

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

**Authors:**

Tugce Ipek<sup>1,2,3</sup>, Petra Hanga<sup>2</sup>, Andreas Hartwig<sup>1,2</sup>, James Wolffsohn<sup>2</sup>, Clare O'Donnell<sup>1, 2</sup>

**Author Affiliation:**

<sup>1</sup>Optegra Eye Sciences, One Didsbury Point, 2 The Ave, Manchester M20 2EY, UK

<sup>2</sup>Aston University, Aston Express Way, Birmingham B4 7ET, UK

<sup>3</sup>Complutense University of Madrid, Madrid, Spain

**Corresponding Author:**

Tugce Ipek [tugce.ipek@optegra.com](mailto:tugce.ipek@optegra.com)

Aston University, Aston Express Way, Birmingham B4 7ET, UK

**Abstract**

**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 48hours post-exposure and the wound closure rates were visualised 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 reduction was observed by 20% in the light

exposed compared to control in p3 cells however, the trend was not observed with p4 and p5 cells.

**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.

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**Conflicts of interest:** None

**Keywords:** Operating microscope, ocular surface, dry eye, conjunctival fibroblast, wound healing, cataract surgery

### Highlights

- The light from operating microscopes might have an effect on the ocular surface in and be a contributory factor in post-operative dry eye
- Wound healing rates may be reduced by light exposure
- Cell viability might be also affected by light exposure.

### 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 multifactorial, 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, anaesthetic agents.[2] Light exposure from the operating light microscope might be also one of the factors leading to dry eye after cataract surgery, although the surgery time is typically 10-15 minutes 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 light 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-1 $\beta$  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 animal for studies have been criticized for ethical reasons. There are also studies that worked with bovine, rabbit and porcine eyes at the same time, found that porcine eyes are the most similar to human anatomically. [13]

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

## Methods

### Cell Culture

Porcine eyes were freshly (within 4 hours 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  $\mu$ g/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% penicillin/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<sub>2</sub> 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.

### Scratch Assay

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

#### Light Exposure and Light Intensity Measurements

Post-scratching, the cells were exposed for 10 minutes 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 used for the experiment from the light microscope which was available to use in laboratory, was around  $10,000 \pm 1000$  lux. With the same lux-meter, the light intensity of an operating microscope used for ophthalmic surgery was measured as  $40,000 \pm 1000$  lux.

Post-exposure, cells at passages 4 and 5 were kept in culture for an additional 48 hours before termination of culture. Images were taken at 0-hour, 6-hour, 12-hour, 24-hour and 48 hours 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 minutes in the dark. Post-incubation, the fibroblasts were washed with PBS and then imaged on a 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  $\mu$ l 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 570nm.

The wound area was calculated by using Image J from each time point at 0-hour, 3-hour, 6-hour, 12-hour and 48-hour. The average of the mean area from each image was calculated at each time point for each condition, control (without light) and light exposed.

Statistical analysis was performed using Student-t Test.

## Results

Live/Dead assay results are indicated in Figure 1, taken by fluorescence microscopy at different time points after light exposure for a test and control sample. Figure 1 indicates the scratch closure over time when comparing light exposed cells to control. The closure rate was similar in test and control sample before the 24-hour time point, seen in Figure 1. However, the closure rate starts to become different after the 24-hour time point. The light exposed cells show a slower wound closing rate in comparison to the control condition

