Development of a 3D Genotoxicity Model for Assessment of Cosmetic Formulations

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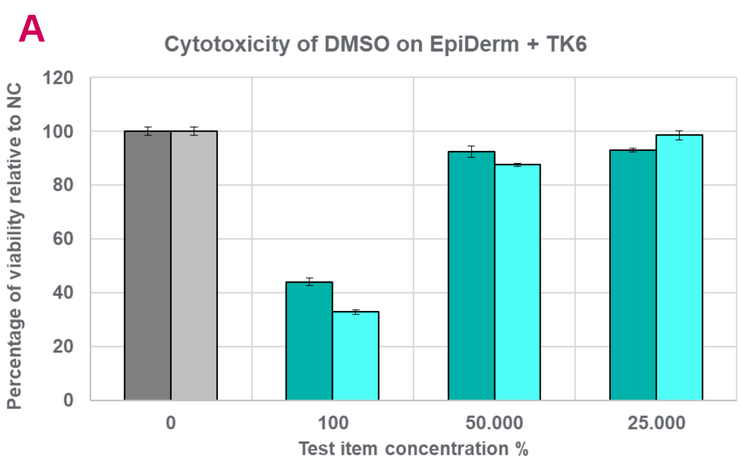
Introduction

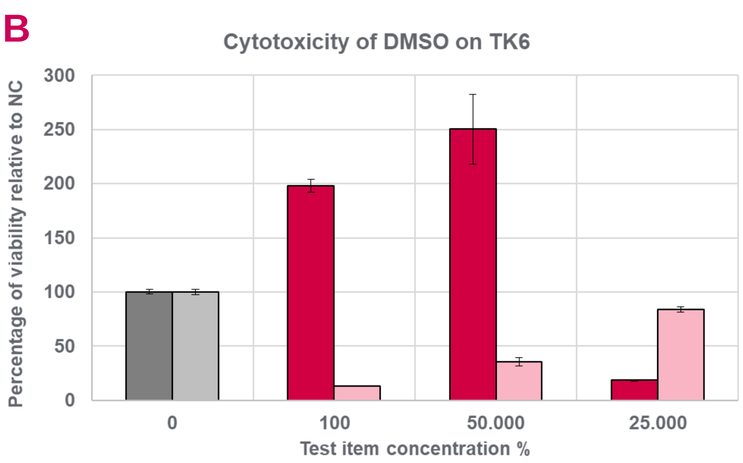
3D tissue models can be effectively combined with a genotoxicity screening assay in order to expand the type of samples that can be tested *in vitro*. Use of the 3D tissue model mimics the skin barrier and allows for absorption to be taken into account when assessing genotoxic potential. This facilitates investigation of whether a positive result in a standard 2D cell-based assay is relevant to products with respect to penetration of the skin barrier. Use of the animal free BlueScreen test as the genotoxicity endpoint allows for identification of all 3 classes of genotoxins; mutagens, clastogens and aneugens[1,2].

