Replacement of the Draize test by a new system: 'ex vivo eye irritation test' (EVEIT)

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Abstract

Objective: By a new construction of ex vivo corneal culture we try to simulate irritation, recovery and healing on cultured animal corneas to improve predictive results on chemicals for human eyes.
Methods: Rabbit corneas freshly prepared from abattoir are mounted in a perfusion system and pre-incubated for 32 hours. After this time we expose the corneas with a modified Low Volume Eye Irritation Test (LVET) or to mechanical abrasion. We monitor the vitality of the corneas by means of continuous glucose lactate measurements in medium and supernatants, microscopic and macroscopic examination of the erosion, endothelial damage and opacification.
Results: Each 16 corneas were exposed to abrasion, no touch or 2n NaOH for 20 sec. Corneas without any touch showed stable epithelium with small rough zones of 2 +- 2% of fluorescein positive staining. Defined corneal abrasions of 34 +- 3% healed within 5 days completely and expositions to sodium hydroxid resulted in persistent corneal erosion of 45 +- 12% . All 36 corneas showed considerable consumption of glucose and production of lactate. The supernatants showed less lactate in case of epithelial damage.
Conclusions: With the presented system we are able to simulate the two main criteria of eye irritation in animal experiments, the acute damage and its regeneration or healing. The system is close to the natural cornea and is ophthalmological evaluated and proofed to replace eye irritation in animals. Additional parameters of tissue repair and inflammation are available in the sampled media. Sponsored by COLIPA Brussels.

Introduction:

Replacing Draize test is one of the major efforts in alternative methods research in the testing of chemicals in the near future. Several items as corneal opacity, cell lysis and LDH release are early reactions that are not completely describing the living animals reactivity on corneal exposures. One major item: corneal healing and the recovery after an exposure should be described within a test replacing Draize test. From corneal culturing in the European eye banking system we know that maintenance of the cornea can be achieved. Our target was to develop an air lift system of in vitro stored rabbit corneas providing epithelial healing.

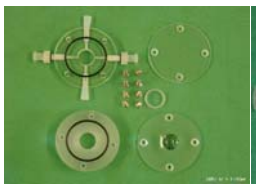


Fig. 1: Disassembled culture chamber

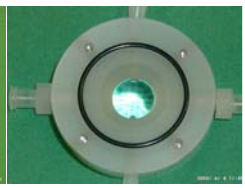


Fig. 2: Assembled culture chamber

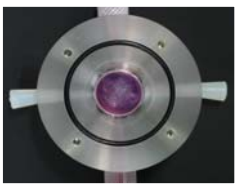


Fig. 3: Rabbit cornea mounted into the culture chamber

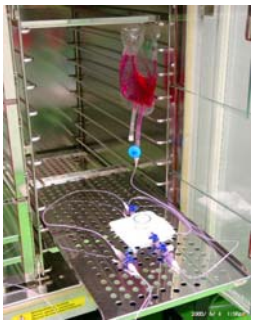


Fig. 4: Medium supply, tubing, mount of the rabbit cornea in the incubator

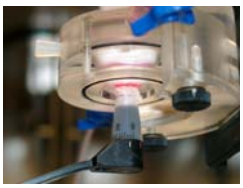


Fig. 5: Exposure to chemicals dlivered 10 ul within a contact lens being mounted on a gold-mann- tonometer head and with defined force exposed to the cornea mounted in the new culture chamber.

Materials and Methods:

Rabbit corneas, derived from an abattoire, were mounted into the newly developed culturing chambers as demonstrated in Fig. 1 and 2 and kept alive in an 32° C air incubator system with medium supply. The special MEM with Earls salts, antibiotics, and pH indicator is regulated to 10 ul per minute via an Ismatec micropump in the outflow line to keep the intracameral pressure securely at 10 cm water height. After pre-incubation of 36 hours to settle the cornea within the system the anterior surface is incubated with 1 ml of MEM which is removed after 5 minutes of incubation and called supernatant medium. Samples of the outflow line medium of the endothelial side of the cornea from the **A**rtificial-**A**nterior-**C**hamber-**M**edium (AACM) and from the **S**upernatant-**M**edium (SM) were analysed on glucose and lactate.
Exposures of the anterior surface were standardized with a special rotating brush to remove a precise 33 % diameter of the epithelium or with a 7.7 mm curvature plexiglas contact lens mounted on an upside down goldmann tonometer with a standardized force of 5 mmHg as shown in Fig. 4. The surfaces were photographed and the epithelial defect measured by planimetry directly and at day 3 and 5 after exposure.

