Suitability of the 3D hemi-cornea eye irritation test for testing of oxidative hair dyes

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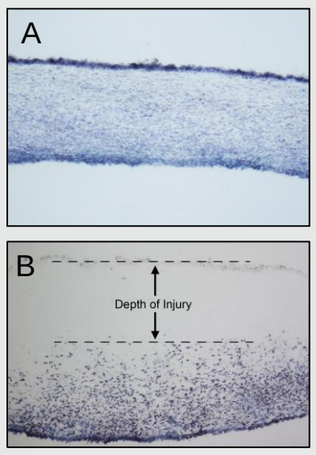
Introduction

Permanent oxidative hair dyes are widely used and account for 70 to 80 % of all hair dyes on the European market[1]. Eyes of consumers may be exposed to oxidative hair dyes accidentally during normal use. Therefore, the evaluation of the eye irritation potential of hair dyes and their ingredients is essential for safety assessment. Major efforts have been made to successfully develop alternative tests in order to replace the Draize rabbit eye test. One of such alternatives is the human open-source (OS) 3D hemi-cornea eye irritation test.

The cell culture based hemi-cornea model can be used for determination of depth of ocular injury (DOI) using the MTT viability staining. The aim of this work was to optimize the existing OS 3D hemi-cornea eye irritation test protocol for assessing hair dye formulations containing hydrogen peroxide (H_2O_2) and/or ammonia (NH_3). The models were produced by the method published by Zorn-Kruppa et al. (2014).

OS 3D hemi-cornea model

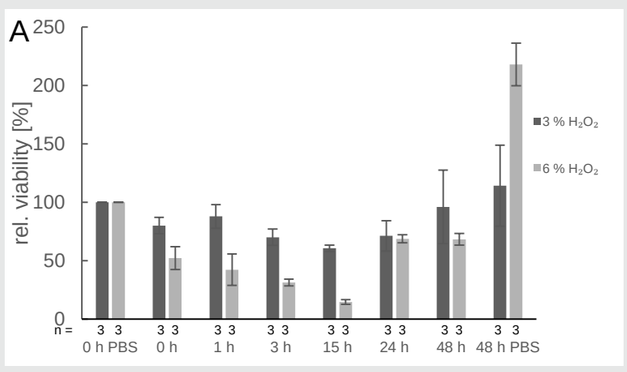
Immortalized human epithelial cells (HCE) were seeded on top of a collagen gel populated with immortalized human corneal keratocytes (HCK). The construct was cultured submersed for six days, followed by seven days air-liquid interface culture to develop a multilayered epithelium. After treatment with the test items tissue damage was quantified photometrically with the MTT assay[2] or by determining the MTT-DOI relative to the total tissue thickness on cryosections[3].

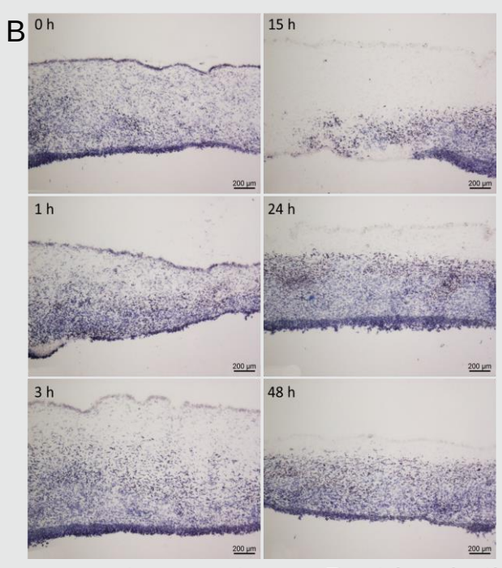


A No tissue damage

B DOI determination after tissue damage

H_2O_2 treatment induces delayed reduction of tissue viability



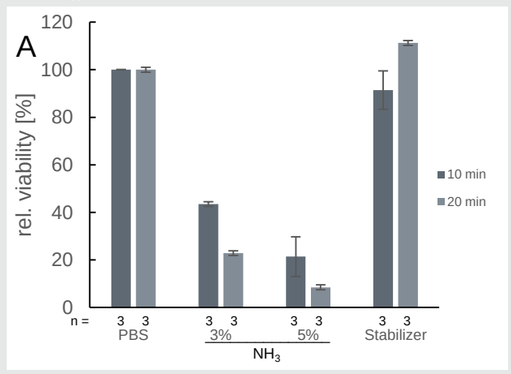


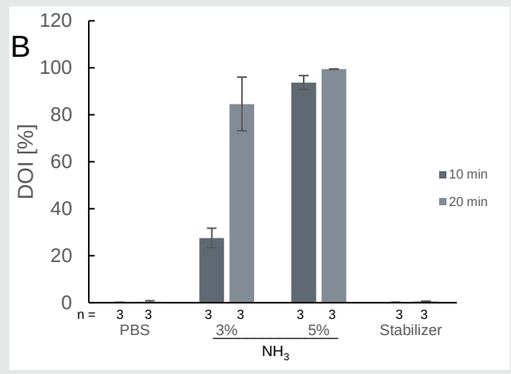
Topical treatment of hemi-cornea models with H_2O_2 reference solutions revealed that tissue damage was manifested up to 15 h after test substance application. Thereafter tissue viability increased again. The apparent changes in viability were more pronounced with higher H_2O_2 concentrations.

