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The initial depth of injury in reconstructed tissue models as an indicator for the eye irritation potential of chemicals

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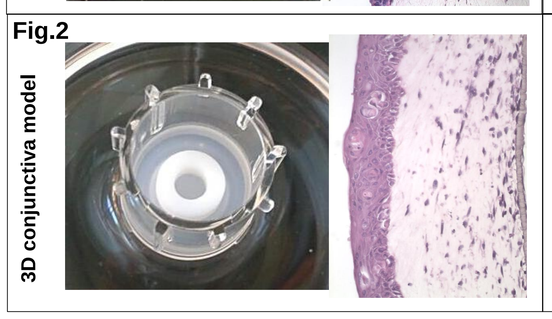
INTRODUCTION

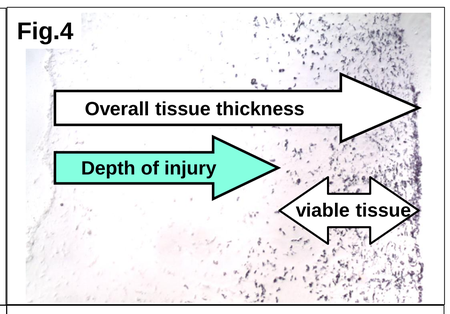
The depth of injury (DoI) is a mechanistic correlate to the ocular irritation response. Attempts to quantitatively determine the DoI in alternative tests have been limited to ex vivo animal eyes by fluorescent staining for biomarkers of cell death and viability of histological cross sections [1]. It was the purpose of this study to assess whether DoI could also be measured by means of cell viability detection based on the MTT viability assay using the sophisticated structure of a previously developed hemi-cornea model [2] and a newly developed conjunctiva model.

EXPERIMENTAL SETUP

Construction of cornea and conjunctiva models: Suspensions of immortalised cornea-derived fibroblasts in collagen gels were cast and subsequently cornea epithelium cells (HCE-T) or conjunctiva epithelium cells (HCjE) were seeded onto the stromal equivalents [2-4]. The tissues were cultivated submerged, until the epithelial cells reached confluence and then lifted to the air-liquid interface for multilayer growth and cell differentiation.

Analysis of DoI in MTT-stained cryosections: Tissues were exposed to different test substances for 10 or 60 min resp. In case of contracted tissues (such as the conjunctiva models) teflon O-rings were used for substance application to avoid spreading. After chemical treatment, tissues were immersed in MTT solution. Then, the tissues were incubated in 20% sucrose and embedded in O.C.T. compound and stored in liquid nitrogen until 30 µm cryosections were collected. Images of transverse sections were quantitatively analyzed by FiJi software program. The DoI was calculated as % of non-viable tissue thickness compared to overall tissue thickness.





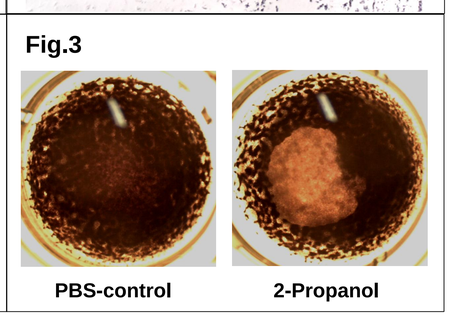


Figure 1: Cornea models (left), H&E stain of paraffin section (right)

Figure 2: Conjunctiva model with fixed teflon O-ring to avoid spreading of test substances (right H&E stain).

Figure 3: Top-view images of MTT-stained conjunctiva models after topical application of PBS and 2-propanol.

Figure 4 shows principle of DoI measurement in MTT-stained cryosections using the example of a cornea model after 2-Propanol treatment: DoI was calculated by subtracting Formazan-stained length (viable tissue) from total length (overall tissue thickness).