Results:

Until the exposure is done stable pH +- 0.3 and glucose - lactate ratios between 0.5 and 0.65 are achieved. After exposure changes occur in pH explained by large epithelial defects with diffusion changes allowing bicarbonate to evaporate from the surface defect. Further severe changes of Glucose lactate ratio in the AACM were observed early but changes were statistically not significant at day 7. All corneas showed lactate producing tissue at the end of the experiment. Corneal erosio healed in the abrasio group and showed enlarging defects in the Tomadol groups. The Hydrogenperoxide and NaOH group enlarged day 0 to day 3 and healed in a certain extend from day 3 to day 5 (Fig. 8 and Fig. 9).

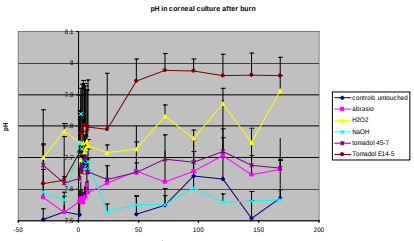


Fig. 6: pH in the AACM before and after exposure to abrasio and chemicals

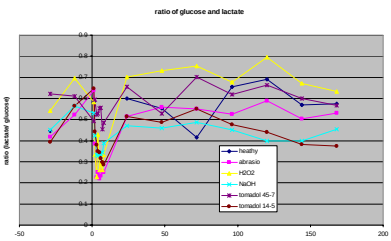
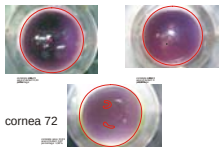
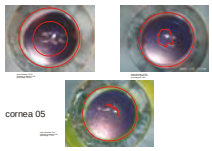


Fig. 7: glucose -lactate ratio in the AACM before and after exposure to abrasio and chemicals





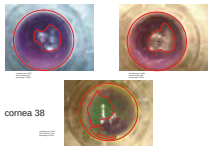
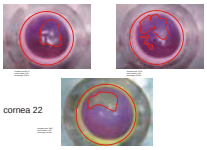
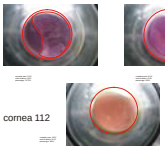


Fig. 8 a-c: epithelial defect and opacity on corneas at day 0, 3 and 5
a: control b: abrasio c: Hydrogenperoxide 3%





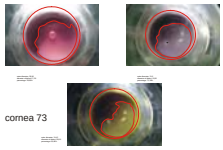


Fig. 8 d-f epithelial defect and opacity on corneas at day 0, 3 and 5
d: Tomadol E 47-7 e: Tomadol E 14-5 f: NaOH 2 mol

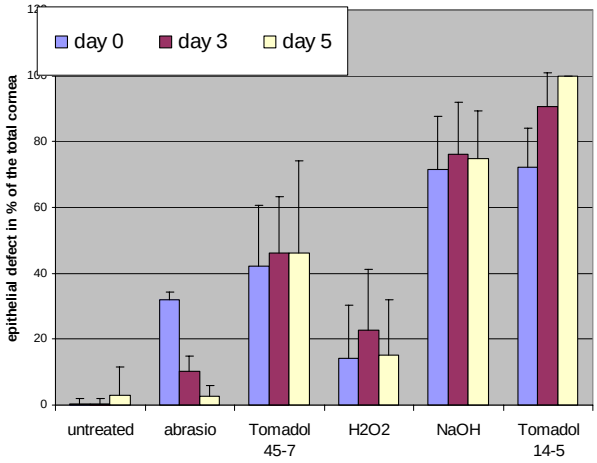


Fig. 9 Healing characteristics of the epithelial defect after different treatments of the corneas in culture. The controls untreated showed stable epithelium, the abrasion group healed and Tomadol 45-7 and NaOH showed stable defects whereas the ionic surfactant Tomadol E14-5 showed increasing epithelial defects.

Discussion:

The in vitro rabbit corneal culture model shows in vitro healing of a rabbit cornea after abrasio and healing patterns known from alive animals. We are able to monitor the biochemical turnover and to evaluate the corneal surface and stroma within this model so that we are convinced of being able to replace corneal exposure in animals by this test system.

For inflammatory mediators released by this system and the here described experiments see poster 226: Inflammation mediator detection in the 'ex vivo eye irritation test' (EVEIT) and oral presentation from N. Schrage et al. in the preceding workshop.