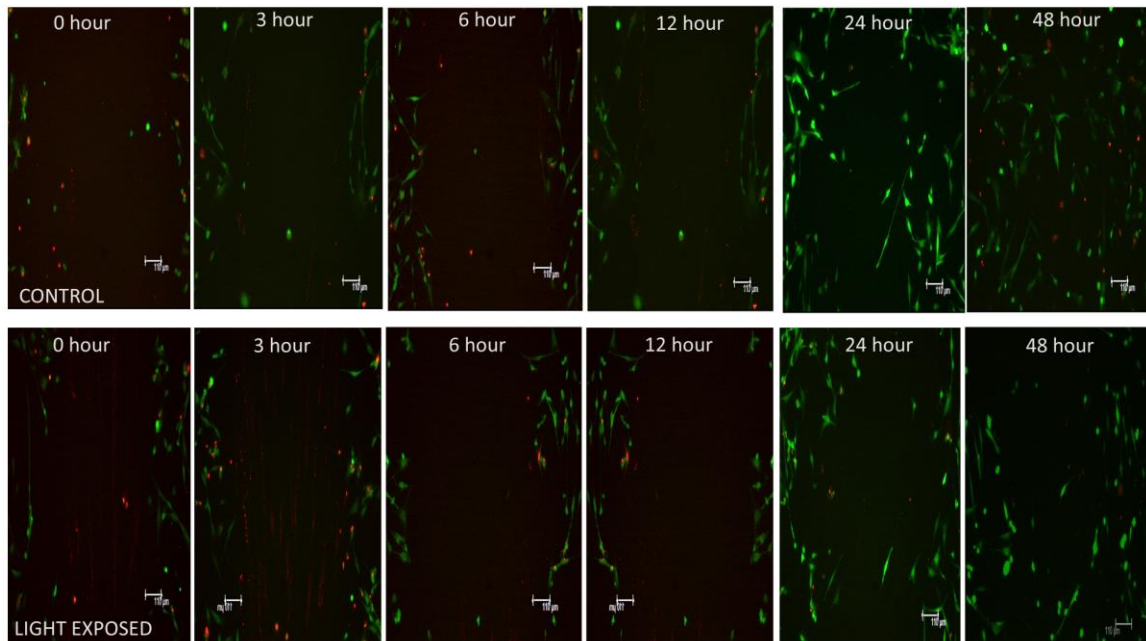


Figure 1. Live-Dead staining images shown at different time points after the scratch was made. Image labelled as control represent the row as control images at different time points as 3, 6, 12, 24 and 48 hours. Image labelled as light exposed represent the row as the light exposed wound closure of cells at 0, 3, 6, 12, 24 and 48 hours.

The MTT results are shown in Figure 2. This assay was performed 24 hours after light exposure. The absorbance of each sample was read at 570nm in the plate reader, and was normalised according to the mean of the control samples. The results in Figure 2 represents p3, p4 and p5 cells. A statistically difference in cell viability was only observed in p3 cells, however this difference was not significant for p4 and p5 cells.

