Development, Optimization and Standardization of an in vitro Skin Irritation Test for Medical Devices Using the Reconstructed Human Tissue Model EpiDerm™

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ABSTRACT

Assessment of dermal irritation is an essential component of the safety evaluation of medical devices. Reconstructed human epidermis (RhE) models have replaced rabbit skin irritation testing for neat chemicals (OECD TG 439). However, medical device (MD) extracts are dilute solutions with low irritation potential, therefore the validated RhE-methods needed to be modified to reflect needs of ISO 10993.

A protocol employing RhE EpiDerm was optimized in 2013 using known irritants and spiked polymers (Casas et al., TIV, 2013). In 2014 a second laboratory assessed the transferability of the assay. Two additional exposure times were tested along with other medical device materials.

After the successful transfer and standardization of the protocol, nine EU and USA laboratories were trained in the use of the protocol in the preparation for the validation. All laboratories produced data with almost 100% agreement of predictions for the selected references.

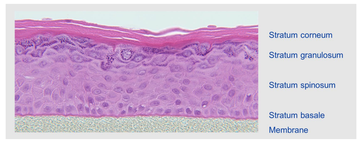
Two of the laboratories performed additional tests with heat-pressed PVC sheets spiked with Genapol X-080 (Y-4 polymer), Vicryl suture, and polymers spiked with heptanoic acid and sodium dodecyl sulfate. All materials were extracted for 24 or 72 hours in both saline and sesame oil at either 37 ˚ C or 70 ˚ C.

Significant irritation responses were detected for Y-4 under all conditions. These results were consistent with those reported by other research groups involved in the upcoming validation study. Vicryl suture was negative and spiked polymers were either positive or negative dependent on the extraction solvent.

We conclude that a modified RhE skin irritation test has the potential to address the skin irritation potential of the medical devices, however, standardization and focus on the technical issues is essential for accurate prediction.

METHODS

Tissue Model: The EpiDerm tissues are cultured at the air-liquid interface allowing for attainment of in vivo-like differentiation and topical application of test articles. Normal human cells derived from tissue explants are used to produce the tissues. The EpiDerm is produced under Good Manufacturing Practices (GMP).



Protocol: The tissues were exposed for 4, 18 and 24 hours to extracts from medical devices. Extraction conditions were either 24 or 72 hours, polar as well as non-polar solvents. Viability was assessed in the MTT assay. In some of the experiments IL-1α was measured to support the viability assay outcomes.

Table 1. Materials used as benchmarks for development of a predictive test for skin irritation of extracts from medical devices

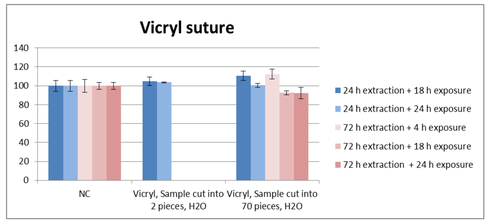
Sample	24 hrs dermal aplication - Rabbit (ISO)	Intracutaneous aplication - Rabbit (ISO Modified)	4h dermal application - Human (ISO)					18 h dermal application - Human (ISO Modified)				
			0h	1-2h	24h	48h	72h	0h	1-2h	24h	48h	72h
Lactic acid	not irritating	irritating	0/30	0/30	0/30	0/30	0/30	0/30	2/30	0/30	0/30	0/30
Heptanoic acid	not irritating	irritating	0/30	0/30	0/30	0/30	0/30	0/30	1/30	0/30	0/30	0/30
Polymer Y-4 extract, saline	Non Irritating, but very slight oedema in one test animal	irritating	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30
Polymer Y-4 extract, sesame oil	Non Irritating	irritating	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30
PC, SDS, 1% solution in saline	irritating	irritating	2/30	9/30	18/30	10/30	9/30	8/12[2]	9/12[2]	10/12[2]	11/12[2]	11/12[2]
PC, SDS, 1% solution in sesame oil	Non Irritating, but very slighterythema in one test animal	irritating	0/30	0/30	1/30	1/30	0/30	09/29[2]	10/29[2]	12/29[2]	12/29[2]	12/29[2]
Saline , vehicle	not irritating	not irritating	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30
Sesame oil, vehicle	not irritating	not irritating	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30
SDS 20% PC in 4h HPT	n.d	n.d	25/30	29/30	29/30	29/30	26/30	n.d	n.d	n.d	n.d	n.d

