

grupo boticário

beleza é o que a gente faz

oBoticário

Ε eudora

quem disse berenice?

THE beauty BOX



Intensifying the application of alternative methods in the development of cosmetic projects

Márcio Lorencini, PhD

Products Evaluation and Regulatory Affairs Manager



Alternative methods for cosmetics evaluation

Safety and efficacy evaluation of cosmetics

Preclinical phase

- Theoretical analysis
- *In silico*
- *In vitro*
- *In vivo (animal)*

Clinical phase

- *In vivo (human)*



Alternative methods for cosmetics evaluation

Safety and efficacy evaluation of cosmetics

Preclinical phase

- Theoretical analysis
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- *In vitro*
- *In vivo* ~~(animal)~~

Clinical phase

- *In vivo* (human)



Alternative methods for cosmetics evaluation

Safety and efficacy evaluation of cosmetics

- Grupo Boticário does not use animal tests for final products evaluation since 2000
- We do not use cosmetic ingredients that were tested in animals according to European ban (2009/2013)
- We study and develop different alternative methods



Alternative methods for cosmetics evaluation

Safety and efficacy evaluation of cosmetics

The importance of alternative methods

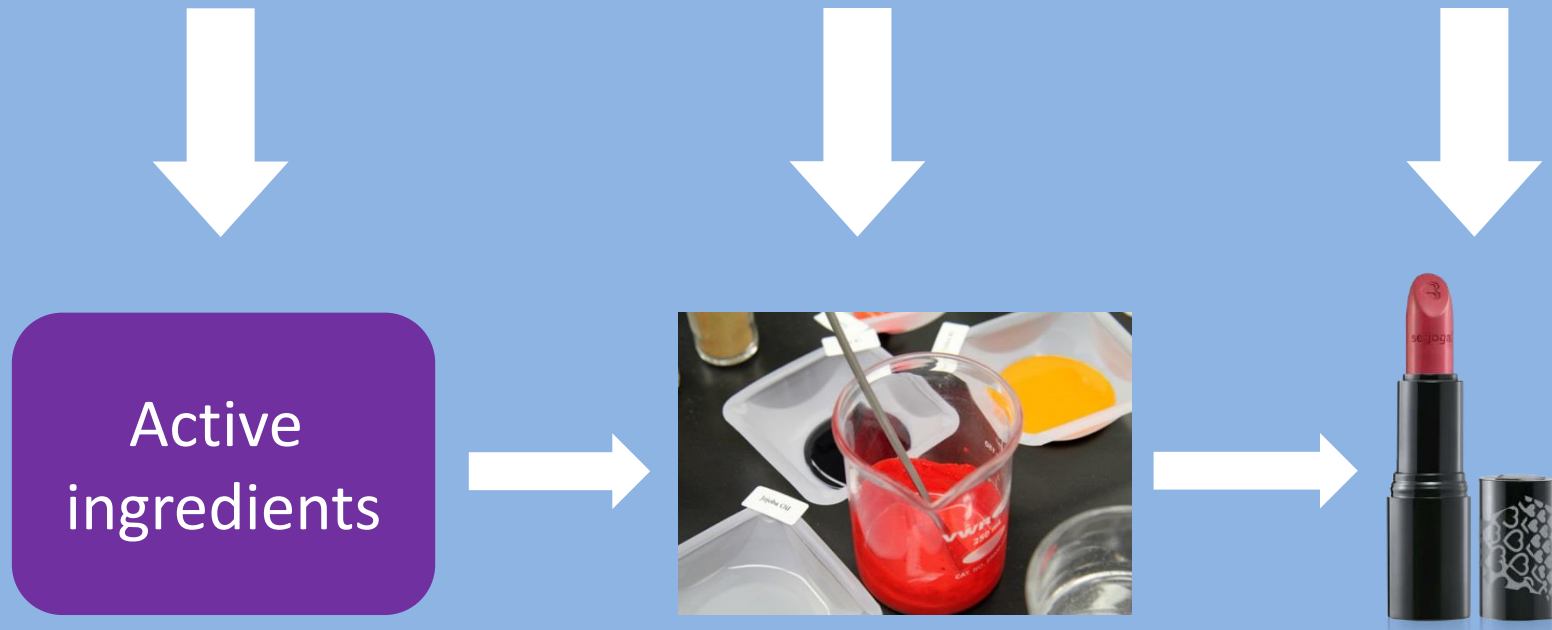
- Preclinical safety assessment
- Optimization of clinical phase
- Reliability and velocity for the process of product development
- New research projects with focus on efficacy evaluation and design of innovative benefits (including the elucidation of mechanisms of action)



