



# Changes in corneal Langerhans cell density during the first few hours of contact lens wear



Yahya Alzahrani, Nicola Pritchard, Nathan Efron\*

Institute of Health and Biomedical Innovation, and School of Optometry and Vision Science, Queensland University of Technology, Kelvin Grove, Australia

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## ABSTRACT

**Purpose:** To determine the impact of contact lens wear on Langerhans cell density (LCD) in the central cornea over an 8 h period.

**Methods:** Ten participants wore a hydrogel lens in one eye (the experimental eye) for 8 h. The contralateral non-lens-wearing eye served as a control. The central cornea of each eye was examined at the level of the subbasal nerve plexus using a laser scanning corneal confocal microscope, at baseline (prior to lens wear), then every 2 h for 8 h.

**Results:** At baseline, LCD was  $18 \pm 19$  and  $20 \pm 19$  cells/mm<sup>2</sup> in the experimental and control eyes, respectively. In the experimental eye, LCD increased to  $36 \pm 32$  cells/mm<sup>2</sup> after 2 h and then decreased gradually to  $30 \pm 31$  cells/mm<sup>2</sup> after 6 h. LCD was greater in the experimental eye than the control eye at the 2, 4, 6 and 8 h time points ( $p < 0.05$ ). LCD remained constant in the control eye throughout the 8 h experiment.

**Conclusions:** LCD increases two-fold within the first 2 h of lens wear, indicating a rapid, sub-clinical inflammatory response to uncomplicated lens wear.

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## 1. Introduction

Langerhans cells constitute a major component of the ocular surface defence system. The appearance of these cells in the cornea has been described in the literature since the earliest microscopic examinations of the ocular surface [1]. Anterior eye disease results in activation of Langerhans cells in the cornea and conjunctiva as part of the immune process [2].

The laser scanning confocal microscope (LSCM) allows the observation of Langerhans cells in the cornea [3–5] and conjunctiva [6] as a result of contact lens wear, thus providing a signal for directly assessing subclinical and overt inflammatory responses to lens wear. In a cross-sectional study, Zhivov et al. [3] observed a greater number of Langerhans cells in the cornea of contact lens wearers versus non-wearers. Sindt et al. [4] noted significant differences in Langerhans cell density (LCD) in the cornea in response to various combinations of contact lenses and contact lens care systems.

Little is known of the time course of Langerhans cell recruitment into the cornea in response to contact lens wear. Characterising this phenomenon is essential for developing a

greater understanding of the inflammatory response to lens wear and for reconciling this mechanism against observed clinical signs and symptoms. The aim of this study was to determine the time course of change in Langerhans cell density in the cornea over an initial 8 h period of contact lens wear.

## 2. Methods

This was a prospective, controlled, observed-masked, randomised, non-dispensing evaluation of LCD in 10 participants who wore a contact lens in one eye while the contralateral eye served as a control. LCD was assessed in both eyes every 2 h for 8 h.

### 2.1. Participants

Ten healthy participants recruited from students and staff of the Queensland University of Technology (aged  $30 \pm 5$  years, range, 23–39 years) provided written informed consent before inclusion in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki and the Queensland University of Technology Human Research Ethics Committee provided ethics clearance.

Individuals with a history of corneal surgery or trauma, diabetes, blood pressure instability, current or long-term topical ocular medication, a history of contact lens wear for six months

\* Corresponding author.

E-mail address: [n.efron@qut.edu.au](mailto:n.efron@qut.edu.au) (N. Efron).

prior to the first examination, being pregnant or breastfeeding, taking oral contraceptives, or with symptoms of dry eye, were excluded.

A slit-lamp examination of the anterior ocular surface was performed before the study commenced to confirm the absence of any pathology.

## 2.2. Experimental protocol

At baseline, the following procedure was performed on each study participant. Prior to lens wear, images of the central cornea were captured using a LSCM (see details below). A lens was inserted into the eye preferred by the participant. At each subsequent 2 h time point over the next 8 h, the contact lens was removed from the test eye immediately prior to assessment of Langerhans cell density in both eyes using a LSCM (see below). A new contact lens of the same power (either  $-0.25$  D or  $+0.50$  D) was inserted immediately following measurement. The reason for inserting a new lens each time was to avoid contamination issues that could arise with reusing lenses. We estimate that on each measurement occasion, the lens was out of the eye for 5–10 min.; thus, the total period of actual lens wear over the 8 h elapsed time frame of the experiment was between 7 h 30 min and 7 h 45 min.

