of various liver-derived cells for cell-based assays.



V-3-424

Assessment of acute, long-term and chronic respiratory toxicity using a long shelf-life 3D model of the human airway epithelium (MucilAir™)

S. Constant, S. Huang and L. Wiszniewski

Epithelix, Plan-les-Ouates, Switzerland

samuel.constant@epithelix.com

Most of the *in vitro* cell models for long term testing of chemicals suffer from at least two shortcomings: 1) The failure of reproducing the *in vivo* physiological characteristics of the corresponding tissues. 2) A limited shelf-life. Herein is reported the use of a standardized 3D air-liquid interface *in vitro* cell model of the human airway epithelium (MucilAir™), which is free of these limitations.

MucilAir™ is morphologically and functionally differentiated and it can be maintained in a homeostatic state for more than one year. The typical ultra-structures of the human airway epithelium, such as tight junctions, cilia, mucus, basal/goblet/ciliated cells can be observed. Classical airway transporters, ion channels and CypP450s are expressed and are functional up to one year. The epithelia react to pro-inflammatory mediators in a physiological manner. The epithelia can be stimulated regularly with inflammatory substances to simulate chronic in-

flammatory reactions for up to several months. A large panel of cytokines/chemokines/metalloproteinases has been detected in MucilAir™.

Due to its unique long shelf-life of one year, this model is used for studying the human respiratory diseases, and for testing the long-term/chronic effects of drugs/chemicals on the respiratory tract *in vitro*. Late effects of chemicals/mixtures (several weeks after exposure) can be observed. Several applications of MucilAir™ will be presented:

- Acute, long-term and chronic toxicity testing (first *in vitro* transposition of OECD TG412 for 28 days repeated dose study will be presented)
- Inflammatory effect assessment
- Assessment of reversible vs. irreversible toxic effects
- Recent advances in the detection of respiratory sensitizers and irritants

V-3-417

A human tissue-engineered vascular substitute with a functional vasa vasorum

M. Tondreau¹, D. Lacroix¹, P. d'Orléans-Juste² and F. A. Auger¹

¹Laval University, Québec, Canada; ²Sherbrooke University, Sherbrooke, Canada

maxime.tondreau@gmail.com

Due to the paucity of available healthy human blood vessel samples and the intrinsic variability they can display, animal models are often necessary. However, we have previously demonstrated the capacity of tissue-engineered human vascular media and adventitia to respond *in vitro* to classic vasoactive agents such as endothelin and sodium nitroprusside. Recently, we were able to add to our model a vasa vasorum, the network of capillaries supplying the external layer of arteries and veins with nutrients and oxygen. We have demonstrated that the addition of a vasa vasorum to our model significantly benefited the inosculation of grafted tissues. However, the functionality of this tissue-engineered vasa vasorum and its contribution to the vascular tone still remain to be established. Four different con-

structs were made, comprised of an adventitia with or without a vasa vasorum and in the presence or not of an endothelium. As expected, immunohistochemical localisation of the endothelial nitric oxide synthase (eNOS) confirmed its expression by the endothelial cells of the vasa vasorum. To induce the production of nitric oxide, rings from the constructs underwent stimulation with various concentrations of histamine and acetylcholine. Our results indicate that the vasa vasorum is capable of inducing a significant nitric oxide-mediated vasodilation in our constructs. In conclusion, a functional tissue-engineered vascular substitute could represent a very useful model permitting the *in vitro* study of human vascular pharmacology.



