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Evaluation of the medical devices benchmark materials in the controlled human patch testing and in the RhE *in vitro* skin irritation protocol

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ABSTRACT

Several irritants were used in the *in vitro* irritation medical device round robin. The objective of this study was to verify their irritation potential using the human patch test (HPT), an *in vitro* assay, and *in vivo* data. The irritants were lactic acid (LA), heptanoic acid (HA), sodium dodecyl sulfate (SDS), Genapol® X-80 (GP), and Y-4 polymer. Dilute saline and sesame seed oil (SSO) solutions of each were evaluated using a 4 and 18 h HPT and the EpiDerm™ SIT-MD RhE assay; results were then compared to existing rabbit skin irritation test data. Results from the 4 h HPT were negative in most cases except for GP and SDS, while the 18 h HPT also identified some LA, HA, and GP samples as irritants. EpiDerm™ SIT-MD correctly identified all irritants except GP in SSO due to limited solubility. Data from cutaneous rabbit irritation tests were negative, while all intracutaneous results were strongly or weakly positive except for the most dilute GP solutions. These findings indicate that EpiDerm™ SIT-MD results correlate with those from the rabbit intracutaneous test and confirm that RhE assays are suitable replacements for animals in evaluating the tissue irritation potential of medical devices.

1. Introduction

Biocompatibility assessment is required for all medical devices in order to minimize potential hazards to patients (ISO, 2009). Animal irritation testing is used to predict whether a patient-contacting device could cause an irritation response as indicated by edema, erythema, and eschar formation. This test is done in compliance with the ISO 10993-10 and ISO 10993-12 standards (ISO, 2010, 2012).

The most frequently used skin irritation test for medical devices is the rabbit intracutaneous irritation test, which is a modification of the primary (cutaneous) rabbit skin irritation test (Draize et al., 1944). Both assays are used in the safety assessment of medical devices and may cause pain and suffering in test animals. Furthermore, the rabbit skin irritation test has been reported to produce false positive and false negative results (Basketter, 1999; Basketter et al., 1977, 2004; Liebsch et al., 2000; Robinson et al., 1999).

The main objective of this study was to establish a direct comparison of *in vivo* (human, rabbit) and *in vitro* datasets with five selected benchmark materials used to develop and optimize the EpiDerm™ skin irritation protocol for medical devices testing (Casas et al., 2013, Kandárová et al., 2015). These five substances were tested in human according to the defined 4 h human patch test (4 h HPT) protocol (Basketter et al., 2004) and by extended protocol with exposure lasting up to 18 h (18 h HPT). Data were compared to the results obtained previously in rabbits (cutaneous and intracutaneous testing according to the ISO 10993-10) and results of the EpiDerm skin irritation test optimized for medical devices (EpiDerm™ SIT-MD) (Kandárová et al., 2015).

The human skin irritation test is very similar to the regulatory accepted primary *in vivo* rabbit skin irritation test OECD Test Guideline 404 (OECD, 2002), but it is designed to limit the intensity of skin reactions in human volunteers. The value of the method is that it provides data for the identification of those substances which should, or should not, be classified as human irritants, and benchmark information for

Abbreviations: 4 h HPT, 4-hour human patch test; 18 h HPT, 18-hour human patch test; ET-50, Exposure time that induce 50% cell viability; DPBS, Dulbecco's phosphate buffered saline; GP, Genapol®; HA, heptanoic acid; I, irritant; ISO, International Organization for Standardization; LA, lactic acid; MTT, 3-[4, 5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide; MDS, methods documentation sheet; NC, negative control; VC, vehicle control; NI, non-irritant; OD, optical density; PC, positive control; RhE, reconstructed human epidermis; SIT-MD, Skin Irritation Test for Medical Devices; SDS, sodium dodecyl sulfate; SOP, standard operating procedure; SO, sesame seed oil; v/v, volume/volume; w/v, weight/volume

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future validations of alternative methods for replacing the rabbit irritation test for biocompatibility testing.

