Toxicology in Vitro xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

SkinEthic™ RHE for *in vitro* evaluation of skin irritation of medical device extracts

Christian Pellevoisin[a,*], Christelle Videau[a], Damien Briotet[b], Corinne Grégoire[a], Carine Tornier[a], Alain Alonso[a], Anne Sophie Rigaudeau[a], Charbel Bouez[a], Nathalie Seyler[a]

[a] *EPISKIN, Lyon, France*
[b] *NAMSA, Chasse-sur-Rhône, France*

ARTICLE INFO

Keywords:
Medical devices
Reconstructed human epidermis
RHE
Skin irritation
In vitro
Biocompatibility
ISO 10993
Alternative to animal testing

ABSTRACT

According to ISO 10993 standards for biocompatibility of medical devices, skin irritation is one of the three toxicological endpoints to be always addressed in a biological risk assessment. This work presents a new protocol to assess this endpoint *in vitro* rather than *in vivo*. The protocol was adapted to medical devices extracts from the OECD TG 439 with the SkinEthic™ RHE model as test system. It was challenged with irritant chemicals, Sodium Dodecyl Sulfate, Lactic Acid and Heptanoic Acid spiked in polar solvents, sodium chloride solution or phosphate buffer saline and non-polar solvent, Sesame Oil. Cell viability measured by MTT reduction after 24 h exposure was used as readout. Quantification of IL-1α release as secondary readout did not increased performance. Samples of heat-pressed polyvinyl chloride (PVC) and silicone sheets infused with or without known irritant (4% Genapol-X80, 6% Genapol-X100 and 15% SDS) were tested after extraction in polar and non-polar solvents. Medical device extracts are classified irritant when the cell viability is inferior or equal to 50%, compared to the negative controls tissues, in at least one extraction solvent. The correct classification of all the samples confirmed the good performance of this new protocol for *in vitro* skin irritation of medical devices extracts with the SkinEthic™ RHE model. Seven naïve laboratories were trained in prevision of the Round Robin Study to evaluate Reconstructed Human Epidermis (RhE) models as *in vitro* skin irritation test for detection of irritant potential in medical device extracts.

1. Introduction

Medical devices cover a wide range of health or medical instruments used in the treatment, mitigation, diagnosis or prevention of a disease. Since the early 1990's, biocompatibility testing and evaluation to ensure safety of medical devices are driven by the ISO 10993 standards. According to ISO 10993 Part 1 for evaluation and testing within a risk management process (ISO, 10993-1 Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process, 2009), skin irritation is one of the three toxicological endpoints to be always addressed in a biological risk assessment whatever the category of the device. Irritation testing of medical devices can be performed with the finished product and/or extracts thereof. The reference test method listed in the ISO10993-10 (ISO, 10993-10 Biological Evaluation of Medical devices - Part 10: Tests for irritation and skin sensitization, 2010) is based on *in vivo* testing even if this standard mentions previous validation by ECVAM Scientific Advisory Committee (ESAC) of an *in vitro* method using reconstructed human epidermis. Indeed, this *in vitro* test for skin irritation has so far been validated only for neat chemicals and not for medical device extracts where potential leached irritants are diluted in a solvent. In order to apply these assays for the testing of irritant potential of medical devices, further validation for this specific area is essential.

Turning toward alternatives to animal testing is driven by ethical consideration but also by scientific and economic reasons. In recent years, new cosmetics regulations, first in Europe (EU, 2003) and then in other parts of the world, have progressively banned the use of animals to ensure the safety of ingredients and cosmetics products. In parallel with these regulatory developments, toxicology is undergoing a profound revolution, with a shift from toxicology based on the observation of effects in animals to mechanistic approaches based on *in vitro* and *in silico* tests to predict potential adverse effects in humans. Significant progress has been made with successful replacements, especially for acute toxicological endpoints, even if systemic endpoints are more complex to replace. Several validated *in vitro* test methods were adopted as OECD test guidelines for these endpoints and Integrated

* Corresponding author.
E-mail address: cpellevoisin@episkin.com (C. Pellevoisin).

https://doi.org/10.1016/j.tiv.2018.01.008
Received 18 October 2017; Received in revised form 10 January 2018; Accepted 11 January 2018

Approaches to Testing and Assessment (IATA) provides guidance on how to integrate and use existing test and non-test data for the assessment of health hazards. Even if successful steps from chemical and cosmetics industry need to be adapted, previous experience from these sectors will definitely accelerate the move in medical devices area (Casas et al., 2013; Coleman et al., 2015).

The purpose of this paper is to present the development and preliminary results of a new *in vitro* method for evaluating the skin irritation of medical device extracts. This new protocol is adapted from the OECD test guideline for skin irritation (OECD TG439) of chemicals and uses the SkinEthic™ RHE model as experimental system. The SkinEthic™ RHE model is reconstructed from human primary keratinocytes and mimics human epidermal morphology and physiology. This model is validated as a full *in vitro* replacement method to animal testing to assess the skin corrosion and the skin irritation potential of chemicals (Alépée et al., 2010) (EC-ECVAM, 2006). The protocol for medical device extracts was developed in preparation of an international Round Robin Study conducted under the umbrella of the workgroup eight of the ISO technical committee 194 (ISO/TC 194/WG 8) irritation and sensitization.

