In vitro irritation of SBMD intended for application to oral, vaginal or rectal mucosae

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Local tolerance is a common pre-requisite before placing dermatological products on the market and the need of Alternatives to animal testing is increasing: Guideline on non-clinical local tolerance testing of medicinal products EMA/CHMP/SWP/2145/2000 Rev.1 Corr. 1* of 22 October 2015) states: *In vivo testing should not be undertaken until all available data relevant to the potential adverse effects of the substance have been evaluated in a weight-of-the-evidence analysis. Such data can include the physico-chemical properties of the product in its intended formulation, literature data, findings from one or more structurally related substances, and results from in vitro or ex vivo studies using accepted assays (see the guideline on regulatory acceptance of 3R testing approaches)".* EU Validated ALTERNATIVES are available to classify chemicals and mixtures, but they are not applicable to assess the irritation potential of complex mixtures, such as topical formulations to be applied on skin and mucosal tissues such as cosmetics toiletries and Substance based Medical Devices (SBMD).
ISO 10993-23:2021 guideline has validated the use of reconstructed human epidermis (RhE) to assess irritation potential of medical device (MD) extracts. Furthermore the applicability of RhE systems to assess Substance Based Medical Device by a direct topical exposure has been demonstrated to be robust and reproducible (1). However peculiar requirements are still necessary to assess local tolerance i.e. "irritation " for medical devices intended to be applied to a specific area, i.e. mucosal, eye, vaginal or rectal epithelia. ISO standard states that "RhE models are not adapted to address the safety of medical devices intended to be applied to specific areas of the body and it is recommended to explore the use of other in vitro models with relevant cells or tissues if qualified for use with medical devices.

ISO 10993-23:2021 (Biological evaluation of medical devices - Part 23: Tests for irritation) states that: *"for special irritation tests (es. for medical devices intended to be applied on mucosal or eye epithelia) it is recommended to explore the use of other in vitro models with relevant cells or tissues if qualified for use with medical devices".* 3 Human Reconstructed Epithelia are commercially available, produced by culturing in an air-liquid interface human cells (immortalized or primary) from the target organs, forming multilayered and highly differentiated epithelia having patterns analogous to those found in vivo thus allowing acute and repeated application of topical products. Their multi stratified morphology mirrors features of a functional epithelial barrier and thanks to this human relevance they represent a suitable model with higher reproducibility and predictive power versus humans compared to cell monolayers.

AIM & EXPERIMENTAL DESIGN

A retrospective analysis of our internal data using human reconstructed tissues has been performed: dataset were generated during the last 10 years based on biocompatibility protocols (OECD area 9.3) developed for medical devices biological evaluation assessment. Medical Devices (blind products) corresponding to different formulation types (emulsions, sprays, gels, surfactant-based formulations, ovules) and intended to be used on oral, vaginal and a first approach on rectal mucosa were included in order to validate the hypothesis that the proposed approach is transversally applicable as previously demonstrated for cosmetics (2-3).

The experimental protocol I described in Tab. I and in accordance with ISO 10993, cell viability was used as an endpoint and determined on the basis of MTT reduction. The acceptance criteria introduced for the biological evaluation of medical devices (ISO 10993-23) were applied: NC meets the acceptance if the mean OD negative control value of the two-three tissues is ≥0.8 and ≤3.0 and if the Standard Deviation value (SD) of the % viability is ≤ 20%.; Positive control satisfies acceptance criteria if mean cell viability expressed as percentage compared to NC is < 40% and Standard Deviation value (SD) is < 20%.

