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Preparation of irritant polymer samples for an in vitro round robin study

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ABSTRACT

A round robin study using reconstructed human epidermis (RhE) tissues was conducted to test medical device polymer extracts for skin irritation potential. Test samples were four irritant and three non-irritant medical device polymers. Five of these polymer samples were developed and two were obtained commercially. The three non-irritant samples were comprised of 100% 80A polyurethane, one-part silicone, and polyvinyl chloride (PVC). The polyurethane samples were made using a hot-melt process, while the silicone samples were created by mixing and casting. The PVC samples were commercially produced sheets. The four irritant samples were comprised of one-part silicone and 25% heptanoic acid (HA), two-part silicone and 15% sodium dodecyl sulfate (SDS), PVC and 4% Genapol® X-100, and PVC and 5.8% Genapol® X-080. The HA, SDS, and Genapol® X-100 samples were produced using the mixing and casting method, while the Genapol® X-080 sheet samples were obtained commercially. During development, irritant polymer samples were extracted using polar and non-polar solvents that were subsequently analyzed chemically. Samples with sufficient levels of extracted irritants were tested on RhE tissues to confirm their irritation potential. Polymers that passed this screening test were used in the round robin study described elsewhere in this special edition.

1. Introduction

Biocompatibility assessment is an important aspect of the preclinical safety evaluation of medical devices. The globally harmonized ISO 10993 series of standards govern this process. As required by ISO 10993-1: 2009, dermal irritation is one of three biological effects that must be addressed for all medical devices regardless of the nature or duration of their body contact (ISO, 2009). Currently the Draize rabbit skin irritation test is used for this purpose (Draize et al., 1944; ISO, 2010).

This special edition of Toxicology In Vitro describes a round robin study designed to determine if reconstructed human epidermis (RhE) models are suitable replacements for the rabbit skin irritation test. Prior to this study, a proof-of-concept pilot project was conducted using medical device polymer extracts spiked with irritant chemicals and dosed on RhE tissues (Casas et al., 2013). Briefly, eleven medical device polymers were evaluated using EpiDerm™ EPI-200 tissues from MatTek Corporation (Ashland, Massachusetts, USA). Saline and sesame oil extracts were prepared for all polymers. Half of the extracts were spiked with two R-38 irritants, lactic acid in saline and heptanoic acid in sesame oil. The reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was used to assess cellular viability in the RhE tissues. The authors reported that the EpiDerm™ EPI-200 tissues were able to accurately identify low levels of the two R-38 irritants in the dilute medical device extracts, which were complex mixtures. Casas et al.'s pilot project results were successfully reproduced by two labs in Europe (National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; MatTek In Vitro Life Science Laboratories (IVLSL), Bratislava, Slovakia). This prompted ISO Technical Committee 194's Working Group 8 (WG8), which is responsible for the ISO 10993-10

Abbreviations: DEHP, Diethylhexyl phthalate; DINP, Diisononyl phthalate; DPBS, Dulbecco's phosphate-buffered saline; ECVAM, European Center for Validation of Alternative Methods; EC_{50}, Effective concentration 50%; ESBO, Epoxidized soybean oil; HA, Heptanoic acid; IVLSL, In Vitro Life Sciences Laboratories; LA, Lactic acid; LC-MS, Liquid chromatography–mass spectrometry; NIHS, National Institute of Health Sciences, Japan; PTFE, Polytetrafluoroethylene; PU, Polyurethane; PVC, Polyvinyl chloride; RhE, Reconstructed human epidermis; RIVM, National Institute for Public Health and the Environment, The Netherlands; SA, Saline; SDS, Sodium dodecyl sulfate; SO, Sesame oil; WA, Water

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standard on irritation and sensitization, to sponsor an international round robin validation study. A key requirement of this study was that the test samples had to be medical device polymers that contained irritants that could be extracted according to ISO 10993-12 criteria (ISO, 2012).

When the round robin began, WG8 was unable to identify any commercially available medical grade skin irritating polymers. Consequently, two working group member companies volunteered to make the needed irritant polymers. These two medical device manufacturers, Medtronic plc (Minneapolis, Minnesota, USA) and Arthrex, Inc. (Naples, Florida, USA), both had polymer laboratories and Medtronic had an analytical chemistry laboratory with experience extracting and testing medical device materials.

This article summarizes studies that were performed to develop and test irritant polymer samples for the round robin validation study. Key findings are presented and discussed.

2. Materials

2.1. Medical device polymers

The test samples made for this study were comprised of the following medical grade polymers:

- A one-part, translucent, solvent-free silicone adhesive that cures at room temperature upon exposure to ambient moisture. Composition: 94% Silicone; 5% Silanetriol, ethyl-,triacetate; and 1% Silanetriol, methyl-, triacetate.
- A two-part, translucent, pourable silicone elastomer that features room temperature and heat accelerable curing. Part A composition: ≤60% Dimethyl siloxane, dimethylvinyl-terminated; and 15–40% Trimethylated silica. Part B composition: > 60% Dimethyl siloxane, dimethylvinyl-terminated; and 10–30% Dimethyl, methylhydrogen siloxane.
- A clear custom-made polyvinyl chloride (PVC) that contained diisononyl phthalate (DINP; 30–60%) as a plasticizer.
- A translucent 80A thermoplastic polyurethane elastomer polyether that may be processed by extrusion or thermoforming.

