Emerging Trend in Use of Skin Care and Sun Care, A Boost to Upgrade *in vitro* Strategies for Local Tolerance Evaluation of Ingredients

SC97

Roso Alicia[1], Puginier Mickaël[1], Bergal Mathilde[1], De Servi Barbara[2], Ceriotti Laura[2], Meloni Marisa[2]
[1] Seppic, 92257 La Garenne Colombes, France ; [2]VitroScreen, 20149 Milan, Italy

Introduction

Formulation trends or new uses are also an opportunity to improve technical knowledge and upgrade practices. In recent years, the popularity of aerosol sprays has increased worldwide. Out of traditional use in fragrances, deodorant/antiperspirant, hairspray and spring waters, **aerosol format expanded into new categories, especially skin care and sun protection**. This trend raises a **new challenge for local tolerance assessment as some ingredients,** not present in traditional aerosol compositions, can be **occasionally or frequently in contact with the upper airways.**

Objectives

- Investigate the tolerance of some key ingredients on a reconstructed upper airway epithelium model
- **Ultimate goal: facilitate, at early development stages, the selection and appropriate dosages of ingredients widely used in skin care and sun care sprays.**

Materials & methods

❶ Investigation of model sensitivity with aerosol sprayable skin care benchmarks (expected good tolerance levels according to marketing claims).

❷ Validation of the experiments with negative and positive controls.

Oxalic acid selected as a known respiratory irritant [1] used at reduced dosage (2mg/mL), intended to induce a slight effect (IC-75=1.16mg/mL; Concentration required to reduce viability to 75%; MTT measurement).

Formula	Type	Main components	Skin target
Benchmark1	Oil in water (O/W) emulsion	*Isopropyl Palmitate, Petrolatum, Dimethicone, Hydrolyzed Hyaluronic Acid, Isohexadecane, C12-20 Alkyl Glucoside, Polysorbate 60, Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, C14-22 Alcohols, Preservative system and Fragrance*	Moisturizing body mist
Benchmark2		*Dipropylene Glycol, Isostearyl Isostearate, Sucrose Stearate, Squalane, Pentylene Glycol, Cellulose Gum, Polyacrylate Crosspolymer-6, 5 Active Ingredients: Plant Extracts & Vitamin, No Preservative, No Fragrance*	Soothing / for sensitive skin
Controls	**Negative**	*Phosphate-Buffered Saline (PBS)*	
	Positive	*Oxalic acid (2mg/mL)*	

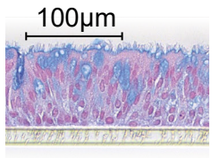
❸ Evaluation of ingredients with key functionality in skin care and sun care formulations selected according to: well known safety and local tolerance profiles, texture and rheology behaviour suitable for aerosol format, versatility in skin and sun care usages.

Category	Code	Structure / INCI
Emollient	**Emo**	*C15-19 Alkane*
O/W Emulsifier	**Emul1**	*C12-20 Alkyl Glucoside, C14-22 Alcohols*
	Emul2	*C20-22 Alcohols & C20 Glucoside*
Rheology modifier	**RM1**	*Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer*
	RM2	*Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Isohexadecane, Polysorbate 60*

❹ Methodology:

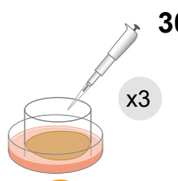
The protocol was developed to mimic limited exposure time on the upper airway.

in vitro model



3D Reconstructed human airway epithelium model
(MucilAir from Epithelix company)

Protocol



30µl of product / epithelium
- Ingredients (▮▮▮) & controls at defined dilution in water
- Benchmarks (▮) pure

x3

Wash off

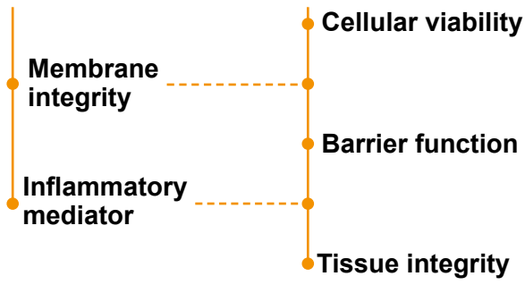
t0 — *Time of contact* → 3h — *Recovery* → 24h

Analysys

- **Cellular viability** (Resazurin assay)
  - Measure of fluorescence
  - Calculation of viability % *versus* negative control

- **Membrane integrity**
  LDH (Lactate Dehydrogenase) release measurement by UV spectrophotometry
- **Inflammatory mediator:** IL-8 release (Immunoassay)
- **Barrier function**
  Trans-Epithelial-Electrical-resistance (TEER) measurement
- **Tissue integrity:**
  - Histology analysis / Alcian blue stained slices / Light microscopy analysis (Leica DMi8 THUNDER).
  - Scoring from score 0 (comparable to negative control) to 3 (severely altered)

Multiple End-Points Analysis



Results & Discussions

Model sensitivity and experiments validation

- Protocol validated on independant experiments (1 and 2) using well known respiratory irritant chemicals (data not shown)
- Significant and reproducible effects obtained for the positive control.
- High sensitivity of the model confirmed by the results on the benchmarks with significant effects:
  - Cell viability not affected (both benchmarks),
  - Benchmark 1 affected cellular membrane integrity at 3 hours and increased inflammatory signal. After 24 hours, inflammation was maintained while LDH release returned to normal level.
  - Benchmark 2 reduced membrane integrity and epithelium barrier functions up to 24 hours.