Alternative methods for cosmetics evaluation

Safety and efficacy evaluation of cosmetics

The application of alternative methods in the different steps of cosmetics development



Alternative methods for cosmetics evaluation

The (r)evolution of alternative methods

From the restriction in the use of animals according to the 3Rs concept:
Reduction
Refinement
Replacement

For the expansion of analyses with the application of different methodologies

Alternative methods for cosmetics evaluation

The (r)evolution of alternative methods

From the restriction in the use of animals according to the 3Rs concept:
Reduction
Refinement
Replacement

For the expansion of analyses with the application of different methodologies

- 1) Different biological models
- 2) Different techniques of analyses

Alternative methods for cosmetics evaluation

The (r)evolution of alternative methods

Animal *in vivo* models



Systemic but
NOT HUMAN



Human *in vitro* models



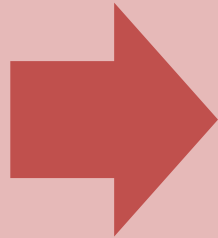
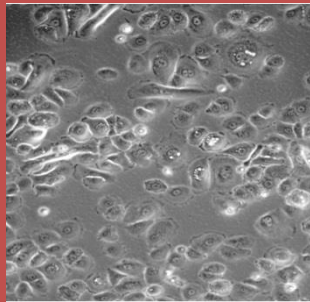
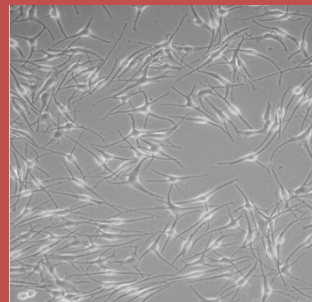
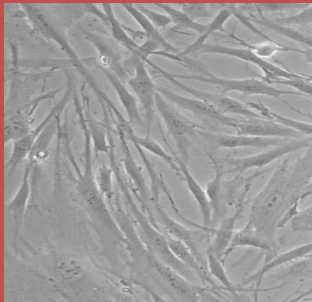
Human but
NOT SYSTEMIC

Alternative methods for cosmetics evaluation

The (r)evolution of alternative methods

Human-based models are attractive, but they might evolve...

2D cell culture models



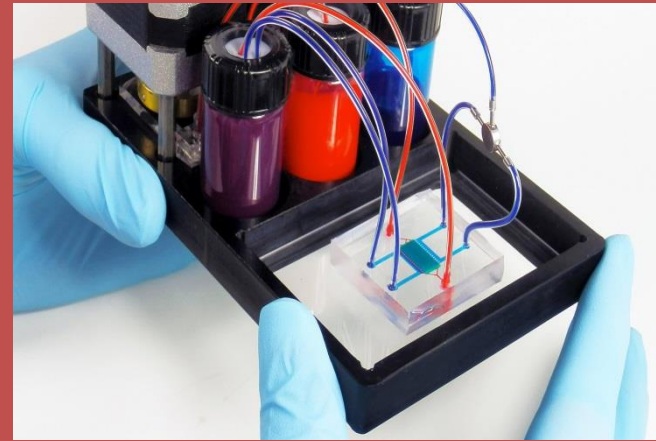
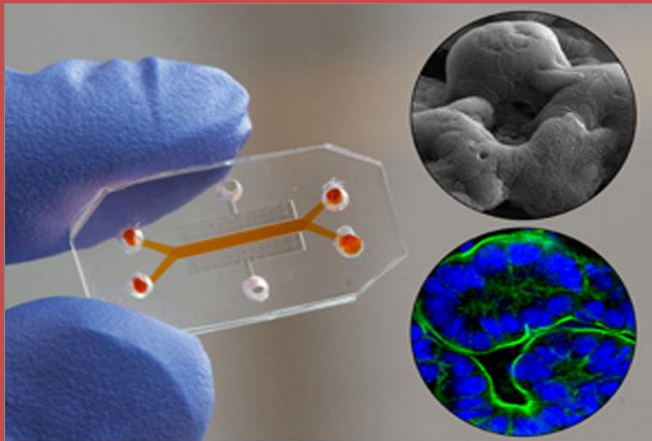
3D cell culture models



Alternative methods for cosmetics evaluation

The (r)evolution of alternative methods

Organs on chips as the next step of human cell cultures...