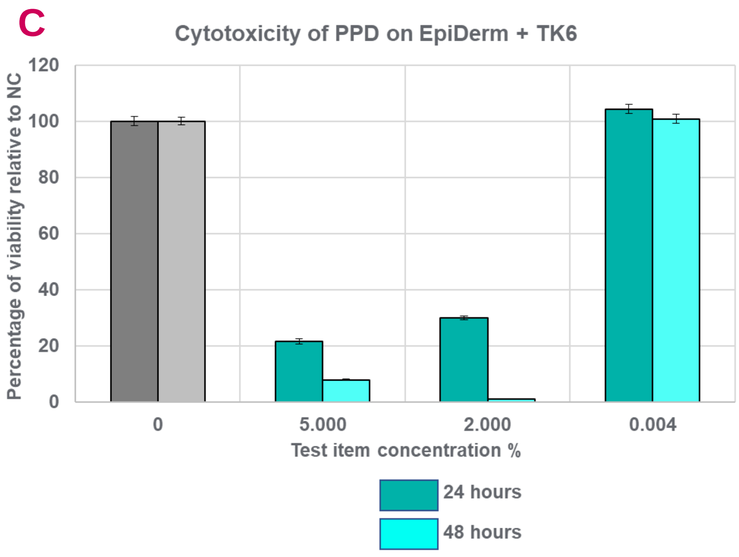
Methods

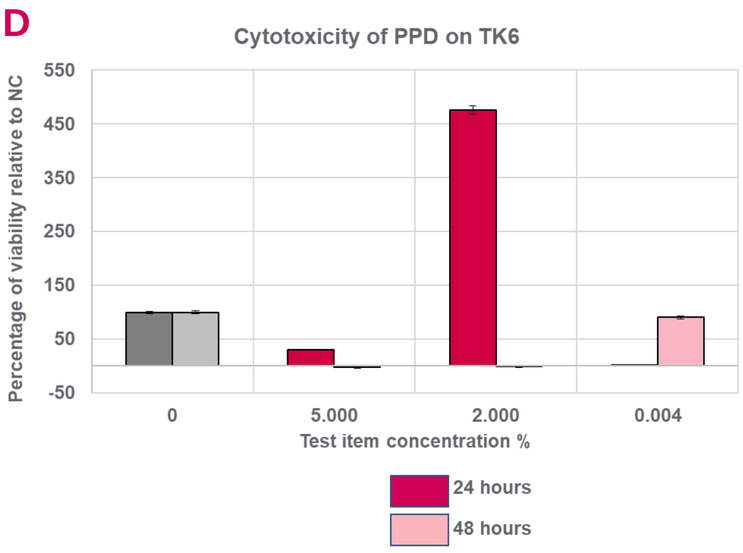
TK6 cells (with GLuc reporter) were seeded into 24 well plates. EpiDerm 3D tissue models were placed into the 24 well plates so that the basolateral side of the insert made contact with the TK6 cell suspension forming a co-culture system. The same experiments were carried out using TK6 cells only as a control. DMSO and Paraphenylenediamine (PPD) were added to the apical side of the tissue models or the cells directly across a dosing range for 24 or 48hrs. Cells were collected at the end of the dosing periods and added to a 96-well plate alongside assay controls for the endpoint measurements. Endpoint measurements for luminescent (genotoxicity) and fluorescent (cytotoxicity) endpoints were collected.

