Latest advancements of *in vitro* evaluation of cosmetics

from regulatory compliance of ingredients to finish products claims

roundtable, in-cosmetics, 16 April 2015 Barcelona

CENTRE EUROPÉEN DE DERMOCOSMÉTOLOGIE

premium beauty news .com

Moderators

Anne Canet

Member of the European Centre of Dermocosmetology, CED Lyon.
CEO ATTRACTION consulting

Vincent Gallon

Publisher and editor, PremiumBeautyNews.com and BrazilBeautyNews.com

CED CENTRE EUROPÉEN DE DERMOCOSMÉTOLOGIE

premium beauty news .com

European Centre of dermo-cosmetology (CED)

Centre européen de dermocosmétologie

- The European Centre of Dermocosmetology is a professional association founded in 1962 in Lyon, France to promote and support research projects, to organize training sessions, to create links with other foreign association, in France and abroad

- Since 1966, and every two years, the CED has been organizing the European Dermocosmetology Days, a two-day conference focusing on scientific issues.

- The 27th European Dermocosmetology Days will take place in Lyon on May 28 and 29, 2015 and will focus on "***New plant chemistry, from safety to efficacy*** »

CENTRE EUROPÉEN DE DERMOCOSMÉTOLOGIE

premium beauty news .com

Latest advancements of *in vitro* evaluation of cosmetics

1. *In vitro* methods for assessing the sensitising potential of ingredients.

2. New *in vitro* models: 3D reconstructed human skin models.

3. *In vitro* tests for supporting cosmetic innovation and marketing claims.

Béatrice Le Varlet

- A skin biologist, cosmetologist and toxicologist, Dr Le Varlet initially focused on academic research in cell-cell interactions (INSERM, Lyon, Paris) then she joined LVMH Perfumes & Cosmetics as a senior scientist in the R&D Biology Department where she was more specifically in charge of the *in vitro* toxicology laboratory.
- She was a member of the Eye Irritation and Skin Tolerance Task Forces of Cosmetics Europe for 10 years.
- Since 2006, she provides consultancy expertise in the *in vitro* testing of cosmetics.

Bart De Wever

- A Doctor in Medical Sciences, Bart has a 25 years experience in *in vitro* tissue engineering, R&D and Business Development of 3D human tissue models. He worked for organisations such as the Johnson & Johnson Research Foundation, SkinEthic Laboratories, Phenion/Henkel or Evonik Healthcare in Europe and in the USA.
- He's President (& Founder) of ALEXANDRA, an NGO focusing on education related to 3D tissue model testing
- In February 2015, he founded ATERA, a company focusing on mass-production of advanced 3D human tissue models

Béatrice Le Varlet Ph.D, ERT, Consultant, In Vitro Cosmetic Consultancy

Bart De Wever, Ph.D., Business Development Services Alternatives to Experiments on Animals

Alternative methods for assessing the sensitising potential of raw materials (1/5)

Allergic contact dermatitis (ACD) is one of the most common occupational and environmental diseases estimated to affect already 20% of the population in Europe. 4,000 chemicals have been identified as skin sensitisers. **The aim of chemical risk assessment is to identify chemicals that can trigger skin allergies.**

The generation of skin sensitisation information represents a standard requirement within pieces of EU legislation. Information on skin sensitisation potency is also needed to satisfy all regulatory needs.

Béatrice Le Varlet Ph.D, ERT, Consultant, In Vitro Cosmetic Consultancy

Bart De Wever, Ph.D., Business Development Services Alternatives to Experiments on Animals

Alternative methods for assessing the sensitising potential of raw materials (2/5)

Compared to other human health endpoints studied such as phototoxicity, eye or skin irritation, no alternative method was available 15 years ago to assess skin sensitisation, except complicated human T cell priming assays detecting only strong allergens. The **great progress made in *in vitro* skin sensitisation testing** is illustrated by current developments of different integrated testing strategies.

Progress has been made in the development of alternative methods that cover some of the key mechanisms involved in sensitisation, e.g., skin bioavailability, protein binding, epidermal inflammation, dendritic cell (DC) activation, DC migration and T-cell proliferation, as documented in the Adverse Outcome Pathway (AOP) for skin sensitisation developed by the OECD.