In the human patch test, 4% w/v of Lactic acid and 2 % w/v of heptanoic acid were tested. Rebbits were exposed to 2,5 % v/v of heptanoic acid and 5% lactic acid (v/v). The test were performed according to the ISO standards.

Table 2. Extracts from Medical Device samples tested in the EpiDerm SIT- MD protocol

Extracts from medical devices	Abbreviation	Extraction conditions (37°C)					
		Extraction solvent				Time	
		DPBS	Saline	Sesame oil	Water	24h	72h
Vicryl Suture	Vicryl	x	x	x	x	x	x
Polymer Y-4	Y-4	x	x	x	x	x	x
Silicone samples spiked with Heptanoic acid - 25%	HA 25 %	x	x	x	n.a.	x	x
Silicone samples spiked with SDS - 17%	SS 17%	x	x	x	n.a.	n.a.	x
Silicone samples spiked with SDS - 15%	SS 15%	x	x	x	n.a.	n.a.	x

RESULTS



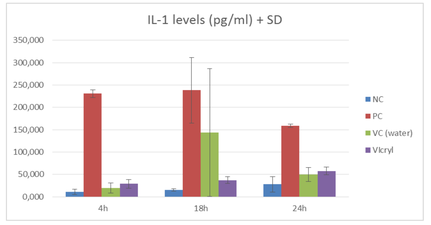


Figure 1. Representative results for Vicryl suture (viabilities % and IL-1a levels): Regardless the extraction time and the extracting conditions tested (DPBS, Saline, Sesame Oil or Water – sample cut into 2 pieces and 70 pieces), Vicryl suture did not produce cytotoxicity nor elevated release of the IL-1a, an inflammatory marker.

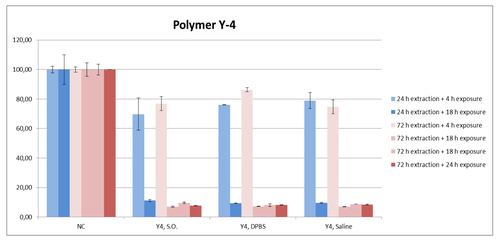
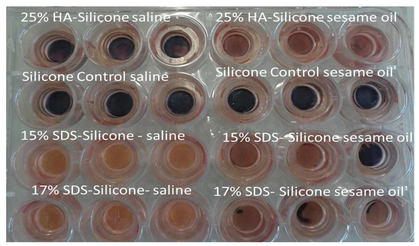
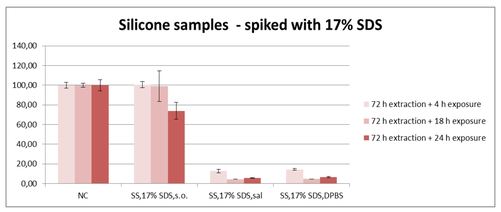


Figure 2. Representative results for Y-4 Polymer (viabilities %): Regardless the extraction time (24 or 72 hours) and the extracting conditions tested (DPBS, Saline, Sesame Oil), Polymer Y-4 produced significant cytotoxicity after 18 and 24 hours of exposure and can therefore be classified as irritating.



Photograph taken during one of the experiments at RIVM showing an experimental design on a 24-well plate and the results before the MTT-extraction and optical density measurements.