Session V-3: Poster presentations

V-3-063

Tissue engineered human bronchial models to study asthma

*F. Goulet¹, J.-P. Lavoie², S. Chabaud¹, F. Simon¹ and V. Moulin¹*¹Université Laval, Québec, Canada; ²Université de Montréal, Saint-Hyacinthe, Canada

The isolation of human bronchial epithelial and fibroblastic cells from biopsies of asthmatic and non-asthmatic volunteers provided unique cellular materials to be used for the production of tissue-engineered bronchial models *in vitro*. The epithelial cells are grown on a mesenchymal layer seeded with fibroblasts. The reconstructed bronchi can be maintained for at least 15 days in culture. Under the culture conditions established, epithelial cells undergo differentiation into ciliated and goblet cells, within a pseudostratified organization comparable to human bronchi. Comparative histologic and functional analyses of

non-asthmatic and asthmatic bronchial models were performed. Our data indicated the maintenance of the asthmatic phenotype of the cells isolated from asthmatic bronchial samples. Desquamation, scarce distribution of cilia and excessive mucus secretion are some of the features observed with the asthmatic bronchial models. These models appear to be powerful alternatives to animal use for the study of the mechanisms involved in asthma *in vitro*.

V-3-171

Replacement of xenobiotic components applied in the culture medium for maintenance of human keratinocytes by human equivalents

*S. Cerejido Altran¹, D. Yoshito¹, C. Isaac², B. da Silva Sufi¹ and M. B. Mathor¹*¹IPEN-CNEN/SP and FMUSP/SP, São Paulo, Brazil; ²FMUSP/SP, São Paulo, Brazil

biancasufi@usp.br

Confluent epithelia of autologous keratinocytes cultivated according to standardized methodology by Rheinwald & Green in 1975, have been used as grafts in different clinical situations. However, the presence of xenobiotic components of the culture medium applied by this method implies the possibility of transmission of zoonoses, prions, and viruses to patients, besides involving ethical issues related to the use of animals to obtain the components. Such concerns have driven researchers to seek alternatives that overcome this deadlock, as the formulations obtained so far are not completely satisfactory. Thus, our proposal in this study was to omit or replace the xenobiotic components traditionally used in the medium for keratinocyte culture with

human equivalents. As a result, we have standardized a culture medium whereby we omitted the use of cholera toxin, replaced fetal bovine serum with human platelet lysate at a 2.5% or 5% concentration, and bovine insulin was replaced by recombinant human insulin at the same concentration as the original method (5 µg/ml). With the results obtained we conclude that the method is viable to cultivate human keratinocytes kept in culture medium free of xenobiotic components.

We are thankful to CNEN (Comissão Nacional de Energia Nuclear) and FAPESP (Fundação de Amparo á Pesquisa do Estado de São Paulo) for the financial support.



V-3-174

Expression and induction of xenobiotic metabolism genes in the StrataTest[®] human skin model

C. Rasmussen, N. Simon, M. Vander Zanden, C. Johnston and L. Allen-Hoffmann

Stratatech Corporation, Madison, USA

crasmussen@stratatechcorp.com

Human *in vitro* skin models have shown broad utility for toxicological testing due to their similarity to the *in vivo* state and ability to evaluate compounds as dosed in actual use or exposure. Numerous studies have confirmed the expression and induction of phase I and phase II metabolic enzymes in keratinocytes and skin models. In the present study, the StrataTest human skin model was evaluated for expression and induction of genes involved in xenobiotic metabolism. The StrataTest full-thickness human skin model, containing both epidermal and dermal components, faithfully recapitulates many of the biological characteristics of human skin. The model is generated using NIKS[®] keratinocytes, a clinically-tested and consistent source of non-tumorigenic, pathogen-free human keratinocyte progenitors. To confirm that NIKS keratinocytes possess metabolic capacity, gene expression profiles of phase I and phase II enzymes from

NIKS and primary keratinocytes were compared. Differences in expression of two-fold or greater between NIKS and primary keratinocytes were considered significant. Concordance for expression of 51 phase I and phase II metabolic enzymes in NIKS and primary human keratinocytes was 98%. In the three-dimensional skin model, baseline gene expression and induction after exposure to 3-methylcholanthrene (3MC) was also examined. Cytochrome p450 1A1 (CYP1A1) and CYP1B1 were weakly expressed, while N-acetyltransferase 1 displayed more robust constitutive expression. Upon exposure to 3MC, CYP1A1 and CYP1B1 expression was strongly induced. Similar to other *in vitro* skin models, StrataTest skin tissues express genes critical to xenobiotic metabolism, further demonstrating the utility of this model for toxicological testing applications.