A Models (n = 3) were treated with 3 and 6 % H_2O_2 reference solutions for 60 min at room temperature. Post-incubation periods ranged from 1 to 48 h. Damage was assessed photometrically after MTT elution.

B Cryosection images of treated models showed a clear demarcation of MTT-DOI after 24 h post incubation.

NH_3 causes immediate reduction of tissue viability





Reference solutions containing 3 and 5 % NH_3 caused a substantial reduction of tissue viability already after 10 min treatment without post-incubation.

A Models (n = 3) were treated for 10 and 20 min with reference solutions comprising of 3 and 5 % NH_3. Tissue viability was quantified photometrically after MTT elution. Viability was heavily reduced 20 min after test substance application.

B Models (n = 3) were treated for 10 and 20 min with solutions containing 3 and 5 % NH_3. The MTT-DOI was determined on cryosection images. Models treated for 20 min were damaged to a much higher extent when compared to models treated only for 10 min.

NH_3 is decisive factor in mixtures containing H_2O_2 and NH_3

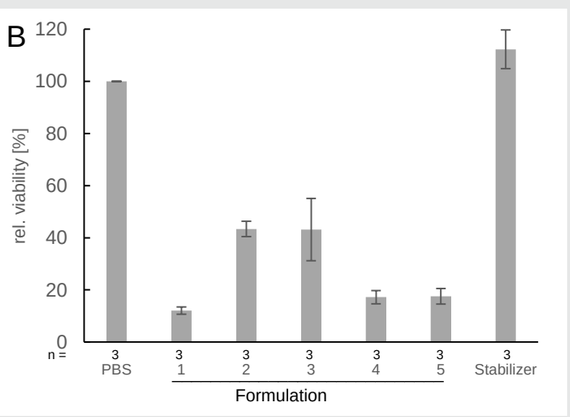
Since oxidative hair dye formulations commonly contain H_2O_2 and NH_3, the toxicological assessment of mixtures containing both ingredients is most relevant. Oxidative hair dye formulations with varying concentrations of H_2O_2 and NH_3 (Table C) were applied onto hemi-cornea models for 10 min without post-incubation. Formulations containing the lowest concentrations of NH_3 induced less damage independent from the their H_2O_2 content. Results obtained either by MTT elution or MTT-DOI clearly show that tissue damage is mainly driven by NH_3.

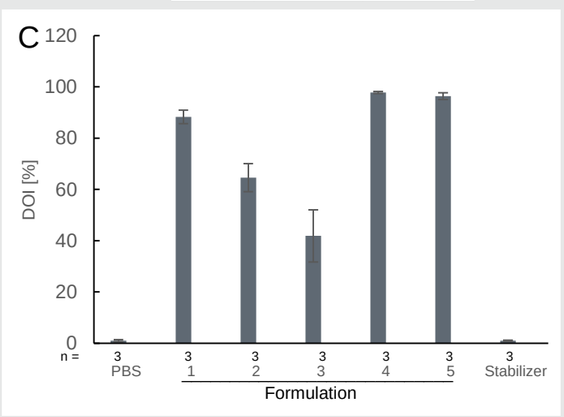
A Relative viability of models (n = 3) treated with oxidative hair dye formulations after MTT elution.

B Tissue damage caused by oxidative hair dye formulations using the MTT-DOI approach. Differentiation of tissue damage was enhanced compared to the MTT elution approach (n = 3) .

Table C

Form.	NH_3 [%]	H_2O_2 [%]
1	4.0	3.0
2	3.0	1.5
3	3.0	6.0
4	5.0	1.5
5	5.0	6.0





Conclusion

- The OS 3D hemi-cornea model is suited for the assessment of the eye irritation potential of oxidative hair dye formulations.
- The MTT elution approach as well as the DOI measurement show concordant results. However, more detailed information can be gathered using MTT-DOI since not only the extent of damage but also localization of effects can be determined.
- H_2O_2 and NH_3 seem to evoke tissue damage differently. The delayed onset of damage induced by H_2O_2 indicates that the reaction of radicals with macromolecules may be an essential mechanism of action. Due to its alkalinity NH_3 causes saponification of the cell membranes, resulting in an immediate reduction in viability. Therefore, individual testing protocols are required.
- Taken together the results demonstrate that the OS 3D hemi-cornea model is suited for testing of chemicals and formulations with different modes of action. However, different chemical or even product classes require individual customization of the test protocol.

References

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