Commentary on assessment of an *ex-vivo* irritation test performed on human skin explants and comparison of its results with those of a 24/48-hours human patch test for the evaluation of cosmetics.

Patrick Matarrese[1], Gallic Beauchef[1], Laurent Peno-Mazzarino[2], Elian Lati[2], Richard Fitoussi[1*], Katell Vié[1]

[1]Department of Dermatology, Laboratories Clarins, 5 rue Ampère, 95300 Pontoise, France

[2]Department of Dermatology, Laboratories BIO-EC, 1 chemin de Saulxier, 91160 Longjumeau, France

Introduction

It is essential to address the irritation potential of compounds, or mixtures of compounds, that come in contact with human skin. The Draize test was developed in the 1940s for this purpose, relying on the apparition of visible alterations on the skin of albino rabbits [1]. It served as a reference for decades [2]. Still, over the years, it has been increasingly criticized due to the subjectivity of its visual grading, low predictivity towards mild irritants and the real accuracy in the extrapolation of results to human. Concomitantly with the rise of public awareness of animal welfare, several studies highlighted its limitations [3-5]. As a result, worldwide legislations endorsed the introduction of alternative test methods.

In vitro Skin Irritation Tests on Reconstructed Human Epidermis

Among the different strategies that were explored, *in vitro* irritation tests taking advantage of Reconstructed Human Epidermis (RHE) became the new standard [6]. RHE are produced from epidermal cells that are allowed to proliferate and differentiate, on a basal inert substrate and at the air-liquid interface [7]. The procedure leads to structures closely resembling real epidermis, with stratification and a stratum corneum. Several features make RHE a system of choice. The stratum corneum has a crucial barrier function. The keratinocytes are instrumental in triggering the inflammatory responses upon stimulation. Being grown in a serum-free medium, they allow accurate detection of inflammation induced by topically applied substances. Results were admitted to be reproducible. Finally, detection of irritation relies on a rapid and easy method: quantification of cell viability upon enzymatic conversion of MTT into a precipitate that is measured by optical density [8].

Some Limits of Irritation Testing using Reconstructed Human Epidermis

The few RHE systems formally validated by the OECD TG439 are commercial [6]. Relying on a few tissue equivalents, their advantages must be balanced with the limits of the system. There can be an interruption in sourcing due to production problems or commercial strategies of suppliers. RHE are expensive and the cost can even be prohibitive as some countries impose high customs barriers to the import of living human tissues. They can also be quality loss due to long shipment. Therefore, the OECD encourages the development of alternatives methods. This led to the development of "open source" reconstructed epidermis that provides greater autonomy as well as enabling better control of culture parameters [9-12].

Despite the opening these "in house" skin equivalents brought, there are technical limits inherent to the use of reconstructed epidermis themselves. Despite highly standardised production process and rigorous quality controls, RHE present inter-batch variability. Their stratum corneum is also not fully mature [13-16], hindering the test of some galenic formulations, such as alcohol-based and pasty products. Finally, scoring of irritation with the MTT assay only gives an "Irritant"/"Non-irritant" type of answer providing very little insight into the irritation potential of the compound tested.

Ex vivo Irritation Tests using Skin Explants

In a recently published article [17], we report on an *ex vivo* irritation test performed on cosmetics, comparing results to those of 24/48-hours human patch tests. There are two originalities in our approach: the use of human skin explants instead of RHE and the quantification of irritation by histological analysis to provide deeper insights into the irritation potential of the compound tested.

Despite this untraditional approach, results show that the test accurately and repeatable detects known irritants. Also, when testing 120 non-alcoholic cosmetics of any galenic form, comparison of data between the *ex vivo* irritation tests and those of a 24/48-hours human patch test reveals accurate prediction of irritancy with a 10% false-positive rate, a situation putting the test on the safe side. If we have no data on the sensitivity of the *ex vivo* irritation test; its specificity the percentage of non-irritants correctly identified is 89.9% and its accuracy the overall percentage of the correct classification of irritants and non-irritant is 89.1%. When testing 49 alcoholic cosmetics, results are similar with a slightly higher false positive rate. Taken together, these values exceed the minimum requirements of high quality standards of OECD TG439.

One of the expected drawbacks of using skin explants is the variability of the irritation response. This is expected due to their diverse origin. Still, the meticulous and thorough scoring of minute histological alterations, as well as the rigorous correspondence scale used to grade irritation, are certainly instrumental in faithfully detecting individual diverse response to irritants. Indeed, results from 52 different skin explants subjected to 0%, 10% or 20% SDS do show variability in the type of histological alterations that were scored. Nevertheless, the resulting irritation score was highly reproducible.