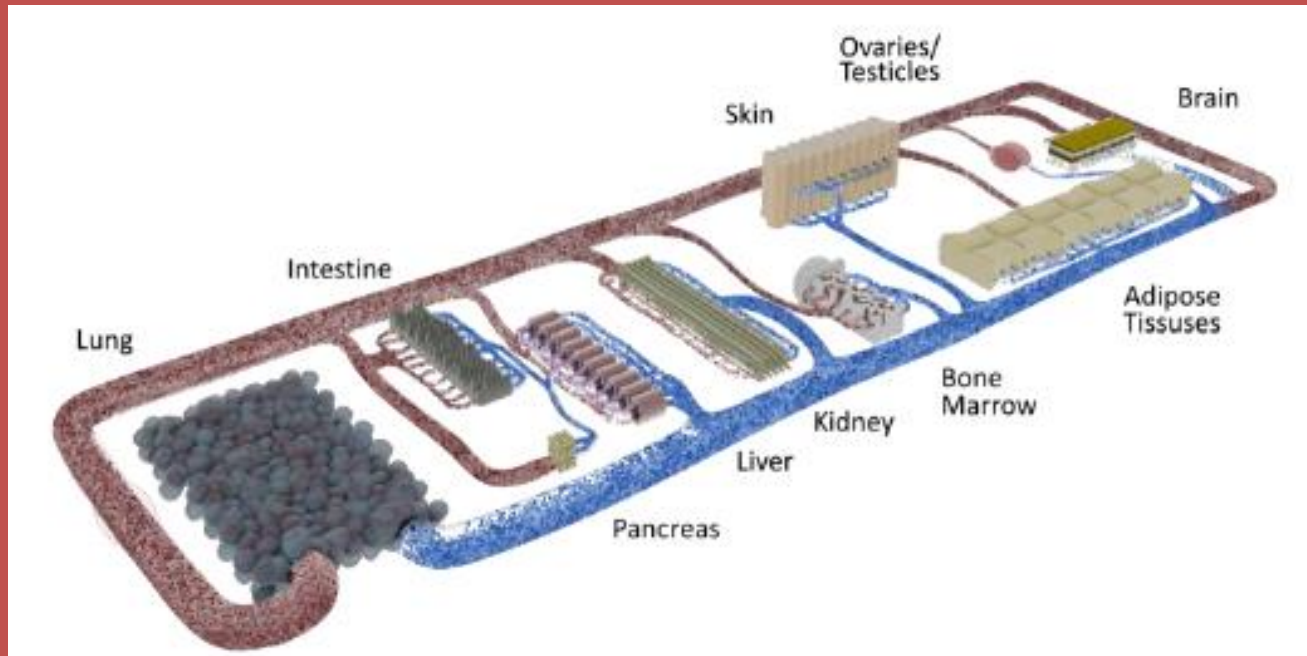


Alternative methods for cosmetics evaluation

The (r)evolution of alternative methods

Human on a chip!

Based on the connectivity of different biological systems *in vitro*



Alternative methods for cosmetics evaluation

Technical considerations

How to address each question to the right model?



- Complexity is not always an advantage
- Sometimes we want to look at biological interactions, sometimes not
- The model must be in accordance with the techniques that will be used for the analyses

