IN VITRO ASSESSMENT OF PERSONAL CARE PRODUCTS FOR VAGINAL IRRITATION USING 3D TISSUE CONSTRUCTS Evans, Eric¹; Priston, Robert¹; Inglis, Heather²; Barnes, Nicole²; Raabe, Hans²; Costin, Gertrude-Emilia² ¹The Kimberly-Clark Corporation, Roswell, GA, USA ²Institute for In Vitro Sciences, Inc. (IIVS), Gaithersburg, MD, USA

Abstract

Three dimensional (3D) human vaginal-ectocervical tissue constructs may be a relevant in vitro model to rapidly screen for vaginal mucosal irritation potential of raw materials and final formulations. Here we report results from a study with an in vitro vaginal tissue construct (EpiVaginal[™] from Mattek Corporation, USA), used to predict irritation responses of a variety of benchmark ingredients (including surfactants and fragrances) and product formulations. Test products were representative body washes, personal care wipes, etc., tested at concentrations relevant to use in final product form. The time-to-toxicity (exposure time to reduce viability of the tissues to 50% of the controls, or ET₅₀) for each test sample was determined. Samples were applied topically to the surface of the tissue constructs for various exposure times. Vaginal irritation expressed as ET₅₀ values showed a close correlation with the expected irritation potential based on previous work, published research results and product market history. In general, our results demonstrated a shortening of the ET_{50} values with increasing sample concentration; as such, the ET_{50} values for a dilution series of the positive control (Triton-X-100) ranged from 8.77 to 4.00 h (for a dilution of 0.1%), from 3.83 to 1.53 h (for a dilution of 0.3%) and from 1.69 to 0.64 h for the 1% Triton-X-100. Furthermore, the ET₅₀ of a currently marketed vaginal cream containing 20% benzocaine ranged from 3.03 to 1.50 h. Three categories of personal care wipes were tested and the ET₅₀ values ranged from 4.64 to 6.64 to >24 h, correlating with their expected irritation potential based on other data. In conclusion, our findings suggest that the time-totoxicity assay using EpiVaginal[™] tissues can be used to screen raw ingredients and final products for potential vaginal irritation.

Introduction

Unless formulated properly, frequent use of feminine hygiene and personal care products may result in irritation of the vaginal mucosa which could lead to other effects such as local infection. Therefore, it is important that compatibility of newly developed products with this mucosal surface be assessed during the research and development stage of product development. The most frequently used test to screen for vaginal mucosal irritation is the *in vivo* rabbit vaginal irritation model. However, the current emphasis and preference in toxicology is to use alternative, in vitro methods that Reduce, Refine, or Replace the use of animals. Such an approach is of particular interest to personal care industries in their effort to reduce animal testing. In this context, The Kimberly-Clark Corporation has an immediate goal to investigate whether a 3D reconstructed tissue model is a useful approach for screening raw materials as well as final formulations. Recent advances in tissue engineering and molecular and cellular biology have significantly contributed to the development of reliable tissue constructs designed for safety and efficacy testing of pharmaceutical, personal care, and cosmetic ingredients and final formulations. The reconstructed tissues exhibit in vivo-like morphological and ultrastructural characteristics that are uniform and quite reproducible from batch to batch. Thus, they provide a reproducible, consistent testing platform. Here we detail the results obtained in a screening study with products/ingredients of interest to The Kimberly-Clark Corporation. This methodology, based on the EpiVaginal[™] (VEC-100) model from the MatTek Corporation, could represent a working model for other similar screening programs.

Materials and Methods

Test System

EpiVaginal[™] Model (VEC-100)

- Supplied by MatTek Corporation
- Tissues are based on normal, humanvaginal-ectocervical (VEC) derived epithelial cells.



Figure 1. Tissue structure of the EpiVaginal[™] Model (VEC-100) and of human native vaginal tissue.

Reagents

- EpiVaginal[™] Assay Medium (VEC-100-ASY): Supplied by MatTek Corporation
- MTT (3-[4,5 dimethylthiazol-2-yl] 2,5 diphenyltetrazolium bromide)
- Ca⁺⁺ and Mg⁺⁺ Free Dulbecco's Phosphate Buffered Saline (CMF-DPBS)
- Extraction Solvent: Isopropanol
- Sterile Deionized Water
- Dulbecco's Modified Eagle's Medium (DMEM) containing 2 mM L-glutamine (MTT Addition Medium)

Assessment of Direct Test Article Reduction of MTT

- Prior to the start of the definitive assay the ability of each test article to directly reduce MTT was determined.
 - 1. A 1.0 mg/ml MTT solution was prepared in warm MTT Addition Medium. Approximately 83 µL of each test article were added to 1 mL of the MTT solution and this mixture was incubated in the dark at 37°C ± 1°C for 1
- The test articles, Fragrance #1 and 20% benzocaine cream were observed to directly reduce MTT in the absence of viable cells.
 - 2. Due to direct reduction of MTT by these two test articles, freeze-killed controls were tested in parallel to the viable cultures and the results from these cultures were used to make further calculation adjustments.