2. Material and methods

2.1. Test materials

2.1.1. Irritant chemicals

The following irritant chemicals that are used in the production of polymers were used:

- Heptanoic acid (HA; CAS No.: 111-14-8; ≥99% purity; Sigma–Aldrich Company).
- Sodium dodecyl sulfate (SDS; CAS No.: 151-21-3; ≥99% purity; Sigma–Aldrich Company).
- Genapol® X-80 (X-80; ethoxylated isotridecanol; CAS No.: 9043-30-5; mixture; Sigma–Aldrich Company).
- Reagent grade DL-lactic acid (LA; CAS No.: 50-21-5; 90–100% purity; Sigma–Aldrich Company).

2.1.2. Extraction solvents

The following extraction solvents recommended by the ISO 10993 were used in the tests:

- Physiological saline (NaCl; CAS No. 7647-14-5; liquid; 0.9%; Sigma-Aldrich Company).
- Super Refined™ Sesame Seed Oil NF-NP, USP grade (SO; Sigma, kat. c.: 85067, Sesame oil from *Sesamum indicum*, tested according to Ph).

2.2. Test material preparation

Dilute solutions of five test materials namely, lactic acid (LA) 4% in saline, heptanoic acid (HA) 2% in sesame seed oil (SO), Y-4 polymer (saline and SO extract solutions), sodium dodecyl sulfate (SDS) 1% (solution in saline and SO) and Genapol® X-80 (solutions in saline and SO; three different concentrations) were prepared because they had either been used by Casas et al. (2013) for their *in vitro* irritation pilot project or during the medical device round robin study. The Y-4 polymer samples (2 mm thick PVC sheets) were extracted at the ratio of 6 cm^2 material per mL of saline (0.9% NaCl) or SO at 37 °C for 72 h. The dilute irritant solutions and extracted samples are summarized in Table 1.

2.3. The human patch test protocols

The 4 h HPT has been described in detail in the literature (Basketter et al., 1997 and 2004, Robinson et al., 1998, 1999, 2001, 2005, York et al., 1996, Jirova et al., 2007, 2010). In addition to the classic 4 h HPT, a modified 18 h HPT was used in our study.

The prolonged exposure aimed the detection of even very subtle or sub-clinical irritation responses to extracts from medical devices, as extracts may contain low levels of highly diluted chemicals (Lucas et al., 2003), so that they may be difficult to detect or to identify (Armstrong et al., 2013; Petrusevski et al., 2016).

All HPT testing was conducted at the National Institute of Public Health (NIH) in Prague, Czech Republic. The studies were performed in compliance with an internal SOP in accordance with ISO 10993-10 (NIPH SOP, 2015). The selection of volunteers and the test method complied with the Declaration of Helsinki (1964) and the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS, 2002). The study was approved by the NIH's Ethical Review Committee. The volunteers were selected on the basis of inclusion and non-inclusion criteria and for this purpose completed a special form. The volunteers were clearly informed regarding the nature of the study, timetable, constraints and possible risks. They gave their written informed consent before participation in the study was permitted.

Thirty volunteers took part in the each of two HPT studies. Study 1 included lactic acid (4%) in saline, heptanoic acid (2%) in sesame seed oil (sso), Polymer Y-4 (extract in saline and sso). Study 2 was conducted with three increasing concentrations of Genapol® X-80 (TMs 5–10). Each study included positive controls, vehicle controls and negative controls.

The epicutaneous study was a single application closed-patch occlusion test. The patch test procedure involved application of the test chemicals (0.4 mL) on 25 mm plain Hill Top Chambers containing Webril pads (occlusive: Hill Top Companies, Cincinnati, Ohio, USA) to the skin of the upper outer arm of 30 human volunteers for up to 18 h. Exposure time increased progressively from 15 or 30 min. Through 1, 2, 3, 4 and 18 h, each progressive application at a new skin site, until a positive irritation reaction was reported by the volunteer and/or recorded by a responsible experimenter. Treatment sites were assessed for the presence of irritation using a 4-point scale (see Table 2) immediately after patch removal, at 1–2 h, 24, 48 and 72 h after patch test removal. Sodium dodecyl sulfate (SDS) at 20% was used as the positive control.