V-3-246

Standardization of the culture of human fibroblasts in medium enriched with platelet lysate

D. Yoshito¹, S. Cerejido Altran¹, M. Roma Herson², C. Isaac³ and M. B. Mathor¹

¹IPEN – CNEN/SP, São Paulo, Brazil; ²Tissue Bank of Victoria, São Paulo, Brazil; ³FMUSP, São Paulo, Brazil

daniele.yoshito@usp.br

Fibroblasts originate from mesenchymal stem cells and are responsible for the synthesis of extracellular matrix proteins and the secretion of growth factors which control the proliferation and differentiation of epidermal cells. The human fibroblasts can be cultured in the laboratory and used in the manufacture of dermo-epidermal substitute for the treatment of burns and chronic ulcers. However, there is a recent concern about the possibility of transmitting prions and animal viruses to transplanted patients, considering that fibroblasts are cultured in medium supplemented with fetal bovine serum. Based on this premise, the present work aims to cultivate human fibroblasts in a medium enriched with human platelet lysate. For this purpose the

lysis of platelet lysate was standardized and tested in human fibroblasts under several concentrations. The results revealed that 10% platelets lysate improved cell adhesion and proliferation of human fibroblasts if compared to fetal bovine serum. Therefore, it was possible to standardize the human fibroblast culture to use these cells for clinical purposes, as well as to eliminate the xenobiotic component.

We are thankful to CNEN (Comissão Nacional de Energia Nuclear) and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the financial support.



V-3-278

A novel approach to assess irritant or respiratory allergenic potential of chemicals *in vitro*

M. Singal¹, D. Vitale¹, L. Smith¹, K. Czollner², L. Farmer², C. Höpfner², H-P. Satzer², S. Schoeffmann², B. Schwartz² and E. Weber²

¹Research Institute for Fragrance Materials, Woodcliff Lake, USA; ²Seibersdorf Labor GmbH, Seibersdorf, Austria
dvitale@rifm.org

There are currently no validated methods for the identification of chemical respiratory allergens, neither *in vivo* nor *in vitro*. Due to increasing health concerns associated with occupational or chemically-induced asthma, and impending directives on the regulation of respiratory sensitizers, standardized test methods are urgently required to identify respiratory allergens.

To establish an *in vitro* test system for the identification of chemical respiratory sensitizers, an immunocompetent, 3D triple cell co-culture system, representing the proximal alveolar region of the human lung, was developed. It is comprised of immature dendritic cells, derived from human peripheral blood monocytes, human lung alveolar epithelial-like cells (A549 cells) and macrophages differentiated from U937 cells.

Employing the Vitrocell[®] system, two well-known respiratory allergens, toluene-2,4,diisocyanate (TDI) and trimellitic an-

hydride (TMA), as well as two irritants, acrylic acid and acetaldehyde, were applied to the model at the air/liquid interface, mimicking the *in vivo* situation in the lung. Since this system allows for the delivery of test materials in their native form, liquid aerosols (TDI, acrylic acid, acetaldehyde) or particle aerosols (TMA) were generated to mimic a real exposure scenario. Aerosol particle sizes and the amount of test substance applied were quantified.

After a 4 hour exposure scenario, cellular viability was evaluated in dose response studies using flow cytometric evaluation of cell cycle analysis. Cytokine release and dendritic cell maturation was investigated to identify whether chemical exposure induces specific inflammatory mediators and/or cellular changes, in order to identify predictive endpoints and biomarkers that may be indicative of potential respiratory allergens *in vivo*.

