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Morphological changes and viability of primary cultured human ocular trabecular meshwork cells after exposure to air

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ABSTRACT

Purpose: To investigate the possible toxic effect of air exposure for an in vitro model of primary human ocular trabecular meshwork cells (HTM).
Methods: HTM were isolated from five donor eyes and cultivated at 37 °C. After reaching confluence the cells were seeded on two well chamber slides. The chamber slides were turned upside down in a Petri culture dish full of culture medium and filled with air using a 5 ml syringe, starting this way the exposure of the cells to the air. Subsequently they were placed in the incubation chamber at 37 °C. Six groups of HTM cultures were set up: group 1 consisted of samples in which HTM were exposed to air for 30 min, group 2 for 1 h, group 3 for 3 h, group 4 for 6 h, group 5 for 12 h and group 6 for 24 h.
Results: At 3 h after exposure, the morphology of the cells was still intact, at 6 h few cells appeared deformed and exhibited characteristics of more senescent cells. At 12 h after exposure to air the HTM cells started losing their typical morphology and appeared enlarged and compromised. Viability was superior to 94% in groups 1–3 while for groups 4, 5, 6 it was 82.7%, 39.5% and 12.7% respectively.
Conclusion: The toxic effect of air exposure for the studied in vitro model of HTM is not significant for the time period of one to three hours. However it starts reducing viability and alternating morphology 6 h after exposure until the time period of 24 h, where the percentage of living cells is drastically decreased. Therefore, we suggest that the use of an air bubble especially in glaucomatous patients should be applied with caution.

1. Introduction

The use of air in the anterior chamber of the eye during cataract operation is common among many cataract surgeons. Injection of a small air bubble in the anterior chamber after the intraocular lens implantation is used to prevent inflow of ocular surface fluid and consequently reduce the possibility of postoperative development of intraocular infection (Sim et al., 2007; Morteza et al., 2010).

Apart from phacoemulsification, the use of air in intraocular operations is significantly increased subsequent to the introduction of lamellar keratoplasty techniques. In Descemet's stripping endothelial keratoplasty (DSEK) and Descemet's membrane endothelial keratoplasty (DMEK) as well, the use of air is vital to support donor tissue adherence to the host cornea stroma and eliminate the need for sutures placement. For situations where graft dislocation after DSAEK (Price and Price, 2006) occurs, reinjection of air bubble is usually performed.

As for any material that is currently being used for intraocular operations, ophthalmologists are also alerted for the possible toxic effect of air for intraocular tissues. There are reports that show increased rate of cataract in phakic patients that underwent endothelial keratoplasty operation with the use of an air bubble (Price et al., 2010; Lee et al., 2009).

Several studies attempted to investigate the toxic effect of air for human cornea cells mostly in animal models (Foulks et al., 1987; Olson 1980; Kopsachilis et al., 2013a,b, Landry et al., 2011). The proposed mechanisms include the separation of cornea and aqueous humor and the mechanical pressure of the air bubble to the interior side of cornea (Kim et al., 1997; Hong et al., 2009).

Even though some studies are focusing on the toxic effect of air for the corneal endothelial cells, there is lack of similar studies for trabecular meshwork cells. The amount of air that is used in lamellar keratoplasty is large enough to affect these cells and an investigation of this interaction would be of great clinical interest. Should the toxicity of air be significant for trabecular cells, one

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could suggest alternative managements such as shorter exposure times.

The propagation of human ocular trabecular cells in culture allows the study of the structural and functional properties of this distinct cell type under reproducible experimental conditions. Human ocular trabecular cells can be effectively grown from dissected explants of trabecular tissue, and the cultured cells can maintain the distinctive morphological and functional features of uncultured trabecular cells (Kahook and Ammar, 2010; Tsaousis et al., 2013). The model of primary human cultured trabecular meshwork cells has been recently used in studies for exploration of their properties and their interactions with other factors (Lin et al., 2010; Kopsachilis et al., 2013a,b). Subsequently it seems to be an appropriate material for the investigation of air toxicity.

The purpose of this study is to investigate the toxic effect of air exposure for cultured primary ocular trabecular meshwork cells (HTM).

