A 3D reconstructed hemi-cornea model for predicting the eye-irritating potential of chemicals: Results of an interlaboratory evaluation study

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Abstract

Our study aimed at the demonstration of the protocol transfer for the hemi-cornea construction of a previously developed hemi-cornea model [1,2] and its quality controlled production according to a standard operation protocol (SOP) in 2 independent laboratories. Furthermore, the hemi-cornea tissues have been treated with twenty chemicals of different eye-irritating potential under blind conditions to assess the performance and limitations of our test system comparing three different prediction models. The results of a quality controlled production of the hemi-cornea models from two independent laboratories and the classification scheme to discriminate between GHS non classified and category 1 and category 2 chemicals are presented [3].

Materials and Methods

Model Construction:

Corneal equivalents were produced independently in two laboratories. Briefly, immortalized keratocytes were embedded into a collagen gel and SV40-immortalized human corneal epithelial cells were seeded on top of the gel. The construct was cultured under submerged conditions. After confluence of the epithelial cells the model was lifted to the air-liquid interface. (**figure 1**).

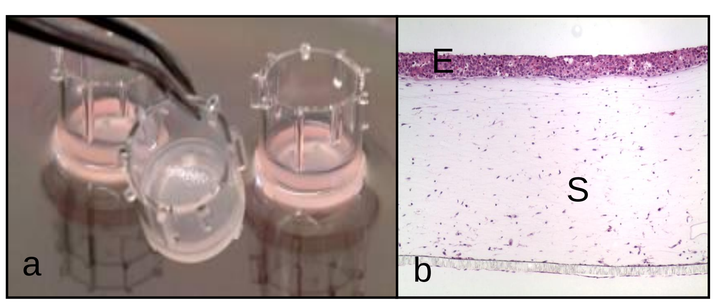

Figure 1a: hemi-cornea model a co-culture insert.
1b:Histological cross section of the hemicornea (H&E staining). E: epithelium, S: stroma

Test Chemicals:

20 chemicals were tested under blinded conditions. The test chemicals comprised different chemical classes with different eye-irritating potentials according to GHS classification (**table 2**).

Tissue Treatment:

Test chemicals were applied topically (3 runs /triplicate tissues). Tissues were incubated for 10, 20 or 60 minutes, respectively, washed with PBS and transferred to the MTT solution (1.5 ml; 1 mg/ml). Formazan was extracted with 2 ml of 2-propanol and the optical density (OD) was determined.

Quality Criteria:

PBS-treated tissues and 0.3% Triton X-100 after 60 minutes incubation time served as a negative control (NC) and batch control (BC), respectively. Only batches with 1.2 >OD_{NC}> 0.5, OD_{BC} of 10-50 % of the respective NC and SD<18% qualified for further analysis.

Data Evaluation:

The viability was calculated from the OD compared to OD_{NC}. Means +/- SDs have been calculated for triplicate tissues. The time-dependent decrease of viability (**figure 2**) could be approximated with a 3-parametrical exponential function:

$$Y=Y0 + a \cdot \exp(-bx)$$

Y_0 (%): asymptote; a (%): amplitude; a + Y_0 ~ 100 %; b (1/min): decay constant

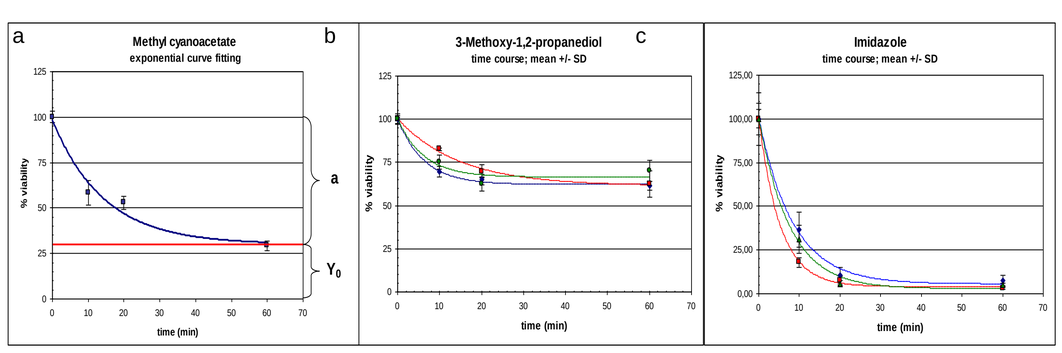

Figure 2 depicts representative examples of curve fittings for 3 chemicals with different eye irritation potentials for 3 independent runs each. In the 1st diagram the main variables of the exponential function are explained.

Prediction Models (PM):

PM1 and PM2 are based on the exponential regression (**figure 2**):
PM1: Y_0/b (% min); PM2 ET_{50} (min).
PM3: is based on the tissue viability cut-off after 60 minutes incubation.