Alternative methods for cosmetics evaluation

Technical considerations

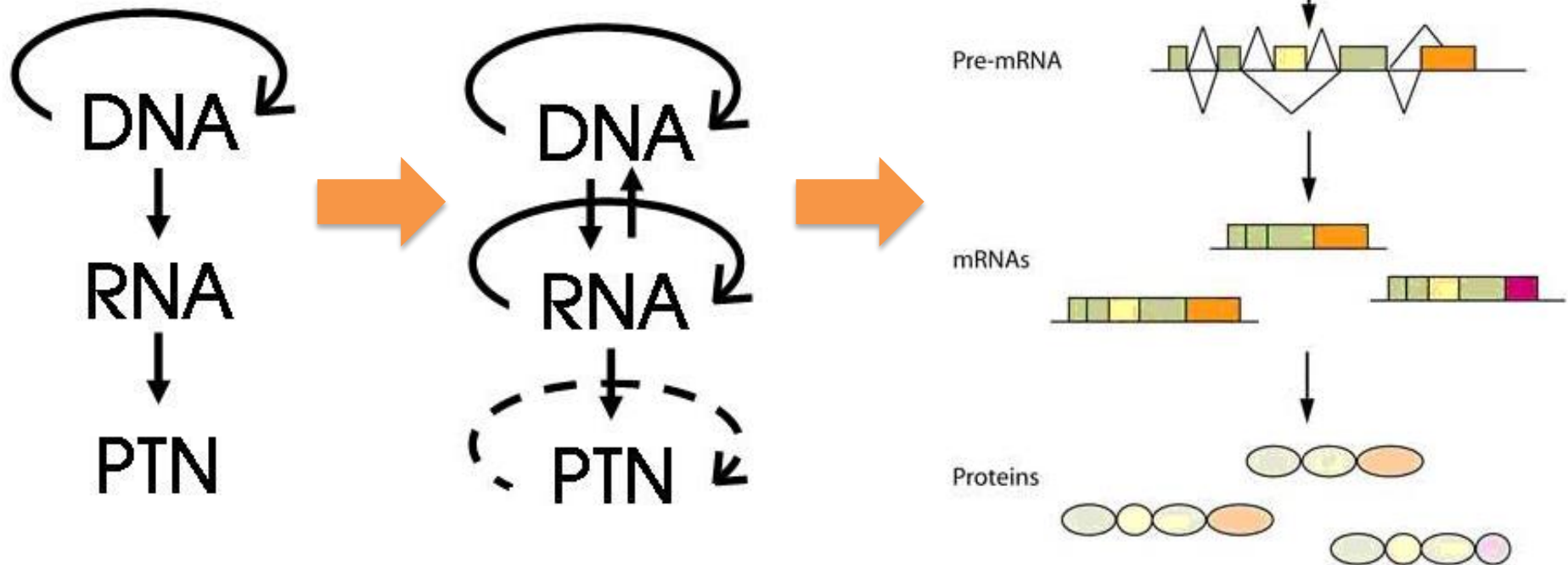
Possibility of working with different techniques

- Light and fluorescence microscopy
 - Flow cytometry
 - ELISA
 - 2D electrophoresis
- Southern, Northern and Western blot
 - PCR
 - Microarray
 - DNA sequencing
 - Bioinformatics

Alternative methods for cosmetics evaluation

Technical considerations

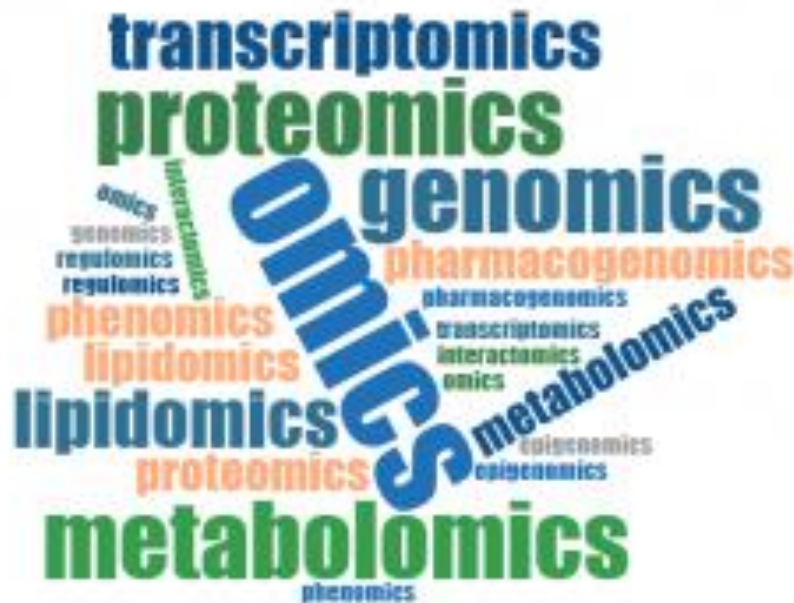
Evolution of biomolecular complexity



Alternative methods for cosmetics evaluation

Technical considerations

Omics approaches



J Invest Dermatol. 2005 Apr;124(4):viii-x.

Skinomics.

Blumenberg M.

Alternative methods for cosmetics evaluation

Examples

Improvement of methods for the evaluation of cytotoxicity

[Toxicol In Vitro](#). 2012 Aug 28. pii: S0887-2333(12)00226-3. doi: 10.1016/j.tiv.2012.08.019. [Epub ahead of print]

A label-free, impedance-based real time assay to identify drug-induced toxicities and differentiate cytostatic from cytotoxic effects.