A volunteer exhibiting a reaction Grade 1 or higher at any of the reading times was considered to have demonstrated a positive irritant reaction and further treatment with that substance did not proceed for ethical reasons. The number of panelists who had developed a positive

Table 1
Preparation of the test materials.

TM	CAS	Test material	Form	Solvent/vehicle	Remark
1	50-21-5	Lactic acid, 4% solution (w/v)	Liquid	Saline	Tested as supplied
2	111-14-8	Heptanoic acid, 2% solution (w/v)	Liquid	Sesame seed oil	Tested as supplied
3	n.a.	Polymer Y-4	Polymer Sheet	Saline	Extract
4	n.a.	Polymer Y-4	Polymer Sheet	Sesame seed oil	Extract
5	9043-30-05	Genapol X-80 0.068%	Liquid	Saline	Tested as supplied
6	9043-30-05	Genapol X-80 0.135%	Liquid	Saline	Tested as supplied
7	9043-30-05	Genapol X-80 0.338%	Liquid	Saline	Tested as supplied
8	9043-30-05	Genapol X-80 0.068%	Liquid	Sesame seed oil	Tested as supplied
9	9043-30-05	Genapol X-80 0.135%	Liquid	Sesame seed oil	Tested as supplied
10	9043-30-05	Genapol X-80 0.338%	Liquid	Sesame seed oil	Tested as supplied
11	151-21-3	SDS, 1% solution (v/v)	Liquid	Saline, positive control in EpiDerm SIT-MD test	Prepared from supplied 20% SDS
12	151-21-3	SDS, 1% solution (v/v)	Liquid	Sesame seed oil, positive control in EpiDerm SIT-MD test	Prepared from supplied 20% SDS
13	7647-14-5	Saline	Liquid	Vehicle	Sterile 0.9% NaCl in H_2O
14	n.a.	Sesame seed oil	Liquid	Vehicle	Pharmaceutical grade
15	151-21-3	Sodium dodecyl sulfate (SDS), 20% (w/v)	Liquid	Positive control in HPT	Tested as supplied

TM = test material; SDS = sodium dodecyl sulfate; HPT = human patch test.

Table 2
Human skin irritation test, grading scale.

Description of response	Grading
No reaction	0
Weakly positive reaction (usually characterized by mild erythema and/or dryness across most of the treatment site)	1
Moderately positive reaction (usually distinct erythema or dryness, possibly spreading beyond of the treatment site)	2
Strongly positive reaction (strong and often spreading erythema with oedema and/or eschar formation)	3

irritant reaction after progressive exposure up to 18 h was determined.

2.4. The EpiDerm SIT-MD protocol

The reconstructed tissue model EpiDerm™ Skin Irritation Test (EPI-200) (OECD TG 439) with modulated dose (100 µL) and exposure period (18 h, no post-incubation) (known as EpiDerm™ SIT-MD) was used in this study. Testing was conducted at MatTek *In Vitro* Life Science Laboratories, Bratislava, Slovakia.

Briefly, after the overnight pre-incubation, the apical surface of the tissues was dosed with 100 µL of the irritant solutions or Y-4 extracts shown in from Table 1. Positive controls ((PC, 1% v/v SDS) in saline and in sesame seed oil), negative control (NC, Dulbecco's phosphate buffered saline (DPBS)) and vehicle controls (VC, saline and sso) were tested concurrently in the EpiDerm™ SIT- MD assays. Incubation time was18 ± 1 h at standard tissue culture conditions which were 37 °C, 5% CO_2, and 90 ± 5% humidity. After the exposure, the tissues were rinsed with DPBS and cell viability was determined by the MTT assay that is based on mitochondrial reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and subsequent conversion to a purple formazan salt that is quantitatively measured after extraction from tissues. The insoluble formed formazan crystals were solubilized by 2 mL of isopropanol and the intensity of the extracted formazan product was measured at 540–570 nm. (Faller et al., 2002; Mosmann, 1983). Tissue viability was calculated in comparison to the DPBS treated NCs. Cell viability reduction below 50% was regarded as a sign of irritation. Since MD polymers are not color releasing materials, nor MTT-reducing chemicals, corrections due to the coloring properties of tested materials or interference with the endpoint were not necessary. For details of the testing procedure see EpiDerm SIT MD SOP (MatTek SOP, 2016 and Kandárová et al., 2018, this issue).

