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A human corneal model for the prediction of eye irritation: progress report

Michaela Zorn-Kruppa, Heike Scholz

German Animal Welfare Federation (Neubiberg) (DE)

e-mail: michaela.zorn-kruppa@tierschutzakademie.de

With the ban on animal testing for development of cosmetics in the EU, the demand for *in vitro* methods for safety and efficacy testing is growing. An extensive number of *in vitro* assays, many of which find applications in industry, have been developed and proposed as alternatives to the Draize eye irritation test. Unfortunately, none of the methods which were included in several validation/evaluation studies was found to meet all the formal validation requirements as prerequisite for replacing the current OECD *in vivo* assay. Hence, success in fully replacing the Draize eye test for the evaluation of acute eye irritation with *in vitro* methods has not yet been achieved. There remains a clearly identified need to define alternative methods that are mechanistically-based, reliably predict the human eye response to chemicals exposure and which can replace the *in vivo* test.

To address development of alternative methods based on mechanistically relevant biological events, we have generated a full thickness human corneal model exclusively based on SV-40 immortalised cell lines. Recently, we have optimised serum-free culture conditions for the maintenance of functional and structural characteristics of the epithelial and the stromal part of the corneal model.

Methods: The human corneal epithelial cell line HCE (Araki-Sasaki et al., 1995) and the human corneal keratocyte cell line HCK (Zorn-Kruppa et al., 2004) were used in attempt to establish a rational basis for the development of serum-free cultivation media for the assembly and long-term tissue culture of a three-dimensional corneal model.

Briefly, we investigated the impact of serum-free cultivation on proliferation, morphology and barrier function of HCE cells: Multilayered epithelia were subjected to histology for the evaluation of tissue morphology and cell proliferation. Barrier functions were investigated by measuring transepithelial electrical resistance (TEER).

We characterised HCK cells in the presence and absence of serum supplements and studied the functional capacity of this cell line to contract a collagen matrix as a result of the myofibroblast differentiation.

Three-dimensional hemicorneal models were constructed by tissue engineering methods using microporous membrane inserts. The resulting hemicornea comprised a stroma of keratocytes embedded into a collagen matrix covered by a multilayered epithelium.

To ascertain applicable endpoints we examined cytokine secretion profiles of corneal cell lines and hemicorneas by antibody array/ ELISA technology and monitored cell/ tissue viability using an MTT-based assay.

Results: Our results revealed that both morphology and barrier function of epithelial constructs were comparable to human *in vivo* corneas under serum-free culture conditions. As well, stromal embedded keratocytes retain their specific characteristics and show an inducible myofibroblast differentiation. By using hemicorneal models the MTT end-point has been shown to be applicable for toxicity testing of relevant reference substances.

Conclusions: Our findings support the biological relevance using immortalised human cell lines for the development of a full thickness corneal model for toxicity tests. Preliminary results revealed that the hemicorneal constructs are suitable models for safety testing of topically applied ocular drugs. However, further work is required to refine these measurements.

Keywords: safety testing, eye irritation, corneal model