[Kustermann S](#), [Boess F](#), [Buness A](#), [Schmitz M](#), [Watzela M](#), [Weiser T](#), [Singer T](#), [Suter L](#), [Roth A](#).

[PLoS One](#). 2012;7(11):e50607. doi: 10.1371/journal.pone.0050607. Epub 2012 Nov 21.

Titanium Dioxide (TiO₂) Nanoparticles Preferentially Induce Cell Death in Transformed Cells in a Bak/Bax-Independent Fashion.

[Zhu Y](#), [Eaton JW](#), [Li C](#).

[Exp Dermatol](#). 2012 May;21(5):370-5. doi: 10.1111/j.1600-0625.2012.01479.x.

Quercetin enhancement of arsenic-induced apoptosis via stimulating ROS-dependent p53 protein ubiquitination in human HaCaT keratinocytes.

[Shen SC](#), [Lee WR](#), [Yang LY](#), [Tsai HH](#), [Yang LL](#), [Chen YC](#).

[Int J Cosmet Sci](#). 2012 Apr;34(2):176-82. doi: 10.1111/j.1468-2494.2011.00698.x. Epub 2011 Dec 30.

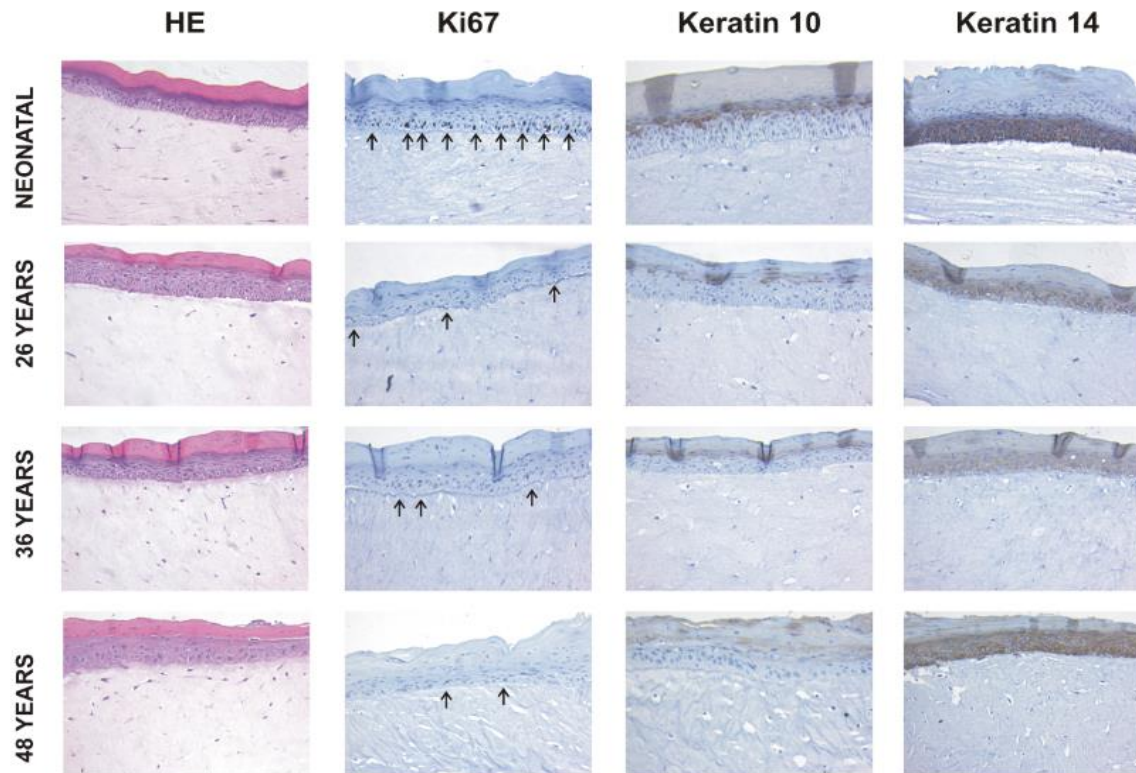
In vitro induction of apoptosis, necrosis and genotoxicity by cosmetic preservatives: application of flow cytometry as a complementary analysis by NRU.

[Carvalho CM](#), [Menezes PF](#), [Letenski GC](#), [Praes CE](#), [Feferman IH](#), [Lorencini M](#).