RESULTS

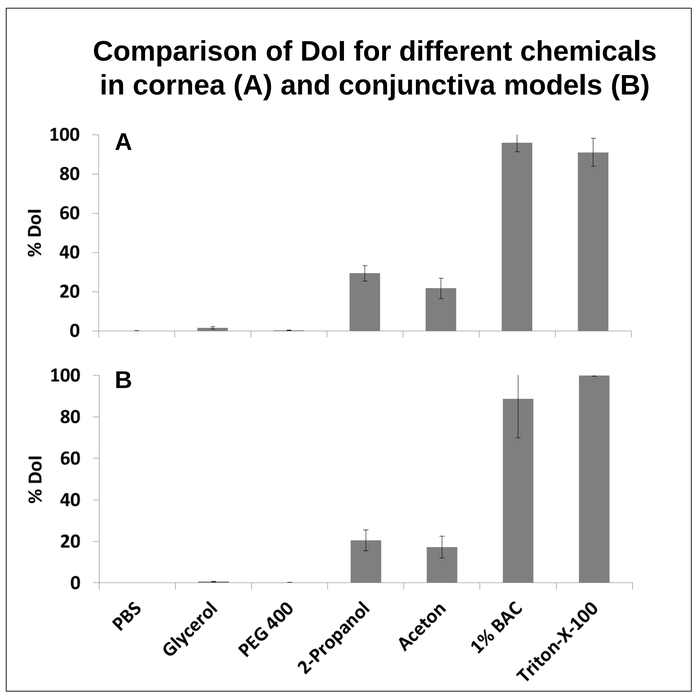


Figure 5: Quantitative analysis of tissue injury in cornea (Fig. 5A) and conjunctiva (Fig. 5B) models. The test substances cover the full range of GHS classification for ocular irritation based on draize data having nonirritant (Glycerol, and PEG 400; GHS no category/NC), slight/moderate irritant (2-Propanol and Aceton; GHS 2A;) as well as severe irritant substances (1% BAC and 10% Triton-X 100; GHS 1). Note, only slight differences in DoI were found between both tissues.

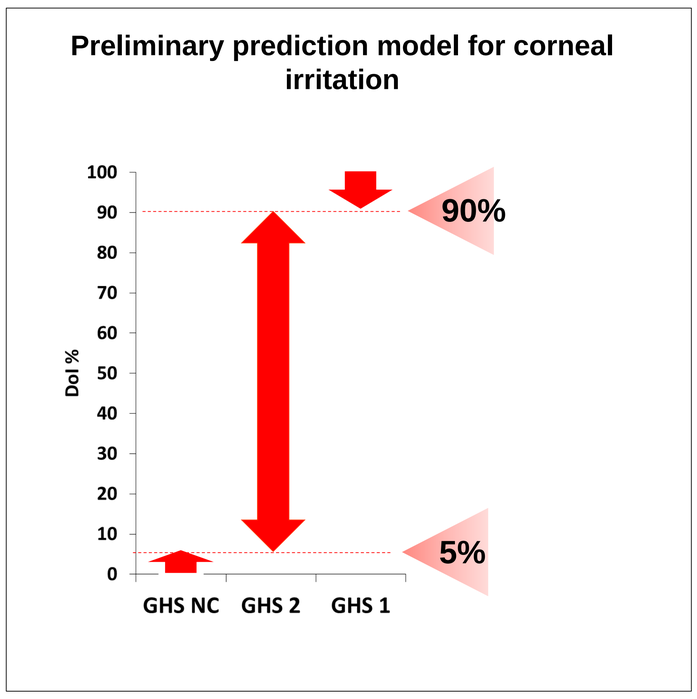


Figure. 6: Scheme summarizing the preliminary parameters for classification of the prediction model based on cornea models

Preliminary parameter for classificaion:

GHS NC = DoI ≤ 5% of total corneal thickness
GHS 2 = DoI > 5% und <90% of total thickness
GHS 1 = DoI ≥ 90% of total thickness