3. Results

Table 3 presents the results of the primary (24 h exposure) and intracutaneous rabbit skin testing, 4 and 18 h HPT, and EpiDerm™ SIT-MD studies conducted using the test materials referred in Table 1. As can be seen, the primary rabbit testing was only able to identify 1% SDS as an irritant, while the intracutaneous rabbit testing identified all materials as strong or weak irritants except for the most dilute Genapol® solutions. The 4 h HPT was able to identify many of the 1% SDS samples as irritants, but scored just eight Genapol® solutions as causing irritation responses. While, the 18 h HPT was more successful at identifying 1% SDS solutions as being irritants, it was only able to categorize ten LA, HA, and Genapol® samples as irritating. However, EpiDerm™ SIT-MD correctly identified all irritants except the three SSO solutions of Genapol®.

Polymer Y-4 was used in this study as a sample of a medical-device material known to cause irritation/inflammation in the exposed individuals. Main component that likely leads to the reported irritation is Genapol® X-80 (Haishima et al., 2014). The material also releases phthalates that may contribute to cytotoxicity. The rabbit test with the topical exposure, as well as the 4 h HPT were completely negative. In the intra-cutaneous test in the rabbits, some responses have been observed in the individual animals, however overall the responses were below the classification threshold. In the EpiDerm™ test, polymer Y-4 has was clearly recognized as a sample that would lead to the cytotoxicity translated to irritation/inflammation. The material was positive in both extracting solutions (saline and SO) providing mean viabilities of 7.7 ± 0.1 (saline) and 8.5 ± 0.8 (SO). In addition, when assessing the IL-1a content which is indicative of inflammatory response, a positive result showing highly elevated IL-1a levels was obtained. The levels were comparable to positive control 1% SDS in the first run (See Fig. 1). The second IL-1a run was however negative. Such a result is typical, if a material causes high cytotoxicity.

Further material tested in the study was Genapol® X-80, a nonionic emulsifier and surfactant for emulsion polymerization, crop protection formulations and component of metal cleaners. It was tested in three increasing concentrations (0.068%, 0.135%, 0.338%) that were considered as hypothetical concentrations extractable from a sheet of the polymer of a size of 6cm^2. In the rabbit text with topical exposure, no reactions were seen in all six samples. In the subcutaneous rabbit test, the two highest concentrations triggered some slight irritation response in SSO and saline. Human patch test revealed positive responses in both 4 h and 18 h exposure times (see Table 3). More panelists reacted on Genapol® in saline than in SSO.

In the EpiDerm™ SIT-MD, clear dose-response effect was observed in both vehicles, with higher intensity of cytotoxicity/irritation in saline. All saline extracts were positive in the EpiDerm™ MD-SIT, the SSO solutions caused less irritation response and only the highest concentration would be considered as possibly irritating based on the borderline tissue viability of 56.7 ± 3.2%. IL-1a assay was not performed for this material.

The positive controls, 1% SDS in saline and in sesame seed oil, were positive in all *in vivo* (human and rabbit) as well as *in vitro* tests, suggesting that this material is correctly selected chemical with desired *in vivo* and *in vitro* response.

