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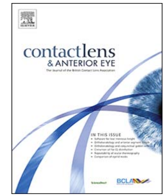

Dry eye following cataract surgery: The effect of light exposure using an in-vitro model

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A B S T R A C T

Purpose: Cataract surgery can lead to the temporary development or worsening of dry eye symptoms. Contributing factors may include corneal incisions, agents used before, during or after surgery, light from the operating microscope, disruption of ocular surface tissues and inflammatory processes. The purpose of this study was to observe the effect of light exposure on conjunctival fibroblasts in order to determine whether light has an effect on wound healing closure, assuming that operating microscopes might have an effect on the ocular surface.

Method: An in vitro scratch assay was performed on porcine conjunctival fibroblasts. Ten minutes of light exposure from a light microscope with halogen bulb was performed after the scratch assay. Fibroblasts were kept in culture for 48 hours post-exposure and the wound closure rates were visualized by live/dead staining. The fibroblasts which were exposed to light were compared to those without light exposure. Cell viability was also analysed by MTT assay.

Results: Slower wound closure rate was found when fibroblasts were exposed to light compared to the non-light exposed controls. Cell viability reduced by 20% with light exposure compared to controls in p3 cells ($p = 0.04$; however, the trend was not observed with p4 and p5 cells ($p > 0.05$).

Conclusions: These results suggest that light exposure might be one of the possible contributory factors for dry eye after ophthalmic surgery. Further evaluation of light effects should be carried out with different ocular surface cells.

1. Introduction

Ophthalmic surgery can potentially lead to temporary dry eye symptoms and it is one of the major reasons for patient discomfort after cataract surgery [1]. The underlying mechanism of dry eye disease (DED) following cataract surgery has been suggested to be multi-factorial, possibly associated with corneal nerve disruption and corneal epithelial defects due to the surgical incisions, intraoperative irrigation of the ocular surface, elevation of inflammatory mediators in the tear film, and active agents/preservatives in pre-, intra-, and post-operative medications, and anaesthetic agents [2]. Light exposure from the operating microscope might be also one of the factors leading to dry eye after cataract surgery, although the surgery time is typically 10–15 min nowadays and light exposure times may be shorter [3]. Photic changes to the ocular structures, through photo-thermal, photo-mechanical and photo-chemical mechanisms have been studied extensively and are well documented in the published literature [4–9]. However until now, there have been few studies investigating the effect of light exposure on the ocular surface tissues in-vitro and in-vivo [10]. The effect of the operating microscope on the rabbit's ocular surface was observed by Hwang et al. [10]. Their analysis of the ocular surface after light exposure showed decreased tear production, devitalized conjunctival and corneal cells, reduced goblet cell density, and an increase in IL-1β as one of the markers of inflammation. Although the rabbit eyes demonstrated a more aggressive inflammatory response compared to the human eye, these findings suggest that light might be a significant factor to consider in the possible aetiology of post-operative dry eye [11].

Porcine eyes have been previously used in research since they are readily available waste products of the food industry and are phylogenetically close to humans [12]. Moreover, sacrificing animals for studies has been criticised for ethical reasons. There are also studies

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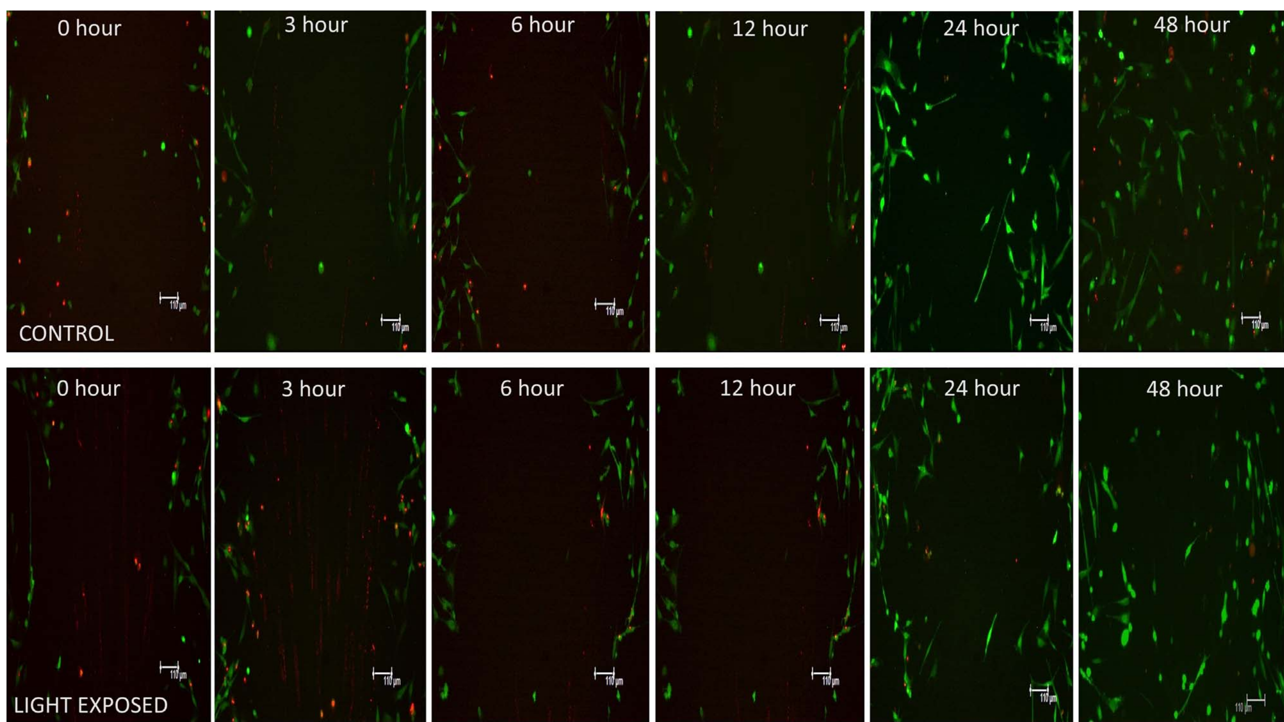