Results and Discussion

Quality controlled production of the hemi-cornea model:

All hemi-cornea models independently produced in both labs matched the quality criteria defined for NC BC and maximum SD (**figure 3**).

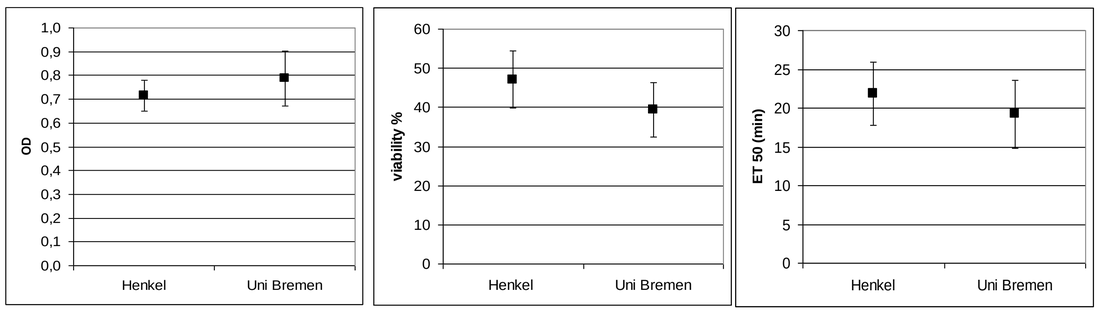

Figure 3: Mean optical density of the NCs (a), viability of BCs (b) and ET_{50} (c) from 12 independent runs (triplicate tissues) produced in each lab.

Performance of the hemi-cornea based test system:

Obtained data were analyzed using Receiver Operation Characteristics. Threshold values which resulted in the highest values for sensitivity, selectivity and accuracy and hence in the best discrimination between non-classified and GHS cat.1 and cat. 2 chemicals were identified and the predictive capacity of all 3 PMs was evaluated in both labs separately. For PM1 and PM2 the optimal threshold values differed between the labs due to an incomplete selectivity of the PMs, whereas a 40 % viability cut-off for the PM3 after 60 minutes incubation was identical in both labs. **Table 1** summarizes the performance of the test system for both laboratories.

Table 1: Performance of the test system for PM3 = cut off at 40 % tissue viability after 60 min treatment. wic %: concordance between the 3 independent runs for each laboratory

	sensitivity %	*specificity %*	*accuracy %*	*Wic %*
Uni Bremen	**77**	**57**	**70**	**75**
Henkel	**77**	**86**	**80**	**70**

In Vitro-In Vivo Concordance:

Table 2: represents the in vivo- in vitro concordance obtained using PM3 for the 20 chemicals tested in both laboratories

Test chemical	GHS[a]	Uni Bremen[b]	Henkel[b]
3,3-Dimethylpentane	NC	NC	NC
Trichloroacetic acid, 3%	NC	I	I
3-Methoxy-1,2-propanediol	NC	NC	NC
2-Heptanone	NC	NC	NC
Ethylene glycol diethyl ether	NC	I	NC
Toluene	NC	I	NC
1- Bromohexane	NC	NC	NC
2-Methyl-1-pentanol	2	I	I
3-Chloroproprionitrile	2	NC	NC
Methyl cyanoacetate	2	I	I
Octan-1-ol	2	I	I
2,6-Dichlorobenzoyl chloride	2	NC	NC
Ammonium Nitrate	2	NC	I
Cetylpyridium bromide, 1%	2	I	NC
Dibenzyl phosphate	2	I	I
Benzalkonium chloride, 1%	1	I	I
Para-fluoroaniline	1	I	I
Cyclohexanol	1	I	I
2-Methoxyethyl acrylate	1	I	I
Imidazole	1	I	I
***in vitro*lin *vivo* concordance**		70%	80%
Between-laboratory concordance		80%	

[a]GHS classification: (United Nation, 2007): NC: no category; 2: irritating to the eye; 1: irreversible effects to the eye
[b]NC (viability > 40 %), I (viability ≤ 40 %)

Conclusion

- The hemi-cornea model can be reproducibly produced in independent laboratories with high quality.
- The classification based on a 60 minutes treatment and 40 % viability cut-off is superior to PMs based on complex data analysis. The in vitro-in vivo concordance was 80% and 70%, respectively.
- The test system reliably predicts GHS cat. 1 chemicals, but it doesn't sufficiently discriminate all 3 GHS categories.

References:
[1] Engelke et al. (2004) Altern. Lab. Anim. 32, 345-53
[2] Zorn-Kruppa et al. (2005) Altern. Lab. Anim., 33, 37-45
[3] Engelke et al. (2012) TIV, accepted; DOI: 10.1016/j.tiv2012.07.11

GEFÖRDERT VOM

Acknowledgement:
This project was funded by the German Federal Ministry of Education and Research, FKZ 0315504

Bundesministerium für Bildung und Forschung

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