4. Discussion

The primary goal of this study was to evaluate skin irritation potential of five benchmark materials used in the optimization of the EpiDerm™ SIT for medical devices testing (Casas et al., 2013, Kandárová et al., 2015). Some of these materials were also used in a training of the laboratories who volunteered to participate in the round robin irritation testing of medical devices extracts (Kandárová et al., 2018; De Jong et al., TIV this issue). The five materials were tested under strictly controlled conditions of the human patch test with 4 h exposure (Basketter et al., 2004) and with the prolonged exposure up to 18 h. Findings of the human patch test were compared to the results obtained previously in rabbits (ISO 10993-10, NAMSA reports) and in the *in vitro* skin irritation assay EpiDerm™ SIT-MD.

The five test materials namely, lactic acid (4%) in saline, heptanoic acid (2%) in SSO, Polymer Y-4 containing Genapol X-100 (extracts pol in saline and SSO), SDS 1% (solution in saline and sso) and Genapo®l X-80 (solutions in saline and SSO in three different concentrations) were tested at the NIH in Prague on a panel of 30 volunteers in two separate studies.

Lactic acid and heptatonic acid had been previously selected for the EpiDerm™ study published by Casas et al. (2013), because they were irritant chemicals used in the manufacturing of medical device polymers. Both are known to cause skin irritation in animals and humans. Sodium dodecyl sulfate is used a mold-release agent in medical device manufacturing and is widely used a positive control in human patch tests (20% SDS) and in *in vitro* validated assays for skin irritation testing (5% SDS, OECD TG 439) (OECD, 2015). Polymer Y-4, is a polyvinyl chloride produced by Japan's National Institute of Health Sciences (Haishima et al., 2014), contains Genapol® X-80 and other components (mainly phthalates) that may lead to irritation/inflammation responses in humans. Genapol® X-80 is also used in the medical device industry,

Table 3
Results of the human patch test in comparison to animal and *in vitro* data.

		Vehicle	*In vivo* rabbit 24 h patch	*In vivo* rabbit intra-cutan	4 h application – human[a]					18 h application – human[a]					EpiDerm SIT – MD[b] (viability ± SD)	
					0 h	1–2 h	24 h	48 h	72 h	0 h	1–2 h	24 h	48 h	72 h		
1	Lactic acid, 4% solution (w/v)	Saline	−	+	0/30	0/30	0/30	0/30	0/30	0/30	**2/30**	0/30	0/30	0/30	36,8 ± 10.6	I
2	Heptanoic acid, 2% solution (w/v)	sso	−	+	0/30	0/30	0/30	0/30	0/30	0/30	**1/30**	0/30	0/30	0/30	6.3 ± 0.7	I
3	Polymer Y-4 extract	Saline	−	+/−	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	7,7 ± 0.1	I
4	Polymer Y-4 extract	sso	−	+/−	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	8.5 ± 0.8	I
5	Genapol X-80 0,068%	Saline	−	−	0/30	0/30	0/30	0/30	0/30	0/30	**2/30**	0/30	0/30	0/30	46,1 ± 7,1	I
6	Genapol X-80 0,135%	Saline	−	+/−	0/30	0/30	0/30	0/30	0/30	0/30	**1/30**	0/30	0/30	0/30	9,4 ± 0,7	I
7	Genapol X-80 0,338%	Saline	−	+/−	0/30	**2/30**	**3/30**	**1/30**	0/30	0/30	0/30	0/30	0/30	0/30	6,5 ± 1,2	I
8	Genapol X-80 0,068%	sso	−	−	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	109,1 ± 15,3	NI
09	Genapol X-80 0,135%,	sso	−	+/−	0/30	0/30	**1/30**	**1/30**	0/30	0/30	0/30	0/30	0/30	0/30	83,7 ± 9,3	NI
10	Genapol X-80 0,338%,	sso	−	+/−	0/30	0/30	0/30	0/30	0/30	0/30	0/30	**3/30**	0/30	0/30	56,7 ± 3,2	NI/I[d]
11	SDS, 1% solution (v/v)	Saline	+	+	**2/30**	**9/30**	**18/30**	**10/30**	**9/30**	**8/12**[c]	**9/12**[c]	**10/12**[c]	**11/12**[c]	**11/12**[c]	3.2 ± 0.6	I
					0/30	**22/30**	**23/30**	**21/30**	**17/30**	0/30	**24/30**	**30/30**	**30/30**	**30/30**		
12	SDS, 1% solution (v/v)	sso	+	+	0/30	0/30	**1/30**	**1/30**	0/30	**9/29**[c]	**10/29**[c]	**12/29**[c]	**12/29**[c]	**12/29**[c]	4.1 ± 1.3	I
					0/30	**21/30**	**19/30**	**17/30**	**10/30**	0/30	**16/30**	**30/30**	**30/30**	**30/30**		
13	Saline	Vehicle	−	−	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	N/A	NI
					0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30		
14	Sesame oil	Vehicle	−	−	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	N/A	NI
					0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30		
15	Sodium dodecyl sulfate (SDS), 20% (w/v)	Pos. control	Not tested	Not tested	**25/30**	**29/30**	**29/30**	**29/30**	**26/30**	–	–	–	–	–	N/A	N/A
					0/30	**18/30**	**14/30**	**12/30**	**3/30**	–	–	–	–	–		