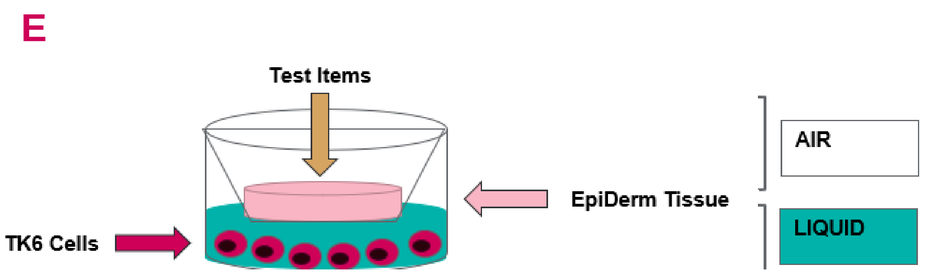
Results





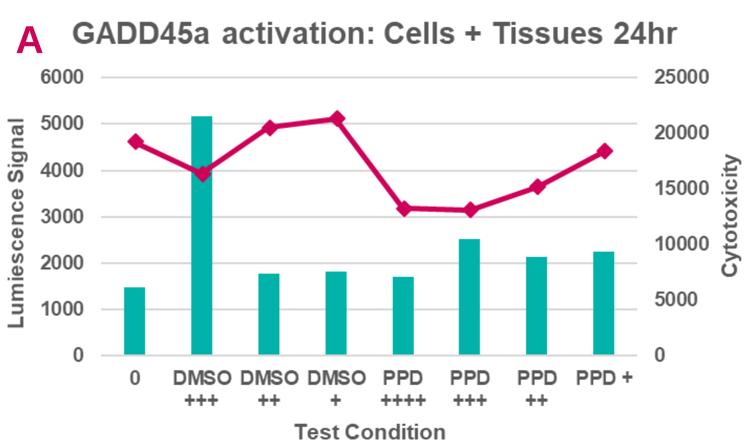


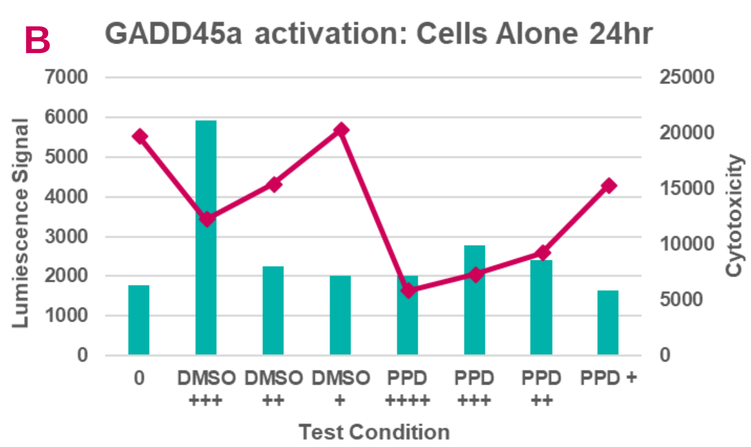


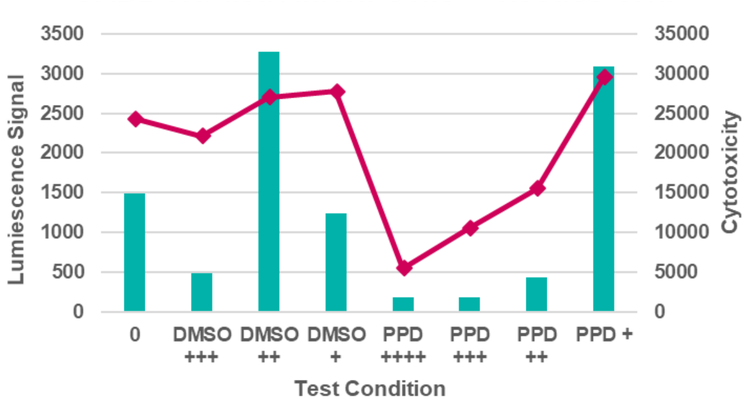


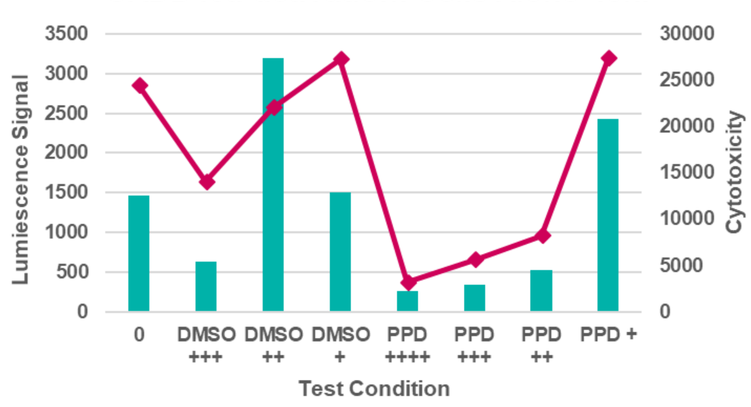
A cytotoxic effect is elicited on TK6 cells when tissues are dosed at the air liquid interface

A. EpiDerm tissues + TK6 cells dosed with three concentrations of DMSO for 24 and 48 hours
B. TK6 cells dosed with three concentrations of DMSO for 24 and 48 hours
C. EpiDerm + TK6 cells dosed with three concentrations of PPD for 24 and 48 hours.
D. TK6 cells dosed with three concentrations of PPD for 24 and 48 hours
E. Scheme depicting co-culture system









Dosing tissue with a test item can elicit a genotoxic response in co-cultured TK6 cells

A. EpiDerm tissues + TK6 cells were dosed with three concentrations of DMSO or four concentrations of PPD for 24 hours. Luminescent (genotoxicity – green bars) and fluorescent (cytotoxicity – pink line) endpoints were collected

B. TK6 cells were dosed with three concentrations of DMSO or four concentrations of PPD for 24 hours. Luminescent (genotoxicity – green bars) and fluorescent (cytotoxicity – pink line) endpoints were collected

C. EpiDerm tissues + TK6 cells were dosed with three concentrations of DMSO or four concentrations of PPD for 48 hours. Luminescent (genotoxicity – green bars) and fluorescent cytotoxicity – pink line) endpoints were collected

D. TK6 cells were dosed with three concentrations of DMSO or four concentrations of PPD for 48 hours. Luminescent (genotoxicity – green bars) and fluorescent (cytotoxicity – pink line) endpoints were collected

DMSO concentrations:
\+ 25%
++ 50%
+++ 100%

PPD concentrations:
\+ 0.004%
++ 0.4%
+++ 2%
++++ 5%

Discussion

Results show that the 3D tissue models and TK6 cells can be effectively combined within a co-culture system and that both DMSO and PPD were able to penetrate through the 3D tissue layer as expected. Cytotoxic dose response effects were observed for PPD and DMSO at both 24hr and 48hr timepoints. PPD elicited an inverted genotoxic dose response effect at 48hrs in the co-culture system and upon the TK6 cells alone with higher doses of PPD causing more cytotoxicity. The genotoxic induction was increased compared to control at the lowest dose. No change in genotoxic induction was observed at 24hrs suggesting that the 48hr timepoint was needed in order to elicit a genotoxic induction. The addition of the 3D model also dampened the cytotoxic effects of the compounds indicating that the skin barrier is effective at preventing a proportion of the PPD from being absorbed, or is detoxified by the skin's metabolic enzymes. This system provides a physiologically relevant model for investigation of genotoxicity for topically applied substances.

References

1. Etter S. Toxicology in Vitro 29 (2015) 1425–1435
2. Hughes, C. et al., Journal of Biomolecular Screening 17(10) 1302–1315