Fig. 1. Live-Dead staining images shown at different time points after the scratch was made. Top row labelled as control represents the row with the control images at different time points 0, 3, 6, 12, 24 and 48 h. Bottom row labelled as light exposed represents the row as the wound closure of cells after light exposure at 0, 3, 6, 12, 24 and 48 h.

with bovine, rabbit and porcine eyes, found that porcine eyes are the most similar to human anatomically [13].

Therefore, the effects of light on porcine conjunctival fibroblast have been assessed in an in-vitro scratch model to establish whether light might have an effect on conjunctival wound healing.

2. Materials and Methods

2.1. Cell culture

Porcine eyes were freshly (within 4 h of enucleation) taken from a local abattoir and transferred in the Dulbecco's modified Eagle's medium (DMEM; Lonza, UK) supplemented with 10% foetal bovine serum (FBS; F7524, Sigma-Aldrich, UK), 1% penicillin (10,000 units/ml) and streptomycin (10,000 µg/ml) (Lonza, UK), 1% L-glutamine (Lonza, UK) and 20% (w/v) Dextran ($M_w \sim 250 kDa$, Sigma-Aldrich, UK). The conjunctival pieces were cut from the porcine eyes and washed thoroughly with 3% pencillin/streptomycin solution. The tissue pieces were then transferred to cell culture flasks and cultured in Dulbecco's modified Eagle's medium containing 10% FBS, 1% penicillin/streptomycin and 1% L-glutamine until the migrated fibroblasts reached confluency.

All cultures were kept in an incubator at a constant 37° C, 5% CO_2 and 95% humidity. The medium of the cells was changed every three days. Porcine conjunctival fibroblasts were cultured from explants for 14 days until they reached confluency.

2.2. Scratch assay

Post-harvest, the fibroblasts were seeded into 96 well plates at 5×10^5 cells/well. The cells were cultured as described above until they reached 80% confluency, which took 48 h. At this point, a vertical scratch from the top to bottom of each well was created using a sterile 200 µl pipette tip. Immediately after scratching, the medium was changed to remove floating cells and debris created by scratching.

2.3. Light exposure and light intensity measurements

Post-scratching, the cells were exposed for 10 min to the light source of a microscope (AE200, Motic, Switzerland). Light intensity was measured with a lux-meter (TV335, Testboy, Germany) while the exposure was carried out.

The light intensity of an operating microscope used for ophthalmic surgery was measured as 40,000 ± 1000 lx. To be conservative, the light exposure from a laboratory light microscope was used which has one quarter of the intensity (around 10,000 ± 1000 lx) as measured with the same lux-meter.

Post-exposure, cells at passages 4 and 5 were kept in culture for an additional 48 h before termination of culture. Images were taken at 0-h, 3-h, 6-h, 12-h, 24-h and 48-h after the light exposure and also from control cells without any light exposure to assess the wound healing rate. Each condition was repeated a minimum of three times.

The viability of the conjunctival fibroblasts was visualised by using a Live/Dead viability kit (Invitrogen, UK) which labelled the dead cells red and the live cells green. Following the manufacturer's protocol, the fibroblasts were incubated with the recommended concentrations of calcein AM and ethidium homodimer (EthD-1) for 30 min in the dark. Post-incubation, the fibroblasts were washed with PBS and then imaged with fluorescence microscope (Leica, UK).

3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide (MTT; Sigma-Aldrich, UK) is a colorimetric assay which is an accurate indicator of cellular viability. MTT is a yellow solution which is converted to dark blue and water-insoluble MTT formazan by dehydrogenase enzymes in the mitochondria of living cells. The formazan crystal's absorbance is directly proportional to the number of metabolically active cells. The MTT assay was used according to the manufacturer's instructions. Briefly, the fibroblasts were incubated with 0.5 mg/ml MTT for two hours. The MTT solution was then aspirated and the cells were washed with PBS. 100 µl dimethyl sulfoxide (DMSO; Sigma-Aldrich, UK) was added to each well for one hour. The plate was then read on a plate reader (Biotek ELx800, UK) measuring absorbance with a wavelength of 570 nm.

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