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WHITE PAPER

Irritation Assessment of PVC-based Polymers with a Reconstructed Human Epidermal Model

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INTRODUCTION

In 1959, Russel and Burch published The Principles of Humane Experimental Technique, which introduced the 3R approach to replace, reduce, and refine testing performed on animal models.[1] This approach has resulted in a movement towards the development of new analytical approaches (NAMs) that implement in vitro, in silico, and in chemico techniques to assess biological endpoints in place of animal models. Internationally, governing bodies are transitioning to the encouragement of in vitro tests over in vivo, with this 3R approach in mind. While the FDA has not fully adopted in vitro over in vivo models for medical device biocompatibility testing, it is stated that a secondary purpose of the ISO 10993 standards is to "utilize scientific advances in our understanding of basic mechanisms, to minimize the number and exposure of test animals by giving preference to in vitro models in situations where these methods yield equally relevant information to that obtained from in vivo models."[2]

The three primary biocompatibility tests all medical devices are required to undergo for FDA biological risk evaluations are cytotoxicity, irritation, and sensitization. Unlike the standard cytotoxicity test, which is performed on an in vitro culture of mammalian cells, the sensitization and irritation tests are typically performed on animal models such as guinea pigs and rabbits. For the intracutaneous reactivity irritation test, extracts collected from the medical device are injected into the subcutaneous tissue of a rabbit; the injection sites are then assessed for observable changes such as erythema, edema, or necrosis. This test often requires a large quantity of medical devices to undergo extraction to obtain the minimum volume of treatment necessary, and may require a 1-2 month turnaround time in addition to the financial and ethical costs of the test. These expenditures increase if, upon completion of the test, the device is found to be irritating, which would require the manufacturer to reassess their product's composition, processing, and manufacturing environment, followed by retesting. With proper screening techniques, a medical device manufacturer can be confident in their product's biocompatibility prior to initiating these tests that are costly economically, ethically, and on production timelines.

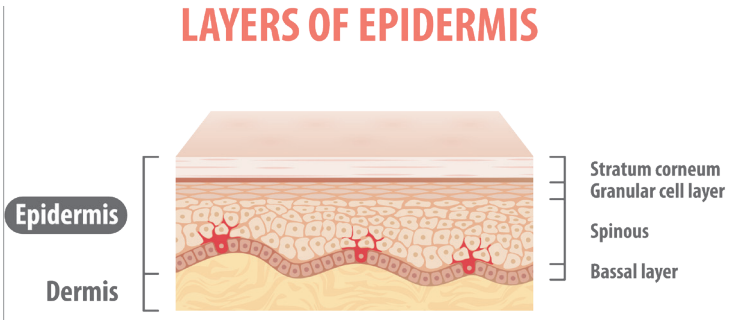

Figure 1: Structure of the Human Epidermis

Additionally, while no regulations outline specific biocompatibility testing method requirements for consumer products such as wearable electronics or cosmetics, the FDA has advised manufacturers of these products to employ whatever testing is appropriate and effective to demonstrate product safety. With the growing popularity of "cruelty-free" products over those tested on animals, and the constant need to protect consumers from risk of skin irritation due to product usage, the need for new analytical methods that replace animal testing for skin irritation is evident.

As such, decades of research have been dedicated to developing in vitro models of human skin that can be used to assess skin irritancy risk of a product. Many models have been validated internationally for neat chemical irritancy testing, such as those used in cosmetics and household cleaners. Some of these models have also been validated for assessment of medical device extracts for irritancy and are mentioned in ISO 10993-23, though not enough historical testing data is currently present for full FDA acceptance of the in vitro method.

To support the movement towards acceptance of in vitro irritation tests for medical devices and consumer products, polymers were developed by Eurofins EAG and characterized by ISO 10993-18 chemical characterization. These polymers were then used for internal validation of the RhE irritancy test by the Eurofins EAG in vitro scientists. The polymers and criteria used to evaluate the model were designed to follow those used in the international round robin validation of the model. Operator proficiency for the method was demonstrated by meeting the published acceptance criteria for RhE irritation testing. Simultaneously, the RhE model met internal validation

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criteria for accuracy, repeatability, precision, specificity, selectivity, range, and robustness, demonstrating its reliability for assessing consumer products, medical devices, and neat chemicals for irritation.