TABLE I: EXPERIMENTAL DESIGN for MTT viability assay

BODY TARGET	TEST SYSTEM	Exposure	Formulation type/ product category	Products	POSITIVE CONTROL
Oral mucosa	Reconstructed human oral epithelium (HOE) Episkin SA	1h + 16h	• Water solution • Tooth paste/gel • pharmaceutical formulations • Medical devices	31	(SDS 0,25% and 1%)
Vaginal mucosa	Reconstructed human vaginal epithelium (HVE) Episkin SA	1h + 16h	• Surfactant based formulation diluted 5%	70	(SDS 0.25%)
			• Emulsion, suppository for gynecological use, ovules	85	

RESULTS

TABLE II: ORAL EPITHELIUM: EXPOSURE 1H + 16H POSITIVE CONTROLS: MTT RESULTS AS RESIDUAL VIABILITY %

STUDY NUMBER	SDS 0.25%	SDS 1%
≠1	42%	5
≠2	35%	5
≠3	28%	5
≠4	5%	5
≠5	5%	5
≠6	20%	5

TABLE III: VAGINAL EPITHELIUM: EXPOSURE 1H + 16H POSITIVE CONTROL: MTT RESULTS AS RESIDUAL VIABILITY %

STUDY NUMBER	≠1	≠2	≠3	≠4	≠5	≠6	≠7	≠8	≠9	≠10	≠11	≠12	≠13	≠14	≠15	≠16	≠17	≠18	≠19	≠20	≠21	≠22	≠23	≠24	≠25	≠26	≠27	≠28	≠29	≠30	≠31	≠32	≠33	≠34
SDS 0.25%	27%	1%	3%	12%	35%	5%	30%	11%	3%	3%	2%	2%	20%	1%	23%	25%	25%	15%	23%	3%	13%	3%	5%	4%	2%	5%	17%	3%	2%	2%	2%	2%	1%	8%

BLIND PRODUCTS: CLASSIFICATION based on 40% viability as cut off value

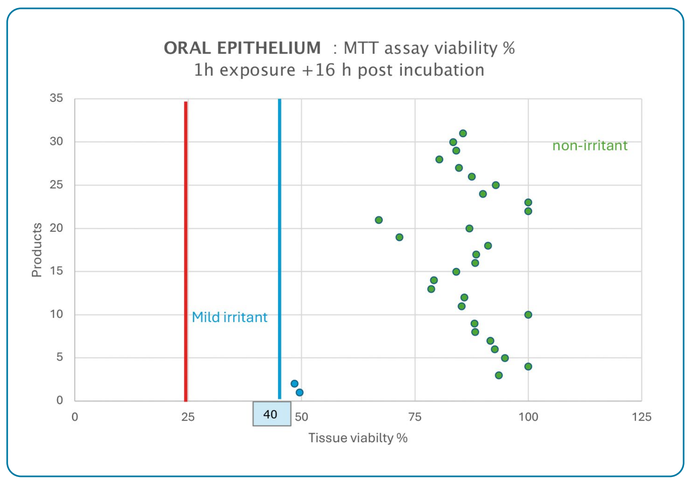


Fig. 1A

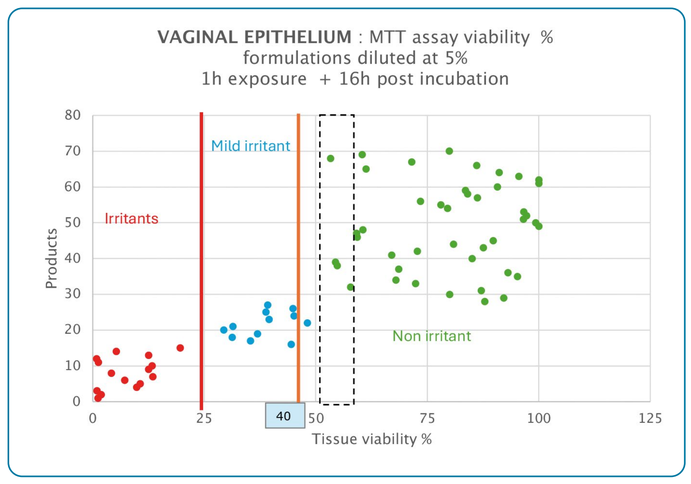


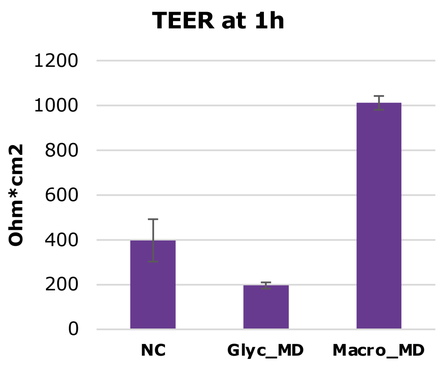
Fig. 1B

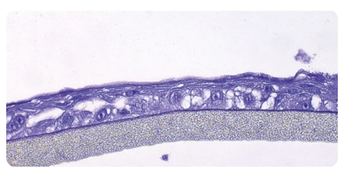
HUMAN RECONSTRUCTED COLON EPITHELIUM: APPLICATION TO ASSESS SBMD MECHANISM OF ACTION BY CHEMICAL-PHYSICAL MEANS