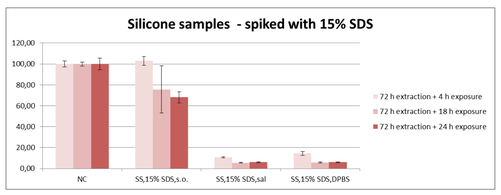


Figure 3. Representative results Silicone samples spiked with 15% and 17% of SDS - SS (viabilities %): Silicone samples were extracted for 72 hours in DPBS, Saline and Sesame Oil. While in the watery extracting solutions the samples produced significant cytotoxicity, in sesame oil the effect was less severe. This can be explained by the affinity of the SDS for the polar solvents and limited solubility (and thus extractability) of SDS in non-polar solvents.

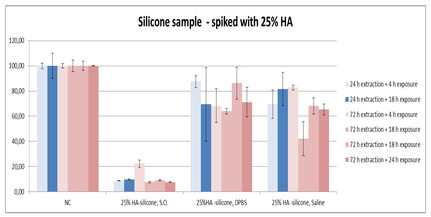


Figure 4. Representative results Silicone samples spiked with 25% of Heptanoic acid – HA (viabilities %): Silicone samples were extracted for 24 and 72 hours in DPBS, Saline and Sesame Oil. While in the sample extracted into the sesame oil produced significant cytotoxicity, the effect in both aqueous solutions was less severe. This can be explained by the affinity of the HA to the non-polar solvents and limited solubility (and thus extractability) of HA into polar solvents.

Table 3. Nine laboratories underwent training in the EpiDerm SIT-MD protocol

Laboratory	Country	Location of the training	Date
RIVM	The Netherlands	EU	October 1-2, 2013
Eurofins	Italy	EU	November 5-6, 2013
NIOM	Norway	EU	November 12-13, 2013
SP Technical Inst	Sweden	EU	July 8-9, 2014
Arthrex	USA	EU	July 29-30, 2014
Nelson labs	USA	USA	November 12-13, 2013
NAMSA	USA	USA	November 12-13, 2013
Boston Scientific	USA	USA	May 20-21, 2014
TOXICON	USA	USA	July 15-16, 2014

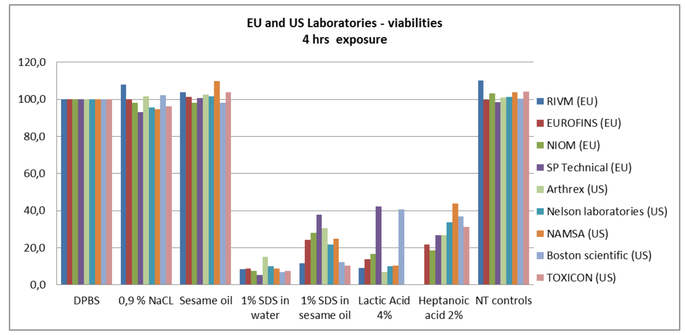


Figure 5. Results obtained by the nine test laboratories during the training sessions with the shortest exposure time of 4 hours. 1% of SDS in polar and non-polar solvents was used as the positive control and 2 chemicals at low concentrations (Lactic acid at 4% and Heptanoic acid at 2%) were used as benchmarks due to their known irritating properties in rabbits and humans. Training was conducted with 4-hour exposure, since at 18 and 24 hours, all the test materials provided viabilities below 25-30%.

SUMMARY & CONCLUSIONS

- We have developed a highly sensitive protocol for testing skin irritation potential of extracts from medical devices.
- The protocol was evaluated for transferability and predictive capacity in several laboratories.
- Initially 4, 18, 24 and 48 exposure times were evaluated (see also Casas et al) and 18 hours exposure was selected as being the optimal in this protocol.
- Nine EU and US laboratories underwent training in in this procedure in 2013 and 2014 in preparation for Round Robin validation.
- When validated, this protocol has potential to reduce or even replace the rabbit skin irritation test for the medical devices.

References

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