Béatrice Le Varlet Ph.D, ERT, Consultant, In Vitro Cosmetic Consultancy

Bart De Wever, Ph.D., Business Development Services Alternatives to Experiments on Animals

Alternative methods for assessing the sensitising potential of raw materials (3/5)

The Direct Peptide Reactivity Assay (DPRA) and the KeratinoSens™ are the first non-animal tests adopted on 5 February 2015 by the OECD to identify skin sensitisers. The OECD Test Guidelines 442C provides an *in chemico* procedure to address the molecular initiating event which is the covalent binding of electrophilic substances to nucleophilic centres in skin proteins. The TG 442D describes the KeratinoSens™ test method, to address inflammatory responses as well as gene expression associated with specific cell signalling pathways such as the antioxidant/electrophile response element (ARE)-dependent pathways.

The European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) has validated and recommended a new method, the human Cell Line Activation Test (h-CLAT) on March 2015. The h-CLAT measures the upregulation of the CD86 and CD54 markers of dendritic cells (DC) in THP-1 cells, a human monocytic leukemia cell line. This addresses the activation of DC within the AOP.

Béatrice Le Varlet Ph.D, ERT, Consultant, In Vitro Cosmetic Consultancy

Bart De Wever, Ph.D., Business Development Services Alternatives to Experiments on Animals

Alternative methods for assessing the sensitising potential of raw materials (4/5)

Due to the **complex cascade of events** underlying skin sensitisation, a combination of methods designed to detect and measure the key events in the skin sensitisation pathway will likely be necessary to achieve replacement of animals.

A systematic evaluation of 16 non-animal test methods for skin sensitisation safety assessment was recently published by the Cosmetics Europe Skin Tolerance Task Force. Sens-IS, SenCeeTox and EE methods are all using 3D reconstructed tissues to predict sensitizer potency. **Combined with bioavailability and skin metabolism data and exposure consideration, a testing strategy is envisaged.** Other Integrated Testing Strategies (ITS) are proposed such as the two out three of BASF.

Béatrice Le Varlet Ph.D, ERT, Consultant, In Vitro Cosmetic Consultancy

Bart De Wever, Ph.D., Business Development Services Alternatives to Experiments on Animals

Alternative methods for assessing the sensitising potential of raw materials (5/5)

The ability to detect pre-haptens and pro-haptens remains a challenge, since these sensitisers are not systematically detected by test methods that lack or have limited metabolic and oxidative capacity.

In vitro and *in chemico* methods used in combination with *in silico* models within Integrated Approaches to Testing and Assessment (IATA) (such as the ITS-2, using a Bayesian network to analyze data, or the Unitis NCS TOX project, aiming to build a predictive database to determine the toxicological profile of Natural Complex Substances, …) may provide sufficient information to allow the identification of potential skin allergens and the characterization of their relative potency.

Finally, these tools could generate more accurate assessments of human toxicity risk than those predicted by animal tests.

Marisa Meloni

- CEO and founder of VitroScreen, a research laboratory committed to in vitro science.
- Marisa Meloni has obtained a PhD in Drug Delivery and Cutaneous Biophysic from the University Renée Descartes, Paris V in 1992 and has been in charge for research and innovation within the dermo-pharmaceutical and cosmetic industry during 20 years. During this period she has also been contract professor of safety assessment and technology of cosmetic products at Milano, Salerno and Padova Universities.
- Past President of Celltox (Italian cell culture association).

Amélie Thépot

- Dr Amélie Thépot (PhD in molecular and cell biology) has been working in cell therapy and tissue-engineering for 10 years. She has worked in various organisations, including the *Laboratoire des substituts cutanés*, the International Agency for Research on Cancer (IARC/WHO) and CellSeed, a biotech firm.
- In 2014, she founded LabSkin Creations, a company that produces customized models of skin equivalents on which ingredients and cosmetics are evaluated. She's also developing new skin models and performing research in tissue engineering with several academic partners.

The value of 3D models (1/2)

Marisa Meloni, CEO and founder of VitroScreen, a research laboratory committed to in vitro science.

As in vitro research laboratory it is on our responsibility to offer to the cosmetic industry the most predictive, relevant and reliable experimental model for efficacy testing.