RHE MODEL

Skin irritation is a complex adverse reaction to substances that penetrate the protective external layers of the epidermis, then elicit cytotoxic effects on the underlying keratinocytes. This reaction may result in transient symptoms such as erythema and edema, or severe reactions such as necrosis and scar formation.[3]

For a chemical to initiate the irritancy response, it must disrupt the cellular membrane and cause macromolecular dysfunction. This results in the dissolution of key components of the stratum corneum, an protective layer of the human epidermis. The RhE model contains differentiated layers of skin cells that parallel the layers found in the human epidermis. Thus, a substance must initiate the same molecular events in the model as it would on the human skin for a measurable response to occur.

Once the irritant damages the stratum corneum, it will initiate cytotoxic effects on the keratinocytes within the epidermis. The differentiated layers in the RhE model that a substance must penetrate make this model more physiologically relevant than standard cytotoxicity methods that are implemented on a single cell monolayer.

In humans, cellular damage from irritant exposure results in local inflammation due to release of cytokines and small signaling molecules. This manifests as the redness, swelling, and blistering commonly associated with skin irritation. In the RhE model, irritating substances (including those that leach out of a medical device or consumer product) are identified by the cytotoxicity they enact on the living keratinocytes in the model. This effect is measured with a cell viability reagent.

The cell viability reagent, MTT, is a well-established reagent used to quantify the proportion of viable cells in a treated population to viable cells in a negative control-treated population. MTT is a yellow tetrazolium dye that is reduced by dehydrogenases found in healthy cells to a purple, colored formazan crystal. Non-viable cells are unable to metabolize the yellow reagent; the intensity of the sample's absorbance at 570 nm can be used to calculate the percent viability of the treated population.

POLYMER DEVELOPMENT AND CHARACTERIZATION

Medical devices and consumer products are carefully developed with materials and methods that are advertised as biocompatible. However, irritants may be introduced to the product in trace amounts due to raw material or environmental contamination, incomplete chemical reactions during processing, or insufficient removal of surface detergents after cleaning of the product. For medical devices or consumer products, these trace chemicals may leach from the device and into the user's skin, eventually eliciting an adverse reaction to the product.

Originally, the RhE model was developed for assessment of neat chemicals for irritancy. To expand this application, an extraction method was developed to obtain leachable substances from the medical device. The product is incubated, under agitation, in saline and sesame oil to emulate the sweat and skin oils. Substances leached from the product are then applied to the RhE tissues for evaluation of irritancy risk.

Y-1 (NON-IRRITANT)	Y-4 (IRRITANT)
61.3% PVC	57.8% PVC
33.7% DEHP	31.8% DEHP
4.9% ESBO	4.6% ESBO
	5.8% GENAPOL X-080

Table 1: Composition of PVC-Based Polymers5

To demonstrate that the sample preparation and extraction conditions leach trace amounts of irritants from a solid product, a PVC-based polymer was developed. This polymer, named Y-4, contains less than 10% Genapol X-080, which is a wetting agent commonly found in industrial cleaners and is used as an emulsifier for acrylates.[4] Generation of this polymer serves as a reference for a finalized product that may have an irritant present due to incomplete chemical reactions after manufacturing. In parallel, a PVC-based polymer, named Y-1, without added irritants was created. These polymers were developed following the recipe of those used in the international round-robin validation of the RhE medical device method.

An exaggerated chemical characterization study was performed in accordance with ISO 10993-18 on the Y-1 and Y-4 polymers. The polymers were extracted at 120°C, and 180°C for 72 hours in 40% ethanol and heptane. This characterization confirmed that the Genapol X-080 was able to be extracted from the Y-4 polymer at a threshold

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detectable by LC-MS and GC-MS. Similarly, this confirmed the lack of Genapol X-080 substance in the Y-1 polymer. This data was vital to determining the accuracy of the RhE model's irritant prediction.