2. Materials and methods

2.1. Cultivation protocol

Cell culture was derived from five human donors (age 57 ± 12 years, post-mortem time 16 ± 8 days (Mean ± Standard deviation)) taken from our Cornea Bank, in accordance with the recommendations of the Declaration of Helsinki. HTM were grown by using a slightly modified protocol (17). Shortly, the eyes were placed in PBS at room temperature for 30 min on arrival and were cut into two parts through the ora serrata with a sterile scalpel. The lens and iris were removed from the anterior segment. The HTM were carefully isolated from the surrounding tissues (Epstein et al., 1997). The tissue was cut into small sections, and the cells were cultured at 37 °C in a 5% CO_2 atmosphere in Ham's F-10 medium with 10% fetal bovine serum, 2 mM L-glutamine, and 0.25 μg/mL gentamicin (Gibco). After 2 weeks, non-adherent cells were removed and trypsinized with 0.25% trypsin and 1 mM EDTA for 10 min. The cells were centrifuged and replated into Petri dishes. Confluent HTM of passage 3 were incubated for 24 h in serum-free Ham's F-10 medium and the medium was changed daily until the cells reached confluency.

After reaching confluency the cells were seeded (5×10^4 per well) on two well chamber slides.

2.2. Sample sorting

After reaching confluency in the two well chamber slides, the culture medium was carefully removed and the trabeculum meshwork cell sheet was gently rinsed with PBS. The chamber slides were turned upside down in a Petri culture dish full of culture medium and filled with air using a 5 ml syringe, (Fig. 1) thus starting the exposure of the cells to the air environment. The chamber slides were then put back in the cultivation chamber at optimal culture environment (37 °C and 5% CO^2). The time point of turning the chamber upside down and filling them with air was considered to be 0 min.

Six groups of HTM cultures were set up and 4 samples were enclosed in each group: group 1 consisted of samples in which HTM were exposed to air for 30 min. Group 2 consisted of HTM exposed to air for 1 h, group 3 for 3 h group 4 for 6 h group 5 for 12 h and group 6 for 24 h. All experiments were repeated three times with three different cell cultures from 3 different donors (see Table 1).

2.3. Cell culture evaluation

Cell viability and morphology were monitored at 30 min, 1 h, 3 h, 6 h, 12 h and 24 h. The confluent HTM were photographed, using an image analysis software (Cell^f; Olympus Soft Imaging Solutions GmbH, Münster, Germany). 4 samples in every group were randomly photographed and examined by 2 independent viewers. Four regions in each culture well, were selected randomly and cells within each area were evaluated at 200× magnification. The difference between the cell morphology measured by the two viewers was found to be not significant.

Microscopic examination was performed using an inverted microscope (Diavert, Leitz Wetzlar, Leica Germany) and images were recorded using a digital camera (F-View, Olympus Soft Imaging System).

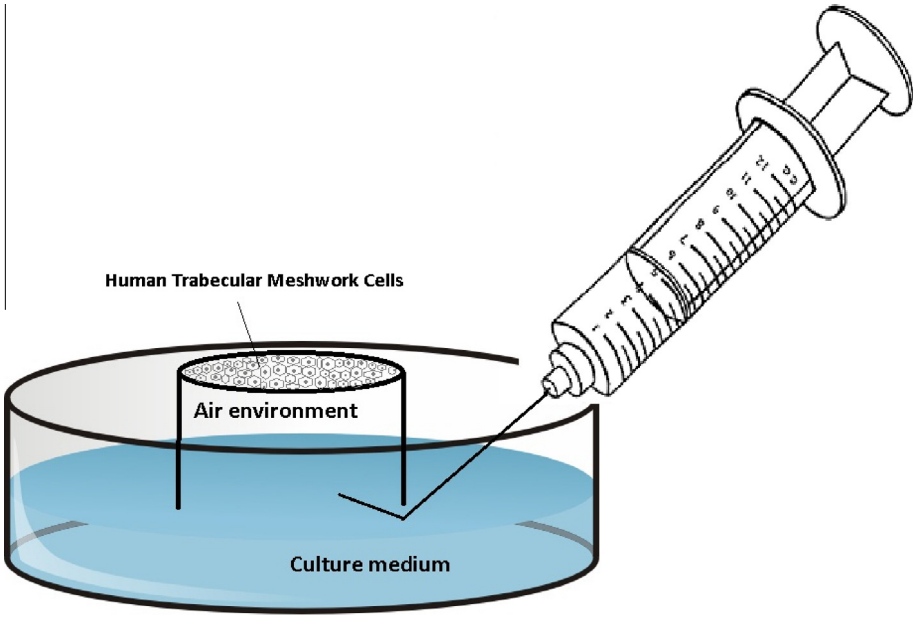

Fig. 1. Experimental setup for testing air toxicity on human ocular trabecular meshwork cells in an in vitro model. After reaching confluency in the two well chamber slides, the culture medium was carefully removed and the endothelial cell sheet was gently rinsed with PBS. The chamber slides were turned upside down in a Petri culture dish full of culture medium and filled with air using an empty syringe, starting this way the exposure of the cells to the air. The chamber slides were then put back in the cultivation chamber at optimal culture environment (37 °C and 5% CO^2).