V-3-341

HepaRG cells: A novel human model for the study of drug hepatotoxicity

M. R. McGill, H. Yan, A. Ramachandran and H. Jaeschke

University of Kansas Medical Center, Kansas City, USA
mmcgill@kumc.edu

In vitro models have reduced the number of animals required for preclinical drug testing. Unfortunately, the usefulness of these models for toxicology is limited due to poor cytochrome-P450 (CYP) enzyme expression in hepatoma lines, as these enzymes are required for conversion of many drugs into toxic metabolites. HepaRG cells, recently isolated, have been shown to express P450s at near physiological levels. For this reason, we tested the usefulness of these cells for the study of acetaminophen (APAP) hepatotoxicity. APAP toxicity begins with formation of a reactive intermediate which depletes glutathione (GSH) and adducts proteins. Mitochondrial protein binding initiates mitochondrial dysfunction and cell death. Treatment with 20 mM APAP (6, 12, and 24 h) depletes GSH levels in HepaRG cells (80, 57, and 26% of control, respectively) and the JC-1 assay revealed

loss of mitochondrial membrane potential (93, 45, and 20% of control, respectively). At later time points, release of lactate dehydrogenase (LDH) was detected (29 and 62% of total LDH at 24 and 48 h, respectively, vs. 5% with control), indicating cell death. A clear dose-response relationship was observed for all parameters. Dihydrorhodamine fluorescence demonstrated increased oxidative stress after APAP treatment. Parallel experiments using HepG2 cells, which lack CYP expression, did not show any cell injury. Therefore, HepaRG cells are a valuable alternative to animal models for studies of xenobiotics requiring metabolic activation. Importantly, the mechanism of APAP toxicity in these cells resembles what is known from rodent studies, while the kinetics of injury are similar to what has been reported in humans.



V-3-347

Use of liver microsome S9 fraction-containing microcapsules to include liver biotransformation processes in cytotoxicity tests

K. Komori¹, N. Yamamoto¹, H. Matsui², S. Takeuchi¹ and Y. Sakai¹

¹University of Tokyo, Tokyo, Japan; ²METI, Tokyo, Japan

kkomori@iis.u-tokyo.ac.jp

Liver microsome S9 fraction obtained from the livers of rats and humans have often been used to include biotransformation processes of the liver in various cytotoxicity tests, but the fraction sometimes causes strong toxicities to the relevant cells due to the self-production of lipid peroxides. Therefore, the final concentration of S9 fraction in cytotoxicity tests should be optimized for each particular test both in terms of its biotransformation effects and original toxicity. To solve this problem, we immobilized rat liver microsome S9 fraction in Ca-Alginate microcapsules whose surfaces were post-coated with poly-D-lysine to retain detoxification enzymes and lipid peroxides produced, while allowing small chemicals and their metabolites to be transported between the capsules and outer culture medium.

Although the initial metabolic rate in terms of CYP 1A1/2 capacity of the S9-encapsulated gel microcapsules was about 70-80% that of the bare S9, such encapsulation completely avoids the original toxicity of S9. Using conventional membrane culture inserts and their accompanying multi-well plates, we cultured NIH 3T3 cells (in the lower compartment) with the microcapsules (in the upper compartment) and observed enhanced cytotoxicity of an indirect mutagen, cyclophosphamide, in almost the same manner as observed in the presence of bare S9 fractions added to the culture. These results clearly show that use of microcapsules containing liver microsome S9 fractions is simple, general and thus practical to include liver biotransformation processes in various cytotoxicity tests.