## 2.3. Contact lenses

All participants were fitted in one eye with a 'Biomedics® 1 day Extra' daily disposable soft contact lens (CooperVision, Pleasanton CA). A hydrogel lens was used in this experiment (the choice of brand was arbitrary) as this lens category will impart greater physiological stress, at least in terms of hypoxia, than a silicone hydrogel lens [7]. The Biomedics® 1 day Extra lenses are made from the hydrogel material 'ocufilcon D'. These lenses had a water content of 55%, diameter 14.2 mm, base curve 8.6 or 8.8 mm, centre thickness (at  $-3.00$  D) 0.07 mm, oxygen permeability (Dk)  $19 \times 10^{-11}$  cm<sup>2</sup> mlO<sub>2</sub>/s ml mmHg, oxygen transmissibility (Dk/t; at  $-3.00$  D)  $27 \times 10^{-9}$  cm mlO<sub>2</sub>/s ml mmHg and light blue handling tint.

## 2.4. Laser scanning confocal microscopy

The cornea was examined using a Heidelberg LSCM (HRT3) in combination with a Rostock Corneal Module (Heidelberg Engineering GmbH, Heidelberg, Germany).

A new disposable Pespex cap (Tomocap™) was used for each participant. The Tomocap™ was filled with GenTeal Gel (Novartis

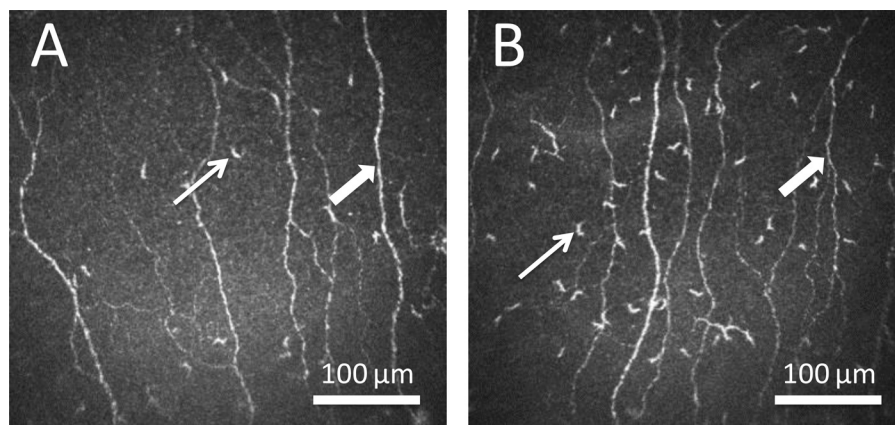
Pharmaceuticals Australia Pty Limited, North Ryde, NSW, Australia) prior to attachment to the Rostock Corneal Module in order to facilitate optical coupling between the objective lens and the back surface of the Tomocap™. An anaesthetic drop (0.4% oxybuprocaine hydrochloride; Chauvin Pharmaceuticals Ltd, UK) was instilled prior to examination.

The cornea was scanned with the head of the participant securely positioned in the chin and brow rest. The face of the Tomocap™ was brought into gentle contact with the central region of the cornea. When capturing images, the participant was advised to fixate on a target located directly in front of the contralateral eye. Accurate positioning of the Tomocap™ on the central region of the cornea was facilitated by a side-mounted CCD camera that transfers a magnified and real-time image onto a computer display screen. The Tomocap™ was moved slightly in a vertical and a horizontal movement with the instrument controls, within a region of about 1 mm<sup>2</sup>, so as to sample LCD in the central corneal region. The LSCM was focused at the level of the subbasal nerve plexus, approximately 60 μm from the epithelial surface, which is the known location of Langerhans cells in the cornea.

## 2.5. Image analysis

Approximately 100 digital images were captured during each examination. A pilot study revealed that the analysis of 5 images from the cornea provided a repeatability for determining LCD of a standard deviation of  $\pm 8$  cells/mm<sup>2</sup> and an intra-class correlation coefficient of 0.95. The first five high-quality images that were overlapping <20% were selected for analysis from a randomised listing (generated with the assistance of a random number generator) of the approximately 100 images captured on each measurement occasion.

The number of Langerhans cells was counted using the in-built general-utility manual counting tool of the Heidelberg instrument. This facility allows elements identified in the image as Langerhans cells, based on their distinctive morphological appearance, to be tagged and automatically counted. Data were expressed LCD, which is the mean of the number of cells per square millimetre observed in the images. The 'L-Method' was used to select which cells were included in the count, whereby those cells partially cut off at the superior and right hand borders of the field were included in the count. The mean LCD of the 5 images analysed at each measurement occasion was taken as the LCD. The operator undertaking image selection and analysis was masked with respect to the assigned group of participants (i.e. lens wearers or controls).



**Fig. 1.** Langerhans cells (thin arrows) imaged at the level of the subbasal nerve plexus of the cornea of a participant (a) at baseline (before lens wear) and (b) after 2 h lens wear. Nerve fibres (thick arrows) traverse each image.

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