– no or very slight effect, +/− some positive response, + positive response resulting into classification, I – irritant, NI – non-irritant, N/A – not analyzed in the current study.

[a] Number of individuals with a positive irritant reaction to the test material/total panel size.

[b] Data in Kandárová et al., 2015, 2016.

[c] Volunteers exhibiting a reaction grade 1 or higher at any of the reading times after 4 h application were excluded from this part of the study as already classified as positive.

[d] According to the prediction model which is based on viability cut of 50%, this material would be classified as non-irritating, however, this testing result clearly indicates need for further testing and assessment of IL-1a release. From further experiments conducted outside of this study, it was confirmed that this sample would cause high IL1a release and would be classified as irritating on a basis of this result.

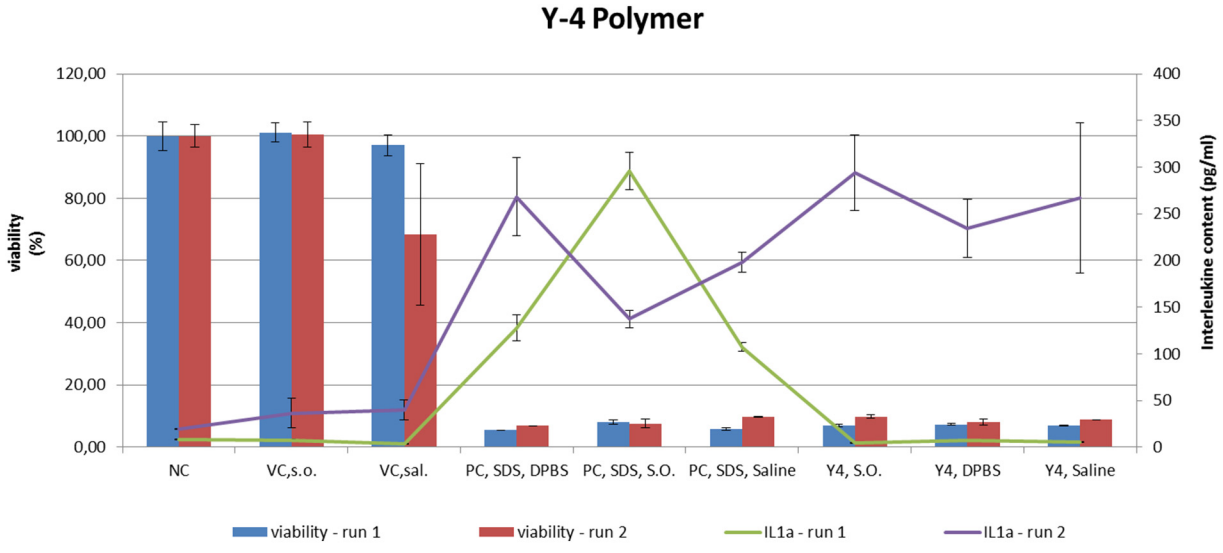

Fig. 1. Cytotoxicity and IL-1a and response to Polymer Y-4 in the EpiDerm SIT MD assay. Positive cytotoxicity response of EpiDerm tissues to polymer Y-4 is showing highly elevated IL-1a in run 1 and almost no IL-1a response in run 2. Such a result is typical, if a material causes high cytotoxicity.

and it was tested at three different concentrations in our study.