V-3-365

Immunohistochemical characterization of a pituitary derived cell line from adult Atlantic salmon, *Salmo salar*

U. Adamcic-Bistrivoda¹, N. T. K. Vo¹, M. S. Mikhaeil¹, H. E. Braid², R. H. Hanner² and L. E. J. Lee¹

¹Wilfrid Laurier University, Waterloo, Canada; ²University of Guelph, Guelph, Canada

uadamcic@gmail.com

Atlantic salmon, *Salmo salar*, are highly valued fish for both fisheries and aquaculture. Growth and reproduction in this species have been widely studied and factors regulating their life cycle, including somatic growth, sexual maturation and reproduction, spawning, smoltification, have been extensively investigated. However, the molecular mechanisms controlling expression of the hormones involved in these processes are largely unknown. Pituitary cell cultures could be valuable for elucidating these mechanisms, and a cell line derived from this teleost's pituitary

could make significant impacts in understanding growth and hormonal regulation as well as endocrine disruption by environmental contaminants. Here we report on the development and histochemical characterization of a continuous cell line derived from adult Atlantic salmon pituitary dubbed ASP309. The cells display a fibroblastic morphology and have been grown for almost 2 years in L15 media supplemented with 10% FBS at 18-20°C. DNA barcoding confirmed the cells as derived from *Salmo salar*.



V-3-415

The development of human skin explant cultures as an *in vitro* alternative to animal testing for translational research in skin care

C. B. Lin, Y. Hu, D. Rossetti, A. Pappas and W.-H. Li

Johnson & Johnson, Skillman, USA

blin1@its.jnj.com

The development of *in vitro* model systems for translational research and skin care applications is of increasing priority, following the increased awareness of the 3Rs concepts and the European Community ban on animal testing for cosmetics. The goals of our studies were: 1) To develop an *in vitro* model system that best represents the physiological complexity of human skin, with metabolically active and structurally intact epidermis, dermis, and subcutaneous fat layers. 2) To create a portfolio of assays for evaluating dermatological and skin care agents for their biological activities, that would enable the prediction of clinical efficacy and expanding new biological insights without animal studies.

We established a human skin explant system using full-thickness human skin biopsies, obtained from healthy donors undergoing abdominal surgeries with informed consent. We

optimized culture conditions that enable maintaining structural integrity and metabolic activity of the epidermis, dermis and subcutaneous fat layers of the skin explant. In addition, biomarkers were identified and a portfolio of assays was developed for examining the epidermal, dermal and subcutaneous adipose functions, and for the evaluation of biological effects of dermatological and skin care agents on pigmentation, skin aging, and subcutaneous lipid metabolism. The skin explants system was validated using TGF- β (an experimental positive control) and several known cosmetic, skin care actives, such as retinol. In summary, a human skin explant system that is viable and metabolically active was established, for better understanding of skin biology, for mechanistic understanding and for predicting efficacy of agents

V-3-486

Evaluation of microbicide toxicity and efficacy using a novel three-dimensional human organotypic co-culture vaginal model

A. L. Radtke, E. M. Jackson and M. M. Herbst-Kralovetz

University of Arizona, Phoenix, USA

aradtke@email.arizona.edu

Recent advances in technology and immunology together with a lack of successful vaccines and microbicides against sexually transmitted viruses (STV), demonstrates a need for alternative investigative models. Human Immunodeficiency Virus (HIV) and Herpes Simplex Virus-2 (HSV-2) are examples of human vaginal mucosal STV that infect a substantial portion of the world's population, with no currently approved interventions. The lack of interventions could be a consequence of utilizing non-physiological relevant animal models, explants, and monolayer cell cultures in preclinical studies. Here we utilize 3D vaginal epithelial cells cultured in a rotating-wall-vessel (RWV) bioreactor to investigate the toxicity and efficacy of various microbicides. We have shown previously that our RWV-derived vaginal model recapitulates *in vivo* structural and functional properties. In this study, we expand our monotypic 3D vaginal model to more closely model the human vaginal

tract with the incorporation of human innate immune cells. Using this co-culture model, we screened a panel of well-characterized microbicides to test their toxicity by measuring mucosal toxicity biomarkers (including TRAIL and IL-1RA). In addition, we evaluated microbicide efficacy by quantifying their impact on HSV-2 replication in our 3D vaginal models. Data generated from our 3D human monotypic and co-culture models are similar to observations in human cervical explants, demonstrating how our *in vivo*-like human models can serve as robust, predictive models for screening for microbicide toxicity and efficacy. Overall, our work supports and illustrates the numerous advantages of utilizing physiologically relevant human models as alternatives to animal-derived models.