Test Material Preparation

Test materials were tested neat or as dilutions in sterile, deionized water.

Human native vaginal tissue





Presentation of Data

- The raw absorbance values were captured.
- The mean OD₅₅₀ value of duplicate blank control wells was calculated.
- The corrected mean OD₅₅₀ values of the individual test article exposure times and of the exposure time controls were determined by subtracting the mean OD_{550} value of the blank control from their mean OD_{550} values.

Corrected test article exposure time OD_{550} = Test article exposure time OD_{550} – Blank mean OD_{550}

• The following percent of control calculations were made:

- A semi-log plot of the exposure time response curves was plotted with the % of Control on the ordinate and the test article or positive control exposure time on the log-scale abscissa.
- The ET₅₀ value was interpolated from each plot.

Criteria for a Valid Test

The assay results were accepted when the ET₅₀ value of the positive control (1% Triton-X-100) fell within two standard deviations of the historical mean:

X 100







Figure 3a. Exposure response continuum for a Triton-X-100 dilution series. 1% Triton-X-100 was used as a positive control.

- Some test articles were tested at several concentrations to cover a range of potential responses
- The test articles and controls were tested by treating two VEC-100 tissues per exposure time with a volume of 83 µL • Cultures were incubated in standard conditions for the appropriate exposure times

 - Positive control: 1% Triton-X-100, tested for 0.5, 1 and 2 hours







Spectrophotometric quantification using a 96-well plate-reader that measures the optical density at 550 nm (OD





Figure 2. Example for interpolation of ET₅₀ values and evaluation of EpiVaginal[™] test results.

- **IIVS**:
- range: 0.50 1.59 h
- mean = 0.99• n = 17
- used during the trials executed in 2009





Figure 3b. Response curves for 20% benzocaine cream – an over the counter final formulation.



Figure 4a. Response continuum of irritation potential of raw materials Figure 4b. Concentration-dependent irritation response for the positive control (Triton-X-100) and two raw materials and final formulations screened in this study. screened in this study

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The Kimberly-Clark Corporation used the time-to-toxicity approach with reconstructed tissues detailed herein to establish the relevant ranges or responses for a series of raw materials and final formulations, intended for vaginal application.

2. Vaginal irritation (expressed as ET₅₀ values) showed a close correlation with the expected irritation based on previous work, published research results and product market history.

3. Our results showed a shortening of the ET_{50} values with increasing sample concentration; as such, the ET_{50} values for a dilution series of the positive control (Triton-X-100) ranged from 8.77 to 4.00 h (for a dilution of 0.1%), from 3.83 to 1.53 h (for a dilution of 0.3%) and from 1.69 to 0.64 h for the 1% Triton-X-100.

4. The ET_{50} of a currently marketed vaginal cream containing 20% benzocaine ranged from 3.03 to 1.50 h.

Three different personal care wipe products were tested and the ET_{50} values ranged from 4.64 to 6.64 to >24 h, correlating with their expected irritation potential based on other data and market history of use information.

Conclusions

1. The reproducibility of the effects of a nonionic ethoxylated surfactant (Triton-X-100) as positive control has been assessed in 4 trials. The data demonstrated a consistent dose-response between trials. The ET₅₀ values for the positive control, 1% Triton-X-100, were 0.64, 0.79, 1.33, and 1.69 h.

2. A currently marketed vaginal cream containing 20% benzocaine was shown to be an appropriate reference control against which to benchmark novel personal and vaginal care product formulations.

3. The experimental protocol used in this study proved useful for the screening and calculation of the concentrationdependent ET₅₀ values of typical personal care product raw materials and final formulations. Furthermore, the irritation responses for currently marketed feminine and personal care products tested in this study were milder compared to a longstanding marketed product, 20% benzocaine vaginal cream.

4. In conclusion, our findings suggest that the time-to-toxicity assay using EpiVaginal[™] tissues can be used to assess the vaginal irritation effects of personal and feminine care product raw materials and final formulations.

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