Due to confidentiality non-disclosure agreements, the brand names and commercial suppliers of these polymers are omitted. In addition, two other polymers were obtained from the National Institute of Health Sciences (NIHS), Division of Medical Devices in Tokyo, Japan. The first polymer, Y-1, was comprised of 61.3% PVC, 33.7% DEHP, and 4.9% EBSO [w/w]. The second polymer, Y-4, was comprised of 57.8% PVC, 31.8% DEHP, 5.8% Genapol® X-080, and 4.6% EBSO [w/w] (Haishima et al., 2014).

2.2. Irritant chemicals

The following irritants were used in the polymer test samples:

- Heptanoic acid (HA; CAS No.: 111–14-8; ≥99% purity; Sigma–Aldrich Company, St. Louis, Missouri, USA).
- Sodium dodecyl sulfate (SDS; CAS No.: 151-21-3; ≥99% purity; Sigma–Aldrich Company, St. Louis, Missouri, USA).
- Genapol® X-080 (X-080; ethoxylated isotridecanol; CAS No.: 9043-30-5; mixture; Sigma–Aldrich Company, St. Louis, Missouri, USA).
- Genapol® X-100 (X-100; ethoxylated isotridecanol; CAS No.: 9043-30-5; mixture; Sigma–Aldrich Company, St. Louis, Missouri, USA) (structurally similar to X-080).
- Reagent grade DL-lactic acid (LA; CAS No.: 50-21-5; 90–100% purity; Sigma–Aldrich Company, St. Louis, Missouri, USA). Composition: 61.5% lactic acid; 38% calcium lactate; 1.2% silicone dioxide; and 1.9% water.
- Food grade lactic acid powder (CAS No.: 50-21-5; 38% purity; Galactic, Milwaukee, Wisconsin, USA).

2.3. Extraction solvents

Physiological saline (NaCl; CAS No. 7647-14-5; liquid; 0.9%; Sigma-Aldrich Company, St. Louis, Missouri, USA) was used as the polar solvent. The non-polar solvent was Super Refined™ Sesame Oil NF-NP, USP grade (Croda, Inc., Edison, New Jersey, USA).

2.4. RhE tissues

The reconstructed tissue model EpiDerm™ Skin Irritation Test (EPI-200) (OECD, 2015) with Modulated Dose (100 µL) and exposure period (18 h, no post-incubation) (known as EpiDerm™ SIT-MD) was used in this study (MatTek IVLSL, Bratislava, Slovak Republic). SkinEthic™ RHE tissues (OECD, 2015) with Modulated Dose (100 µL) and exposure period (24 h) were also used (EpiSkin, Lyon, France).

3. Methods

The irritant and non-irritant polymer samples were prepared by the following processes.

3.1. Irritant samples

3.1.1. Heptanoic acid – one-part silicone

The first irritant polymer was made as follows: the silicone was placed in a 20 g polypropylene mix cup and then enough HA was added to make a final sample that contained 25% HA by weight. The cup was capped and placed on a Speed-Mixer DAC 150 FV (FlackTek, Inc., Landrum, South Carolina, USA) where the following sequence occurred: (1) mix at 1000 rpm for 30 s to initiate blending, and then, if needed, (2) use a metal spatula and mix by hand to facilitate better contact between the oily irritant and liquid silicone; and then (3) mix at 3500 rpm for 1 min. This process was repeated as necessary until the mixture appeared to be fully blended, which never exceeded four repeats. After the material was completely mixed, it was cast into uniform-sized samples. Large Teflon® casting blocks with a dozen surface cutouts measuring 1 cm × 1.5 cm × 1.5 mm deep were used for this purpose (Fig. 1). Enough HA-silicone was transferred with a metal spatula into the surface cutouts on a casting block so that it was flush with each block's surface. The blocks were placed in a laminar flow laboratory fume hood and allowed to cure overnight. Once cured, the samples were removed from the cutouts and any flash was trimmed off. The cured samples were placed into 20 mL borosilicate amber glass vials with hard plastic caps lined with PTFE (Part number: 02-993-253. Thermo Fisher Scientific, Waltham, Massachusetts, USA).

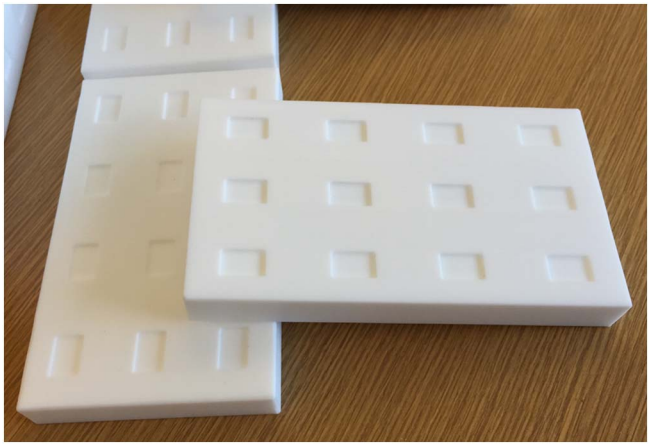

Fig. 1. Teflon® blocks for casting polymer samples.