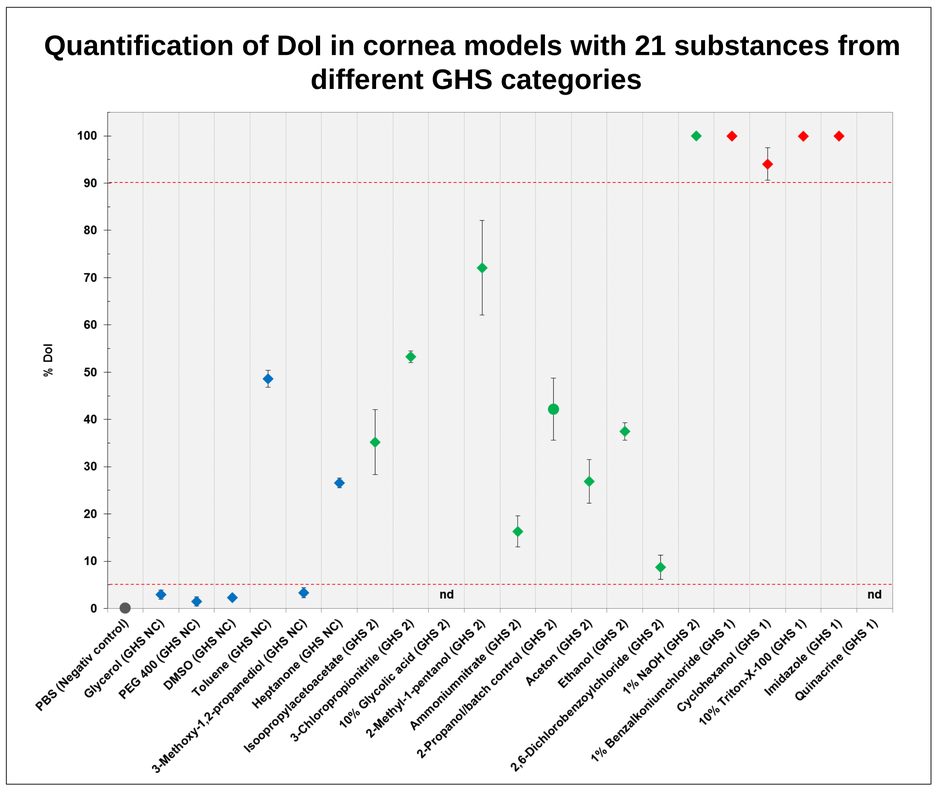


Figure 7: DoI values of 21 chemicals after 60 min exposure in cornea models (data points represent mean± SD of 3 independent batches). The chemicals are are supported by high quality *in vivo* data [5]. Note that 4 of 6 NC chemicals (blue symbols) result in a DoI ≤ 5%, and all 4 evaluable GHS cat. 1 chemicals (red symbols) result in DoI ≥ 90%; whereas 8 of 9 evaluable GHS cat. 2 chemicals (green symbols) lead to DoI between both threshold values. nd: DoI is not detectable/not evaluable due to interference with collagen matrix (10% Glycolic acid) or irregular Formazan stain (Quinacrine).

CONCLUSION

- 3D cornea and conjunctiva equivalents have been developed from immortalized cells originally derived from the human eye. The 3D models comprise multi-layered epithelia on top of a stromal matrix consisting of collagen-embedded fibroblasts [1, 2]
- A modified MTT method for the determination of tissue viability in cryosections was applied to both 3-D models to assess the tissue damage induced by topically applied chemicals. By using this method we could clearly distinguish the eye irritation potential of reference substances by means of their depths of injury (DoI).
- Only slight differences were found between the predictive capacity of the corneal and conjunctival tissue.
- A preliminary prediction model was developed based on the corneal tissues and the modified MTT-method and uses a 60 min exposure period. 21 reference chemicals were tested of which 16 compounds were correctly predicted, and 3 compounds were over-predicted. For 2 compounds DoIs were not evaluable due to chemical interference with the 3D collagen matrix or due to irregular Formazan stain.
- This new approach represents a promising histology-based *in vitro* method to quantify eye irritation reactions and in addition may also offer the possibility of assessing toxic effects in other non cornified tissues.

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