2. Materials and methods

2.1. Functional reconstructed human epidermis model SkinEthic™ RHE

The SkinEthic™ Reconstructed Human Epidermis (RHE) model (EPISKIN Laboratories, France) consists of normal, human-derived keratinocytes, cultured to form a fully differentiated three-dimensional epidermis on a 0.5 cm^2 surface inert polycarbonate filter at the air–liquid interface in a chemically defined medium (Rosdy and Clauss, 1990; Rosdy et al., 1993) (Fig. 1). SkinEthic™ RHE is produced under ISO 9001 certification. Quality control data sheet is provided with every batch of tissue including histology, viability and safety data.

2.2. Exposure protocol

The day of receipt the SkinEthic™ RHE inserts are transferred into a 24-wells plates filled with 0.3 mL fresh medium of culture and maintained a minimum period of 2 h in an incubator (37 °C, 95% humidity, 5%CO_2). This pre-incubation step could be extended to 24 h by using 6-well plates filled with 1 mL of fresh medium. For the experiment, 100 µL of each sample is topically applied concurrently on three tissues replicates (n = 3) for 18 ± 2 h or for 24 ± 2 h at 37 °C. Attention should be made to ensure a correct distribution of the solution onto the surface of the tissue. The surface of the stratum corneum being hydrophobic, surface tension mechanisms can induce peripheral repartition of polar solvent. This can be solved by slightly tapping the insert on the bottom of the well. Solutions of SDS 1% (w/v) in polar solvent, sodium chloride (NaCl 0.9%) or phosphate-buffered saline without Ca ++ and Mg++ (PBS) and non-polar solvent (sesame oil) are used as positive controls (PC). Negative control (NC) are PBS-treated tissues. The solvents alone are tested as vehicle extraction controls (VC). At the end of the exposure step the SkinEthic™ RHE inserts are rinsed 25 times with PBS (1 mL by 1 mL) using a multistep pipette and are manually dried. Samples of the incubation medium are collected and frozen at −20 °C for potential future cytokines quantification.

2.3. Cell viability

The cell viability is determined by measuring in the reconstructed epidermis the formation of insoluble blue formazan crystals by the dehydrogenase enzyme after addition of 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT, Sigma–Aldrich) to the medium of culture (Mosmann, 1983; Korting et al., 1994). The tissues are incubated with 0.3 mL MTT solution (1 mg/mL) for 3 h (± 15 min). The formazan crystals are extracted using 1.5 mL isopropanol for 2 h (± 15 min) at room temperature. Then 200 µL are transferred three times per tissue into a 96-well plates and the concentration of formazan is quantified by measuring the optical density (OD) through

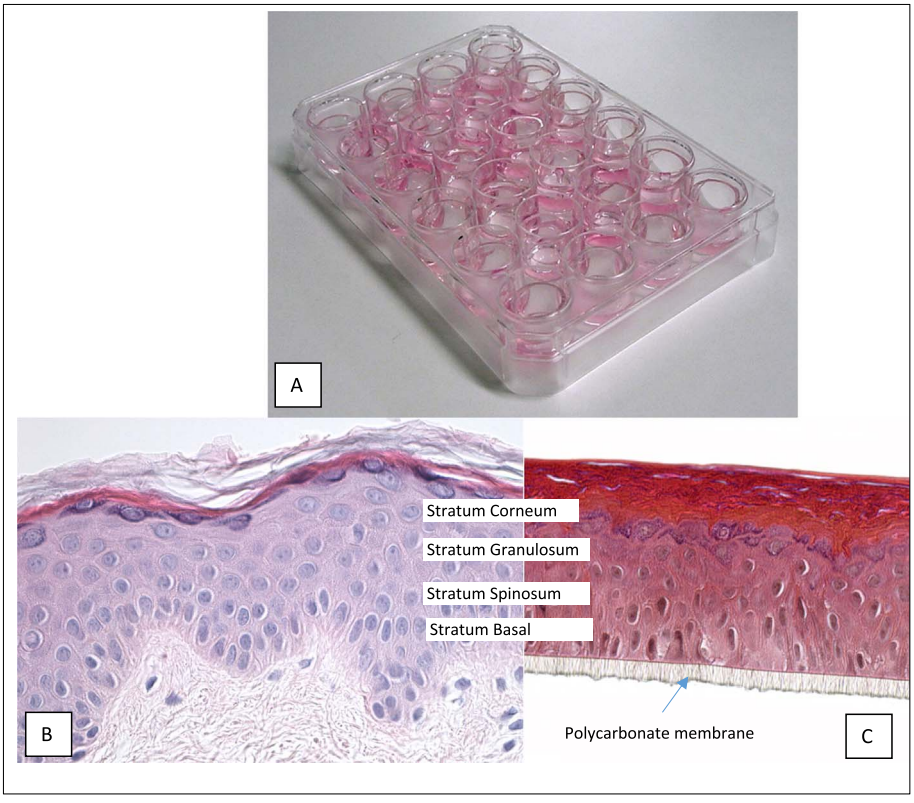

Fig. 1. SkinEthic™ RHE model.
SkinEthic™ RHE model is produced and shipped every week in 24 well plates (A). Hemalun Eosine-Saffron staining (HES) show in the reconstructed epidermis (C) the typical layers of human epidermis (B).