3D tissue models possess a multilayered structure and display functionality close to the in vivo mechanism and are predictive of human responses either at cellular and molecular levels.

Against the use of 2D monolayers and the indiscriminate, non ethical use, of human volunteers, 3D human tissues allow to demonstrate the product efficacy according to the dynamic of the biological response, the interference with the homeostasis at molecular level and the dynamic of the genomic response that is not quantificable in vivo and finally in vitro 3D techniques are the most promising future because there are not alternative to alternatives if the mechanism of action has to be quantified to proof a claims.

The value of 3D models (2/2)

Marisa Meloni, CEO and founder of VitroScreen, a research laboratory committed to in vitro science.

In order to comply with the new European Cosmetics Regulation (EC) N° 1223/2009 more and more specialized and predictive 3D models will be requested : 2 examples of successful experimental models will be presented :

- **How to perform a predictive active ingredient's screening by using realistic dose and exposure ?** It is not really possible on 2 D monolayer because of the multiple limitations of this model : scaffold-free 3Dculture systems better reflect the biological, physical and bio-chemical environment of the natural extracellular matrix compared to cell monolayers (example of microdermis will be given).
- **Human living colonized 3D tissues.** Specialized, colonized human 3 D tissues are really an innovative tool because they provide new insights on skin and mucosae biology taking into account the interaction between bacteria and host; what is really innovative is to to show that skin has a a different development, immune response or barrier formation because of the presence of the commensal bacteria.(example of 3D colonized vaginal mucosa and biofilm formation).

Customized 3D models (1/3)

Amélie Thépot ,
Founder and CEO of
LabSkin Creations

Since the 80's, we are able to reconstruct in vitro epidermis and dermis. These models are known very well, characterized and standardized. However, to reconstruct all the layer of the skin, including hypodermis, cutaneous appendage… is a technological challenge. We know that the different compartments of the skin are essential for cross-talks and play an important role in controlling homeostasis.

In LabSkin Creations, we have a scaffold allowing to reconstruct a full thickness skin equivalent. **The real benefit we offer our clients is the development of skin models exactly tailored to their needs and the issues to be addressed.** Our model is flexible and adaptable to the needs; it is a constant source of innovation and scientific inspirations. The cell types used, the age and anatomical origin of cells (young vs. aged / photo-exposed vs. photo-protected), the culture time, the moment and type of application of the active (topical vs. systemic) are all possible variables just like the different stresses to apply such as pollution, UV exposure, mechanical stress...

Customized 3D models (2/3)

Amélie Thépot ,
Founder and CEO of
LabSkin Creations

With this approach, we can propose very sophisticated models to answer to news claims. For example, we know that skin offers protection against several factors, such as pollution or climatic stress. Products can claim to protect against these conditions by reinforcing the skin's natural barrier. To test the efficacy of this kind of products, it is now possible to develop some skin models cultured under stress condition. Moreover, heat chock and pollution are different depending of ethnicity and geographic area. Thus we can tailor skin models with cells extracted from ethnic donors and then modulate skin equivalent models with defined stress. And we know how much the market for cosmetic products aimed at consumers of ethnic origin is fast growing

In the anti-ageing area, one of our latest advances relate to models mimicking chronological aging over very long cultivation times, up to 120 days, while standard cultures stop after 42 days. We are therefore able to test actives throughout the whole tissue reconstruction and substantiate their efficacy on dermal and epidermal reconstruction from a mechanistic and biological point of view.

Customized 3D models (3/3)

Amélie Thépot ,
Founder and CEO of
LabSkin Creations

Evolution of skin equivalent models which are more and more complete and similar to the physiological skin, are promising and bring to research, as well as to dermocosmetology and to the pharmaceutical industry, a wide range of products such as pigmented, endothelialized, immunocompetent, and now adipose skin equivalent models. They have become essential tools to better understand the mechanisms of action of active substances and to test the efficacy of finished products.

Latest advancements of *in vitro* evaluation of cosmetics

THANK YOU FOR YOUR ATTENTION

www. ced-lyon.com

www.premiumbeautynews.com

www.brazilbeautynews.com

CENTRE EUROPÉEN DE DERMOCOSMÉTOLOGIE

premium beauty news .com