Funding: Alternative Research Development Foundation (ARDF)



V-3-489

Functional studies of proteins: towards a human knock-down model using tissue-engineered skin substitutes

C. Simard-Bisson¹, R. Blouin² and L. Germain¹

¹Université Laval, Québec, Canada; ²Université de Sherbrooke, Sherbrooke, Canada
caroline.simard-bisson.1@ulaval.ca

Knock-out mice and RNA interference in cell culture are useful tools for functional studies of proteins. However, those methods may be less appropriate to study proteins expressed in specific contexts such as in human epidermal differentiation. Indeed, the differentiation of epidermal cells is incomplete in monolayer culture and many differences exist between mouse and human skin. The Dual Leucine zipper-bearing kinase (DLK), expressed in normal human skin, has been reported as an inducer of terminal differentiation of human epidermal cells into corneocytes, which form the protective layer of the epidermis: the cornified layer. To better understand the role of DLK in the cornified layer formation, we developed a Skin Substitute (SS) model underexpressing DLK. SS were transduced at day 0 of air-liquid interface culture with lentivirus vectors containing a short hairpin

RNA sequence against DLK. Biopsies were harvested 14 days later. Immunoperoxidase staining showed a reduction of DLK expression. Immunofluorescence staining revealed reduction of filaggrin and transglutaminase 1, two proteins of the cornified envelope. The decrease of DLK expression was associated with cell detachments and the reduction of desmocollin expression, suggesting defects in desmosome assembly. Finally, microarray data indicate that DLK may contribute to the cornified envelope assembly by regulating expression of the Tazarotene Induced Gene 3, a potential inducer of transglutaminase 1. The development of this kind of tissue-engineered model should provide an interesting alternative to animal testing and contribute to a better comprehension of processes involved in human tissues.

V-3-531

Cutaneous wound healing in the EpiDerm-FT™ full-thickness *in vitro* human skin model: Role of serum growth factors

A. J. Armento, G. Stolper, M. Li, M. Klausner and P. J. Hayden

MatTek Corporation, Ashland, USA
phayden@mattek.com

Cutaneous wound healing involves interactions between dermal fibroblasts and epidermal keratinocytes, as well as cell and extracellular matrix interactions. This poster describes new progress in wound healing experiments conducted with a full-thickness *in vitro* human skin model (EpiDerm-FT™). Normal human epidermal keratinocytes (KC) and dermal fibroblasts (FB) are cultured to produce the highly differentiated full-thickness skin model. Small wounds were induced in the epithelial model by means of a battery operated cauterizer or a dermal biopsy punch. The wounded EpiDerm-FT™ cultures were fixed at various times and H&E stained sections were prepared to evaluate the wound healing process. The effect of human serum (HS) on the rate of wound healing was evaluated over a time course of 9 days. KC migrated over the wounded area to regenerate the epidermis within several days. FB proliferation and

dermal matrix repair were also observed. HS produced a concentration dependent increase in the rate of cutaneous healing. Gene expression profiling and immunohistochemical staining of the wounded area showed temporally regulated increases in expression of basement membrane components, collagens and genes involved in extracellular matrix remodeling. Increased FB proliferation in dermal areas directly adjacent to migrating KC was observed. FB proliferation and epidermal healing were severely impaired in the presence of an EGFR tyrosine kinase inhibitor or a TGF α neutralizing antibody. These results demonstrate that EpiDerm-FT™ is a useful animal alternative skin model for investigating dermal-epidermal interactions during wound healing, and for development of new dermal wound healing therapeutics.