Alternative methods for cosmetics evaluation

Examples

Evaluation of epidermal markers using 3D skin model



Alternative methods for cosmetics evaluation

Examples

Safety assessment of cosmetic preservatives

SAFETY ASSESSMENT OF COSMETIC PRESERVATIVES

CANAVEZ A.D.P.M., BROHEM C.A., LORENCINI M.
Department of Safety and Efficacy Assessment, Botnicario Group.

INTRODUCTION

Consumers are seeking products developed with attentive to the potential health hazards of ingredients. Preservatives are important to prevent microbial growth, however some aspects must be considered for a complete risk assessment, such as concentration limits defined by legislation, toxicological concerns depending on exposure, and the potential to cause adverse reaction according to scientific literature. In USA, women use an average of 12 personal care products a day, which generates an aggregate exposure that should be considered for effective formulations.

OBJECTIVE

The aim of this study was to develop a matrix of preservative toxicology to indicate safe limits according to each product's category.

METHODOLOGY

The rationale was based on a comparative hazard and risk/exposure assessment for 14 preservatives commonly used in cosmetics, considering available results from in silico, in vitro, clinical, and epidemiological studies. Aggregate exposure was also evaluated for finished products. Two correlated dimensions composed the toxicological matrix: 1) local or systemic exposure, considering the endpoints skin sensitization and irritation, eye irritation, phototoxicity, oral acute toxicity, carcinogenicity, mutagenicity, reproductive toxicity; and 2) toxicity level, defined as low, moderate, or high in accordance with the main toxicological guidelines.

Risk assessment considered the margin of safety (MoS) and estimated exposure, with basis on concentration, frequency of use, route, and target population such as children, pregnant, or sensitive skin. Aggregate exposure was calculated for a complete product line, considering combined and sequential use of cosmetics. Risk and hazard assessments generated threshold recommendations for the application of preservatives in different cosmetic categories.

CONTACT



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RESULTS AND DISCUSSION

CAS	INGREDIENTS	HAZARD ASSESSMENT LOCAL AND SYSTEMIC EXPOSURE								RISK ASSESSMENT			
		skin sensitization	skin irritation	eye irritation	phototoxicity	acute oral toxicity	carcinogenicity	mutagenicity	reproductive toxicity	NOAEL repeated dose oral toxicity studies mg/kg bw/d	maximum concentration allowed by EU legislation or usual concentration	SED (mg/kg bw/day)	MoS (margin of safety)
100-51-6	BENZYL ALCOHOL	H	L	H	L	M	L	L	L	500	0,60	1,614	309,7893432
26172-55-4	METHYLSOETHAZOLINONE (MIT)	H	H	L	L	L	L	L	L	2,8	0,001500	0,004035	693,9281289
2682-20-7	METHYLCHLORISOETHAZOLINONE (CMT)	H	H	L	L	L	L	L	L	2,8	0,001500	0,004035	693,9281289
61789-71-7	BENZALKONIUM CHLORIDE	M	M	H	N/A	H	L	L	L	82	0,10	0,269	304,8327138
122-99-6	PHENOXYETHANOL	B	B	H	B	M	L	L	L	357	1,00	2,69	132,7137546
1317-86-8	CAPRYLYL GLYCOL	B	B	H	B	B	L	L	L	300	0,50	1,345	223,0483271
532-33-1	SODIUM BENZOATE	B	B	M	B	B	L	L	L	1000	0,50	1,345	783,4844238
24634-61-5	POTASSIUM SORBATE	B	B	H	B	B	L	L	L	1000	0,60	1,614	619,5786655
70445-33-9	ETHYLHEXYLGLYCERIN	M	B	H	B	M	L	M	M	800	1,00	2,69	297,2877995
15465-53-6	NOODROPYRIN BUTYLCARBAMATE	H	B	H	B	H	L	L	M	20	0,01	0,0269	783,4844238
2682-20-4	METHYLSOETHAZOLINONE	H	H	H	B	H	L	L	L	19	0,01	0,0269	706,3397026
520-45-6	DEHYDROACETIC ACID/ SODIUM PHYPRACETATE	L	L	M	B	H	L	L	L	100	0,01	0,01614	6195,786655
104-29-0	PHENYLENESULFONIC ACID	L	L	M	B	L	L	L	L	100	0,30	0,807	133,9157373
90-80-2	GALLONACTONE	L	L	L	N/A	L	L	L	L	594	0,70	1,883	315,4840627
60177-38-8	SORBITAN CAPRYLATE	L	L	L	N/A	L	L	L	L	1000	1,00	2,69	371,2472119

Margin of safety was calculated by use of the following equation NOAEL/SED to each ingredient. Dermal Absorption was assumed a 100% considering the worst-case scenario and relative daily exposure was considered 269 mg/kg bw/day. According, SCCS Notes Of Guidance For The Testing Of Cosmetic Ingredients And Their Safety Evaluation 9th Revision, 2015. This calculation allows to extrapolate from a group of test animals to an average human population. A default value of 100 (10x10) is generally accepted and a MoS of at least 100 therefore indicates that a cosmetic ingredient is considered safe for use.