A panel of human volunteers (males and females, age 20–65 years) was exposed to the materials listed above (see also Table 1). As expected, most of the human volunteers exposed topically in the 4 h HPT did not react to the very low irritation potential of materials and this response correlated well with the primary (cutaneous) irritation rabbit test results reported by NAMSA.

Biocompatibility testing of medical devices must be conducted in ways that are relevant to the device application. Therefore, for medical devices used as implants or materials coming into the contact with wounded tissues, the intracutaneous rabbit assay is performed. In this testing scenario, materials are injected intracutaneously on the shaved backs of rabbits (five intracutaneous injections of the extract are tested and five injections of the control solution, each of 0.2 mL, are conducted concurrently). The degree of irritation is scored at 4, 24, 48, 72 h after injection and irritation score is calculated.

For ethical reasons, it would not be acceptable to expose human volunteers to the same conditions as those in the intracutaneous *in vivo* test in rabbits (*i.e.*, to inject the materials intradermally), however, we tried to simulate these conditions by extending the exposure time in human volunteers up to 18 h. As shown in Table 3, for 4 out of 5 tested materials (LA, HA, Genapol and SDS 1%), the extension to 18 h brought some positive responses in humans that were seen also in the animals in the intracutaneous test and in the *in vitro* EpiDerm™ test. Responses were slightly more frequent and stronger in the saline solutions compared to sesame seed oil, which can be explained by the protective nature of the oil.

Interestingly, there were no positive responses seen in the human volunteers to the polymer Y-4 that is known to cause irritation in sensitive persons and is also recommended as one of the positive benchmark materials (Haishima et al., 2014). It is possible that the amount of the extractable Genapol® X-80 and plasticizers was not sufficiently high to penetrate the human epidermis and cause inflammation in the human skin. In the EpiDerm™ test the material is clearly positive, and even supported by the high IL-1a release in one run (see Fig. 1). As seen from Table 1, the response of the EpiDerm model to Genapol tested in three different concentrations provide clear concentration-response effect in both solutions. Stronger response was obtained for saline solutions and weaker for sesame seed oil solutions, which can be explained by protective nature of the oil.

When comparing the *in vivo* rabbit responses to topical and intracutaneous exposure, it is clear that the latter test is far more sensitive. The intracutaneous test results correlate well with the *in vitro* data obtained in the EpiDerm™ SIT-MD assay. The five benchmark materials were in almost all cases predicted as *in vitro* irritants. It is expected that if human volunteers would be exposed intracutaneously to the same materials, we would encounter more positive responses. Consequently, it is essential that new *in vitro* tests are calibrated and validated against human and animal data from relevant exposure scenarios if practically and ethically feasible.

The results show that the selected chemicals are suitable to be used as positive reference samples for the evaluation of the RhE *in vitro* irritation test. Lactic acid, heptatonic acid, sodium dodecyl sulfate, and Genapol® were selected are suitable irritants to be incorporated into the polymers prepared for the medical device *in vitro* irritation round robin study (Coleman et al., 2017; De Jong et al., 2017).

5. Conclusion

Our study's human patch tests confirmed that RhE assay results correlate well with those from intracutaneous rabbit tests when evaluating irritant solutions. Based on the results presented here we conclude that the EpiDerm™ SIT-MD assay has been successfully developed and optimized for identifying low levels of medical device irritants at dilute concentrations. These findings indicate that RhE assays are suitable replacements for animals in evaluating the tissue irritation potential of medical devices.

Transparency document

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