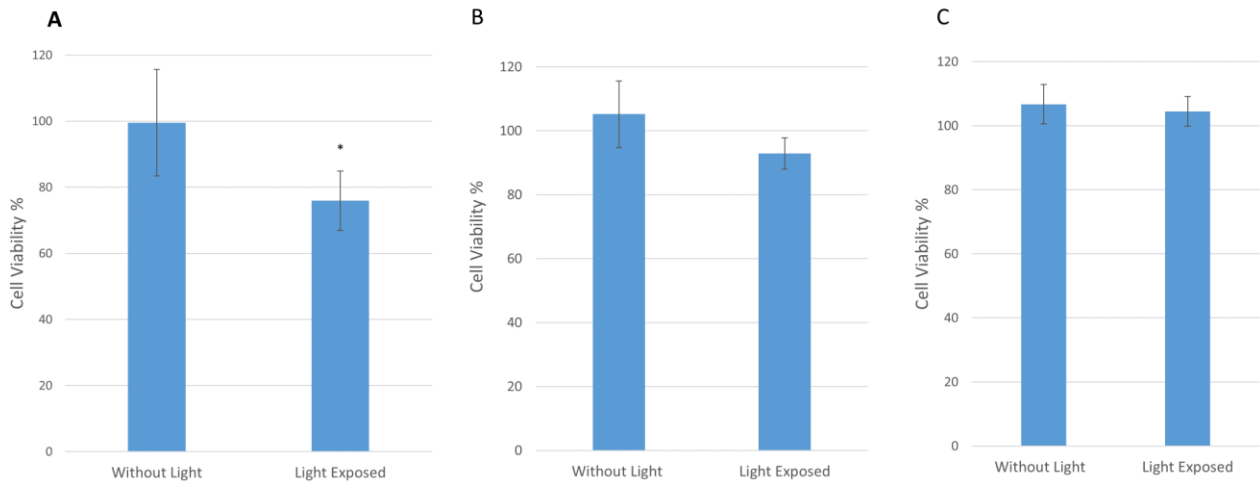


Figure 2. MTT results is shown for A) p3 cells and B) p4 and C) p5 cells, indicating the effect of light on cell viability. Cells in the light exposed group were exposed to light for 10 minutes and both groups were tested with MTT assay after 24 hours. All absorbance values were normalised to control. Data represent the percentage of mean viable cells. Student t-test was performed to compare the groups (\* $p < 0.05$ ;  $n = 3$ ).

Wound closure images were used to calculate the area of the wound using Image J at different time points; 0 hour, 3 hours, 6 hours, 12 hours, 24 hours after scratch both in light exposed cells and the cells without any light exposure, seen in Figure 3. A significant difference between light-exposed and control cells was found at the 24 hour time point.

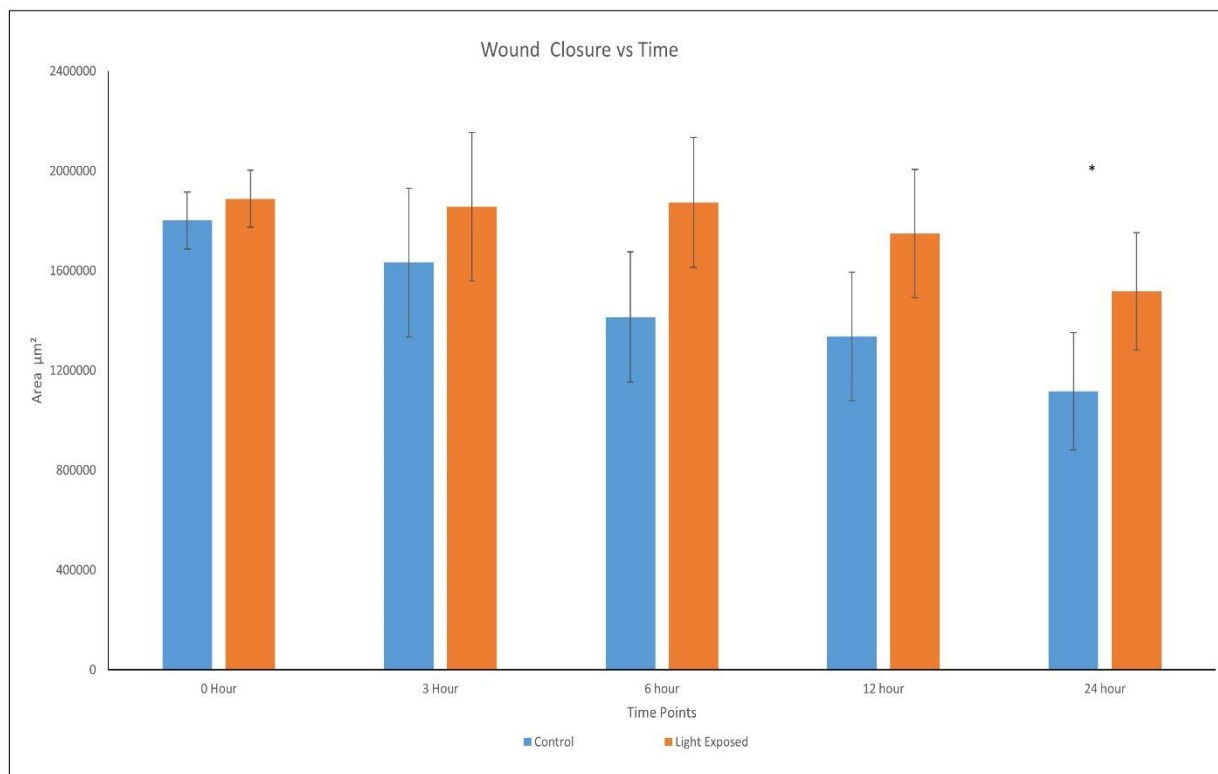


Figure 3. The wound area difference between the light exposed and control samples at different time points are shown. The average of the mean area for each time points for each conditions is shown (\*p<0.05).