Items	Viability (NC %)	LDH release (mU/mL) 3h	LDH release (mU/mL) 24h	TEER (NC %)	IL-8 release (pg/mL) 3h	IL-8 release (pg/mL) 24h	Histology (score)
Negative control (Experiment 1/ Experiment 2)	100	0/1	0/26	100	2 800/2 002	7 300/10 600	0-1/0
Positive control (Experiment 1/ Experiment 2)	78*/73	27*/46*	99*/184*	10*/18*	15 900*/13 700*	60 000*/50 200*	2-3/3
Benchmark1 (Experiment 1/ Experiment 2)	100/100	5*/9*	24/11	86/66	5 354*/ND	20 953*/ND	0-1/ND
Benchmark2 (Experiment 1/ Experiment 2)	100	13*	63*	15*	748	12 138	2

Controls & Benchmarks results * p<0,05 T-test compared to negative control; ND: Not Done

Items	Viability (NC %)	LDH release (mU/mL) 3h	LDH release (mU/mL) 24h	TEER (NC %)	IL-8 release (pg/mL) 3h	IL-8 release (pg/mL) 24h	Histology (score)
Negative control (Experiment 1/ Experiment 2)	100	0/1	0/26	100	2 800/2 002	7 300/10 600	0-1/0
Emo 50% in paraffin oil (Experiment 1/ Experiment 2)	77*/100	2/5	3/0	114/148	ND/0	ND/2075	ND/1
Emul1 2% in water (Experiment 1)	93	3	34	99	3 420	21 884*	0-1
Emul2 2% in water (Experiment 1)	100	4	5	66	2 052	4 871	0-1
RM1 2% in water (Experiment 2)	65	5	51	105	2 529	18 259	1
RM2 2% in water (Experiment 2)	79/100	2/3	8/7	131/92	ND/863	ND/10 328	ND/1

Ingredients results * p<0,05 T-test compared to negative control; ND: Not Done

Limited effects of ingredients considering controls.

Emollient did not significantly affect the evaluated parameters. Decrease in viability in experiment 1 considered as biologically non significant (variability within replicates and experiments).

Emulsifiers, differing only in the length of the fat chain, preserved cellular viability, tissue organisation and barrier function. Emul1 slightly increased the LDH release and inflammatory response after 24h.

Only RM1, at 24h, affected viability and LDH release (non-statistically) and IL-8 expression ($p<0.05$). RM1 and RM2 provided same histology score of 1 (preserved overall morphology *versus* negative control, visible cilia, goblet cells with regular shape, distribution and mucus).

Discussion: Attempted tolerance scale

Viability selected as entry parameter for irritation, others equally weighted to determine tolerance levels.

- **Good:** Parameters not significantly different from the negative control
- **Acceptable:** Viability comparable to the negative control. One or several parameters with significant effects (acceptability according to Benchmarks)
- **Poor:** significant decrease in viability and marked effects on one or several other parameters.

Items	Tolerance conclusion
Negative control	Good
Positive control	Poor
Benchmark1	Acceptable
Benchmark2	Acceptable
Emo	Good
Emul1	Acceptable
Emul2	Good
RM1	Acceptable
RM2	Good

Tentative tolerance conclusion

Emul1, included in Benchmark 1, provided the same type of effects with expected similar conclusion. Sensitivity of 3D reconstructed epithelia to surfactants is well recognized [2] and found both in Benchmarks 1 and 2. Even if Emul1 provided an acceptable conclusion, Emul2 can be preferred for such aerosol sprays.

Higher polymer concentration of RM1 compared to RM2 can partly explain the different conclusions. Mucoadhesive properties of RM1 [3] could have contributed to wash-off disturbance ► overestimated effects. On the contrary, RM2 was easily washed off (more fluid gel/quick break effect).

Conclusion

- **This 3D in vitro model represents a promising screening tool to assess the local tolerance of ingredients that may frequently or occasionally come into contact with the upper airway, first line of defense in the respiratory system.**
- Multiparametric analysis increases model sensitivity and opens perspective to determine suitable well-tolerated ingredient doses. Further investigations on diverse chemical structures covering all ingredients categories should be carried out as well as dose-response effects. Due to its high sensitivity, this model could be used to predict the tolerance level of ingredient combinations, from the very beginning of aerosol formulations development.

References:
1. Maione AG, Jackson GR, Vinall JL, Simpson H, Storey EL, Debatis M, Klausner M, Roper C, Hayden PJ ; *(2018) Comparative inhalation toxicity testing using in vitro organotypic rat and human airway epithelial models*. 20th ESTIV Congress, poster 135, Berlin, Germany.
2. Roso A, Puginier M, Bergal M, Nunzi F, Alonso A ; *(2021) In vitro Approach to Assess Local Tolerance of Ingredients Dedicated to Specific Topical Care Applications*. J Dermatol & Skin Sci. 3(1):30-48.
3. Denis A, Coudert C, Despax S, Ben Arous J, Bulcourt C, Roso A ; *(2019) Innovative Rheology Modifiers for Mucosal Formulations. Skin and Formulation 5th Symposium Reims, France.*

10169- GB - 01 July 2021 - Design: Doc@lyne