Using human skin explants to perform irritation tests offers several advantages. The most obvious is that, being plastic surgery wastes, they are easily available and very affordable. Skin explants are also closer to skin physiology than RHE. They are also more robust and have a fully mature stratum corneum with its full barrier function, a feature that enabled us to successfully test cosmetics encompassing all galenic formulation of cosmetics, including alcohol based cosmetics, pasty products, and solid ones like nail polishes, something that is hardly possible using RHE.

Discussion and Conclusion

The *ex vivo* irritation test suffers some drawback. Grading of histological alterations is time consuming and requires highly trained histological experts. In our case, two who grade independently, randomly and blindly and who confront their observation to avoid deviation from the initial scoring grid. Besides, like any *in vitro* system, the timespan during which a compound can be tested depends on the time the skin explant can be maintained in culture without losing its characteristics. These limitations still do not enable testing chronicle exposure or long-term effects.

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References

1. Draize JH, Woodard G, Calvery HO. Methods for the study of imitation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Therap. 1944;82:377-90.
2. OECD. Test No. 404: Acute Dermal Irritation/Corrosion. OECD Guidelines for the Testing of Chemicals, Section 4, Éditions OCDE, Paris. 2002.
3. Basketter DA, Chamberlain M, Griffiths HA, et al. The classification of irritants by human patch test. Food Chem Toxicol. 1997;35:845-52.
4. Robinson MK, Whittle E, Basketter DA. A two center study of the development of acute irritation responses to fatty acids. Amer J Contact Dermat. 1999;10:136-45.
5. Basketter DA, York M, McFadden JP, et al. Determination of skin irritation potential in the human 4-h patch test. Contact Dermatitis. 2004;51:1-4.
6. OECD. Test No. 439: *In vitro Skin Irritation: Reconstructed Human Epidermis Test Method, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. 2019.*
7. Netzlaff F, Lehr CM, Wertz PW, et al. The human epidermis models EpiSkin, SkinEthic and EpiDerm: an evaluation of morphology and their suitability for testing phototoxicity, irritancy, corrosivity, and substance transport. Eur J Pharm Biopharm. 2005;60:167-78.
8. Mosmann T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays, J. Immunol. Methods. 1983;65:5563.
9. De Wever B, Goldberg A, Eskes C, et al. "Open source"-based engineered human tissue models: A new gold standard for nonanimal testing through. Appl. In Vitro Toxicol. 2015;1:5-9.
10. Mewes KR, Fischer A, Zöller NN, et al. Catch-up validation study of an in vitro skin irritation test method based on an open source reconstructed epidermis (phase I). Toxicol in vitro. 2016;36:238-53.
11. Groeber F, Schober L, Schmid F.F, et al. Catch-up validation study of an in vitro skin irritation test method based on an open source reconstructed epidermis (phase II). Toxicol In Vitro. 2016;36:254-61.
12. Pedrosa T.D.N, Catarino C.M, Pennacchi P.C, et al. A new reconstructed human epidermis for in vitro skin irritation testing. Toxicol In Vitro. 2017; 42:31-37.
13. Lebonvallet N, Jeanmaire C, Danoux L, et al. The evolution and use of skin explants: potential and limitations for dermatological research. Eur J Dermatol. 2010;20:671-84.
14. Thakoersing VS, Danso MO, Mulder A, et al. Nature versus nurture: Does human skin maintain its stratum corneum lipid properties in vitro? Exp. Dermatol. 2012;21:865-70.
15. Andrade TA, Aguiar AF, Guedes FA, et al. Ex vivo Model of Human Skin (hOSEC) as Alternative to Animal use for Cosmetic Tests. Procedia Eng. 2015;110:67-73.
16. Frade MA, Andrade TA, Aguiar AF, et al. Prolonged viability of human organotypic skin explant in culture method (hOSEC). An Bras Dermatol. 2015;90:347-50.
17. Matarrese P, Beauchef G, Peno-Mazzarino L, et al. Assessment of an *ex vivo irritation test performed on human skin explants and comparison of its results with those of a 24-/48-h human patch test for the evaluation of cosmetics. Toxicol in vitro. 2021;70:105030.*

*Correspondence to

Dr. Richard Fitoussi

Department of Dermatology

Laboratories Clarins

5 rue Ampère

95300 Pontoise, France

Email: richard.fitoussi@clarins.com