### Discussion

The multifactorial origin of dry eye is still not fully understood.[14] Ophthalmic surgery may have the potential to temporarily induce or worsen dry eye conditions, typically during the short post-operative period.[1,15–17] Cataract surgery for instance, might lead to disruption of homeostasis of the ocular surface due to the speculum, the surgical incisions and the pre-operative and/or intra-operative agents used. Light exposure from the operating light microscope may also have an effect on the ocular surface, which is ‘stressed’ under the conditions of surgery, possibly contributing to the signs and symptoms of dry eye.

To our knowledge, there have been few studies investigating the effects of light of operating microscopes on the ocular surface [10]. It is not clear precisely how the ocular surface is affected by light exposure. Retinal damage due to light exposure is known to occur through photo-

mechanical, photo-thermal and photo-chemical mechanisms, photo-chemical damage being the most common mechanism of photic changes due to operating microscopes.[4,18] The photo-chemical disruption occurs from the generation of free radicals or reactive oxygen species which can disrupt the poly-unsaturated fatty acids located on cellular membranes.[4] Hwang and Kim [10] have recently shown the effects of the operating light microscope on the rabbits ocular surface and they also found an elevation of IL-1 $\beta$  levels in the rabbit's tears, which is thought to be due to the generation of reactive oxygen species as described above. IL- $\beta$  is known to be responsible for stimulating the production of reactive oxygen species.[19,20] The authors of this study suggest that the effect is most likely photo-chemical, however further research is required in order to confirm the actual mechanisms in vitro.

In the present study, it was shown that exposure to light from a microscope could potentially slow down the wound healing process. In this model created using a scratch assay, it was assumed that alterations to the conjunctival cells might occur during surgery perhaps due to the incisions and the speculum preventing blinking leading to cell desiccation. The results suggest that light exposure might slow down the wound healing and also might reduce the viability. This can be seen at different time points post-exposure to the light. The live/dead staining images show the differences in wound closure rate when comparing cells that have been exposed to light from the microscope and the controls (Figure 1). In addition, cell viability was reduced significantly when using earlier passage cells (p3), but not in p4 or p5 cells. This apparent inconsistency in cellular behaviour might be due to the aging of the cells with the increasing passage number. It has been known that cellular health declines and so may the cell proliferation rate, as the passage number of cells increases. [21,22] Therefore, the cell response to light exposure might not give the same result with higher passage number. The reduction of the wound area was found to be different with longer culture time between light exposed and control conditions, which might be due to the high passage number of cells which might need more time to respond to such treatment. Therefore, it would be useful to study the effects after longer culture time.

In order to understand the biological processes how the light might affect the cells, molecular studies such as gene expression, immunophenotypic analysis are required. A shortcoming of the present study is that the intensity of the light of the microscope used for the experiment is lower than that of microscopes used for ophthalmic surgery. The light intensity used in this experiment was around 10,000  $\pm$  1000 lux which was less than that of a typical operating light microscope



40,000 ± 1000 lux. Therefore, future studies should include the light exposure whose intensity is more similar to operating microscope.

Although, the exposure time is usually relatively short during cataract surgery nowadays (10 minutes) [3], steps might need to be taken to minimise light exposure during ocular surgery by reducing the light levels, minimising the duration of exposure and/or by using inbuilt safety filters.

The results of the present study suggest that light exposure potentially is a contributory factor for dry eye seen after ophthalmic surgery. In this study, only conjunctival fibroblasts were used; additional studies incorporating corneal cells would be helpful, since corneal cells also play a vital role in dry eye pathogenesis.[23–25]

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