Exposure
1-hour topical treatment to assess 2 MDs with laxative action in modulating water flux across the colon epithelium by osmotic mechanism.

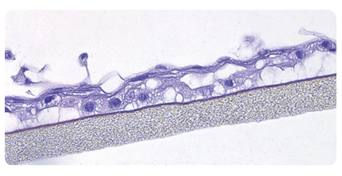
End points
- Tissue morphology by **Hematoxylin and Eosin (H&E)** staining
- Barrier function via Trans Epithelial Electrical Resistance **(TEER)**

Results
- Glycerin based MD: tissue de-hydration with reduced tissue thickness and reduced epithelial integrity (TEER reduction) via **chemical action (hygroscopic)**
- Macrogol based MD: increased tissue thickness and water accumulation within the ECM (TEER increase), resulting in a **mechanical and lubricant barrier (high MW)**

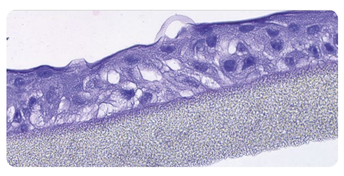




Untreated NC



1H GLYCERIN BASED MD



1H MACROGOL BASED MD

CONCLUSION

This retrospective analysis confirms the relevance and predictivity of in vitro approaches using 3D human tissue models, as well as their flexibility and applicability to formulations regardless of their regulatory status. By using exclusively MTT assay as defined for ISO 10993-23 it has been possible to discriminate products irritation potential compared to a well know irritant SDS which response at 0.25% was found reproducible on oral and vaginal epithelia.

We have has previously demonstrated the interest to adopt a more predictive testing strategy where no validated Alternatives exist or are applicable: MEA, Multiple Endoint Analysis was adopted by our Laboratory since 2002 (4) for testing topically applied formulations and a further communication appeared in 2010 (5) demonstrating that it allows to obtain multiple levels of quantitative information other than cell viability by MTT assay : the measure of barrier function impairment by TEER measure, histological scores and inflammatory response by qRT-PCR.

An encouraging nice correlation with human data has been established for 1 product: a cream (STP) characterized in vivo on humans by means of several but contradictory clinical data. The in vitro results on RHE have shown a significant decrease of cellular viability (residual 45% and 17% respectively), a severe modification of TEER values corresponding to a significant barrier permeability modification and a significant over expression of TNF-α gene.

In Vitro Results (24h)	MTT [% viability]	TEER [Ohm*cm²]	PCR (TNFα) [RQ Study]
CN	100	362,981481	1
STP	45,024	56,5685425	9,07

Human data	Patch Test (48h)	Patch Test (96h)	20 days
STP	Not Irritant	Not Irritant	Slightly irritant

REFERENCES

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2) **R.Roguet et al.** Toxicology in Vitro 12 Toxicology in Vitro 12 (1998) 295-304 *An Interlaboratory Study of the Reproducibility and Relevance of Episkin, a Reconstructed Human Epidermis, in the Assessment of Cosmetics Irritancy.*
3) **Faller et al.** Toxicology in Vitro 16 (2002) 557-572 *Predictive ability of reconstructed human epidermis equivalents for the assessment of skin irritation of cosmetics.*
4) **Meloni M. et al.**: Proceedings INVITOX Congress October 2002-Formia (Italy) *The importance of Multiple Endpoint Analysis (MEA) using reconstituted human tissue models for irritation testing.*
5) **M. Meloni** et al In vitro assessment of skin and mucosae tolerance of cosmetic products: Proceedings of 16th EUSAAT Meeting 2010 -LINZ (Austria).