IN VITRO IRRITATION METHOD

Sample Preparation:

Extracts of the Y-1 and Y-4 polymers were prepared in parallel in saline and sesame oil at 6 cm2/mL for 72±2 hours in a humidified incubator set to 37°C.

The positive control treatment, 1% SDS, and the negative control treatment, DPBS were supplied by the manufacturer of the tissues. These controls have published acceptance criteria for the model, and are used to demonstrate the tissues respond appropriately when exposed to irritating and non-irritating substances.

Test System Preparation and Treatment:

One day prior to completion of the extraction period, two operators independently prepared 24 RhE tissues by pre-incubation in fresh media overnight.

On the day of treatment, the two operators collected aliquots from the same set of Y-1 and Y-4 polymer extracts and treated three replicate tissues with each test article and control extract. Treated tissues were then returned to the incubators. This was designed to demonstrate the precision of the method.

Upon completion of the treatment period, the tissues were thoroughly rinsed with DPBS to stop the treatment.

Analytical Method:

MTT viability reagent was prepared and divided between the two operators. Each tissue was transferred into a multi-well plate and incubated with the MTT reagent; once this incubation period was completed tissues were transferred to a multi-well plate filled with isopropanol to solubilize the purple formazan crystals. After another incubation period, the tissues were punctured with a needle to drain the solubilized formazan. Two replicate aliquots were collected from the solutions drained from each tissue and transferred to a 96-well plate for analysis at 570 nm in a UV-Vis plate reader. Treatments that resulted in a 50% reduction in tissue viability are categorized as irritants.

RESULTS

This method was performed twice by two operators over two independent testing periods to assess several key validation parameters. The acceptance criteria for each parameter was set to parallel the published literature for the international round-robin validation of this method.

In each replicate test, the RhE model correctly identified the negative control and vehicle controls, as well as the Y-1 polymer extracted in polar and non-polar solvents, as non-irritants. The model also identified the 1% SDS positive control and the Y-4 polymer correctly as irritants. These results were repeated by independent operators with freshly prepared extracts over multiple independent testing periods.

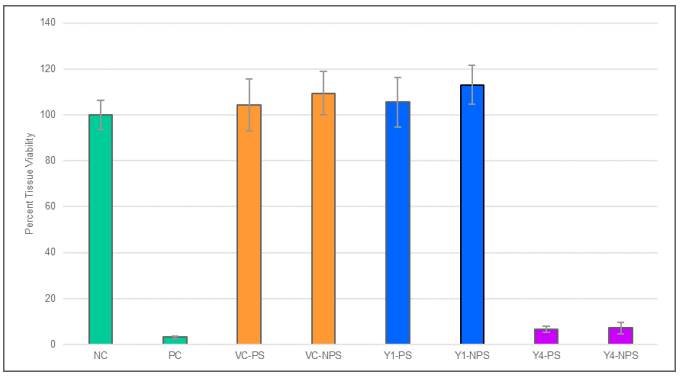

Figure 2 Overall results of the Eurofins EAG internal validation of an RhE model for irritancy testing of test article extracts. NC, PC, and VC indicate the negative, positive, and vehicle control results. The suffix of -PS reflects samples extracted in saline (polar solvent) and -NPS is samples extracted in sesame oil (non-polar solvent).

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CONCLUSION AND APPLICATIONS

The data collected in this method validation indicates that the use of reconstructed human epidermal tissues provide an ethical alternative for assessing extracts collected from a product, including wearable technology and medical devices, for irritancy potential. This is in addition to the intended function of testing neat chemicals, gels, and creams for irritancy, as published in OECD 439. While this in vitro method is not currently accepted by the FDA for the biological evaluation of medical devices, there are several applications it could be used for, including:

- Screening of raw materials
- Evaluation of new manufacturing environments for contaminants
- Confirmatory testing of biocompatibility prior to in vivo/ clinical trials
- Marketing (Cruelty-Free label)
- Ensure customer safety
- Risk mitigation
- Prototype evaluation
- Reformulation testing compared to predicate products
- Investigating new sterilization or packaging methods
- Lot release testing

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