Tipo de produto	Preservatives	relative daily exposure mg/kg bw/day	product concentration (%)	SED (mg/kg bw/day)	NOAEL	MoS (margin of safety)
SKIN CARE PRODUCTS	Exfoliating Wash	269	0,25	0,0725	337	500,8502188
	Cleansing Mousse	269	0,25	0,0725	300	783,4844238
	Skin Tonic	269	0,5	1,345	2,8	2,081248827
	Night cream	269	0,25	0,0725	300	619,5786655
	Eye cream	269	0,3	0,807	300	819,5786655
	Serum	269	0,5	1,345	300	783,4844238
	AGGREGATE EXPOSURE	269	1,26	2,826	300	177,0224835
	AGGREGATE EXPOSURE	269	1,8	5,118	300	60,8044805
	AGGREGATE EXPOSURE	269	1,26	2,826	300	177,0224835
	AGGREGATE EXPOSURE	269	1,8	5,118	300	60,8044805

CONCLUSION

Despite the possible overestimation, aggregate exposure showed to be an efficient tool for the prediction of cumulative risk, allowing to make the best choices of preservative systems during the development of new products. Identification and use of ingredients with lower intrinsic hazard is an important and effective way to reduce overall potential health concerns.

Alternative methods for cosmetics evaluation

Examples

Development of a human dermal papilla in vitro model

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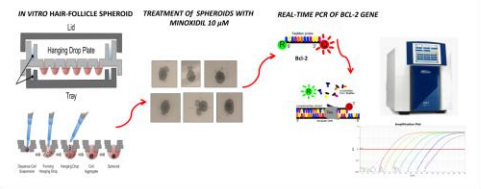
Neopapila for hair care ingredients evaluation: efficacy model.

Desirée Cigarán Schuck¹*, Bruna Bastos Swirka¹, Aline Raquel Leck¹, Márcio Lorencini¹, Carla Abdo Brohem¹

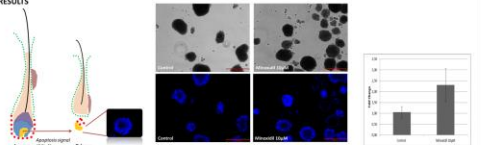
ABSTRACT
The hair sector of cosmetic industry is a promising and competitive market. Considering this scenario, development of new products and aggressive claims become essential in hair market. In vivo testing for development of new products have a high cost besides ethical implications, thus in vitro methodologies provide a quick and practical screening of new ingredients. In this work we developed a spheroid of human hair dermal papilla (HHDP) in vitro, forming an organoid with anatomical similarities to hair bulb. The spheroids were formed using the hanging drop technique, where HHDP are grown in droplets of culture medium for 24 hours. We tested the ability of these spheres to respond to Minoxidil, known ingredient that promotes hair growth. After 24 hours of incubation, RNAs were extracted from treated and non-treated spheres and BCL-2 expression was evaluated by real-time PCR. BCL-2 is responsible for apoptosis control, inhibiting the start of this process. Sasse et al (1994) showed that this protein is increased in the anagen phase, reduced in the catagen and non-existent in the telogen stage, showing the central role in the development of the hair cycle. Minoxidil increase BCL-2 (fold change= 2.28, p=0.001) showing potential to inhibit apoptosis in HHDP spheroids. It is well established that Minoxidil enhances hair growth by prolonging anagen phase, blocking apoptosis signal in in vitro models. Our result corroborates with Minoxidil mode of action showing that the dermal papilla spheroid model can be an important tool in the search of new products for hair treatment.

OBJECTIVE
To evaluate the neopapillae model originated from HHDP spheroids as a method and understand its ability to respond to minoxidil, a standard ingredient described in the literature for hair growth stimulation.

