Abstract

Three-dimensional (3D) human vaginal-ecocellular tissue model: In this study, we present a reliable 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 3D vaginal tissue constructs (EpiVaginal™ from MatTek Corporation) used to predict irritation response of a variety of resources (including surfactants and fragrances) and product formulations. Test products were represented by body wash, personal care, and package undergoes. Each test was conducted at multiple concentrations relevant to use in silicone. The test results were compared to historical range data for a currently marketed vagina cream containing 20% benzocaine. This research provides a new methodology for understanding the role of surfactants and fragrances in vaginal care product formulations.

Materials and Methods

Reagents

Epithelial™ Assay System (VEC-100)/ASSY, supplied by MatTek Corporation. (WST-3 or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Cari- and Mile- Free Donor Medium (DME)/MEM containing 2 ml-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 0.1 mg/mL MTT solution was prepared in MTT Addition Medium. Appropriately, 3 µL of each test article was added to 1 mL of the MTT solution and this mixture was incubated in the dark for 15 min. The absorbance of the mixture was measured at 570 nm.

The test materials, including 20% benzocaine creams, were observed to directly reduce MTT in the absence of visible color.

Presentation of Data

The raw absorbance value was captured as the mean absorbance of duplicate blank control wells. The corrected test article exposure time OD was obtained by subtracting the mean OD of the blank control from the mean OD values of the corrected test article or positive control exposure time on the log-scale abscissa.

The corrected mean OD values of the individual test article exposure times and of the exposure time controls were determined by subtracting the mean OD0 of the blank control from their mean OD values.

In conclusion, our findings suggest that the time-to-toxicity assay using EpiVaginal tissue tests can be used for drug-screening purposes and for product formulations for potential vaginal irritation.

Materials and Methods

Test System

Epithelial™ Model (VEC-100)

• Supplied by MatTek Corporation

• Tissues are based on normal, human-derived vaginal-ecocellular (VEC) epithelial cells

Figure 1. Tissue structure of the Epithelial™ Model (VEC-100) and human vaginal tissue.

Reagents

• Epithelial™ Assay System (VEC-100)/ASSY, supplied by MatTek Corporation

• WST-3 (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

• Car- and Mile-Free Donor Medium (DME)/MEM containing 2 ml-L-glutamine (MTT Addition Medium)

Test Material Preparation

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

Results

The raw absorbance value was captured as the mean absorbance of duplicate blank control wells. The corrected test article exposure time OD was obtained by subtracting the mean OD of the blank control from the mean OD values of the corrected test article or positive control exposure time on the log-scale abscissa.

The corrected mean OD values of the individual test article exposure times and of the exposure time controls were determined by subtracting the mean OD0 of the blank control from their mean OD values.

A serving 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 ETn value is interpreted from each plot.

Criteria for a Valid Test

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

Conclusions

The reproducibility of the effects of a cosmetic ethylhexyl salicylate (THB-10X) as positive control has been assessed in 4 trials. The data demonstrated a consistent dose-response between trials. The ETn values for the positive control (THB-10X) were 0.64, 0.79, 1.32, and 1.48.

We have marketed vaginal cream containing 20% benzocaine shown to be an appropriate reference control against which to benchmark raw materials and final formulations.

The standard protocol used in the study provided the necessary and calculation of the concentration-dependent ETn values of typical personal care product final formulations. Furthermore, the irritation responses for currently marketed feminine and personal care products tested in this study were comparable to a long-standing marketed product, 20% benzocaine vaginal cream.

In conclusion, our findings suggest that the time-to-toxicity assay using Epithelial™ assays can be used to assess the vaginal irritation potential of femine care products and final formulations.

References


1. The Kimberly-Clark Corporation used the time-to-toxicity approach with reconstructed tissue models 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 ETn 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 ETn values with increasing sample concentration; as such, the ETn values for a dilution series of a currently marketed feminine and personal care product formulation ranged from 8.77 to 4.00 (for a dilution of 0.1%), from 3.83 to 1.53 (for a dilution of 0.3%) and from 1.69 to 0.64 h for the 1% Triton-X-100. Furthermore, the ETn of a currently marketed vaginal cream containing 20% benzocaine ranged from 3.03 to 1.59 h. Three categories of personal care products tested and the ETn values varied from 4.86 to 1.04 h, consistent with their expected irritation potential based on other data and market history of use information.

4. Three different personal care products were tested, and the ETn values ranged from 0.49: 0.64 to 0.74 h, correlating with their expected irritation potential based on other data and market history of use information.