METHODS
IN VITRO HAIR-FOLLICLE SPHEROID **TREATMENT OF SPHEROIDS WITH MINOXIDIL 20 µM** **REAL-TIME PCR OF BCL-2 GENE**



RESULTS



CONCLUSION
The development of in vitro methods is helpful to clarify mechanistic approaches of substances. The neopapillae in vitro model represents an important opportunity to study physiological and pathological parameters of hair development. This method can be an useful tool for the understanding of morphological and molecular aspects associated to different hair disorders.

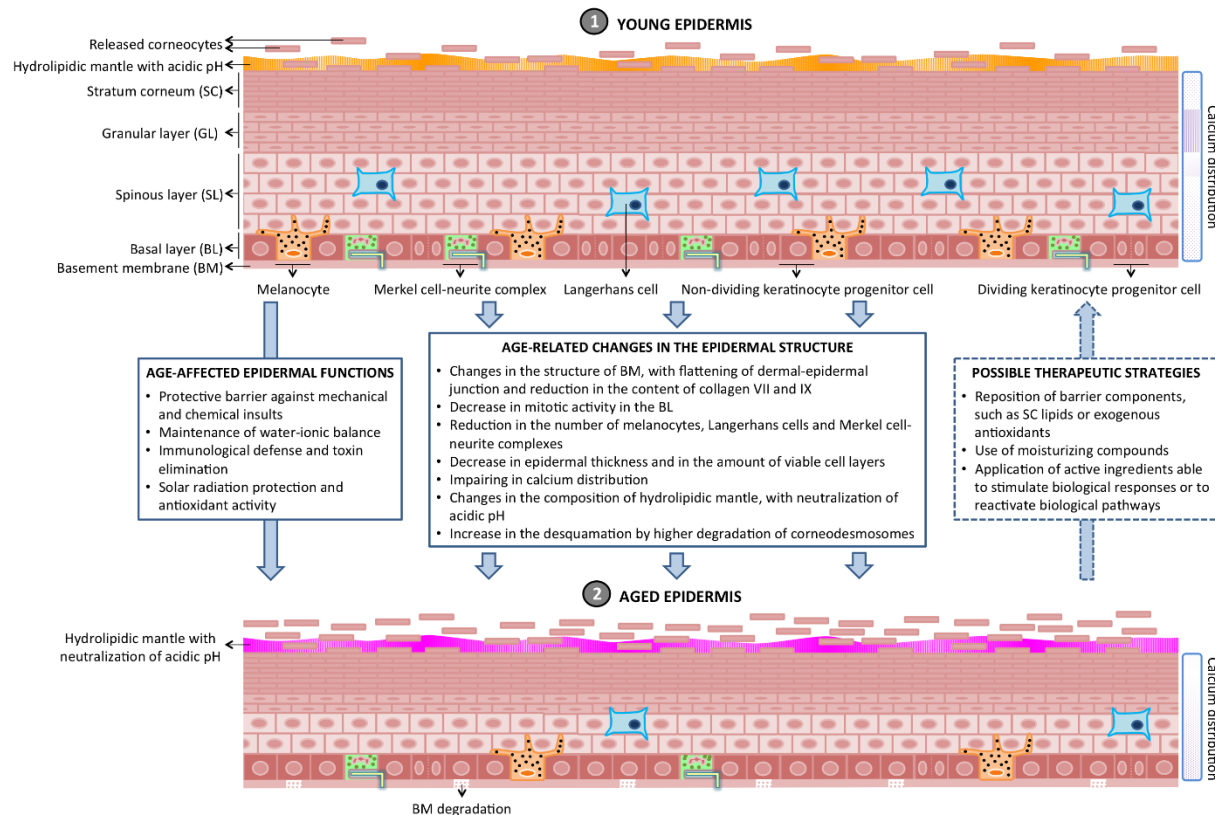
REFERENCES
* Han JH, Kwon GS, Chung JH, Cho KH, Eun HC, Kim KH. Effect of minoxidil on proliferation and apoptosis in dermal papilla cells of human hair follicle. *J Dermatol Sci*. 2004 Apr;24(2):93-8.
* Stern RS, Lawrence L, Vohr B, Kohnmeyer S, Seiberg M. Expression of the bcl-2 protooncogene in the cycling adult mouse hair follicle. *J Invest Dermatol* 1994;103(1):107-11.

This work was conducted with the support of Grupo Boticário.

Alternative methods for cosmetics evaluation

Examples

Mechanistic approach for treating epidermal aging





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Thank you!

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