A SEM-study of a keratoconus and an artificially aged human cornea

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Abstract. The substructure of both the epithelial and endothelial surfaces of a keratoconus and an artificially aged cornea was compared with that of a healthy cornea by investigating them with a scanning electron microscope.

From the depressions around the protruding centre of the epithelial surface of the keratoconus cornea, and from the whole epithelial surface of the artificially aged cornea, cells detached themselves, assuming a more or less rounded shape.

The endothelial surface of both the keratoconus and the aged cornea showed areas of cells with an almost completely disintegrated cell membrane, exposing the cell contents.

On the endothelial surface of the keratoconus cells were found with a missing cell-nucleus and a perforated cell membrane, due to a 'Kammerwasser Einbruch' effect.

Introduction

A keratoconus removed from the eye of a juvenile Dutch patient and a fresh human cornea, suitable for transplant purposes, were brought in for SEM-examination.

Accidently the fresh cornea was kept too long at too high a temperature (20–25 °C) in TC-199 culture medium prior to processing for the SEM. The cornea looked edematous on visual inspection after that period. Bacterial infection was initially considered to be the cause of the white aspect of this cornea. Observation of the possible changes at the corneal surfaces caused by this infection was the purpose of the examination of this cornea. Examination showed that there was no bacterial infection, but the initiation of an aging process.

The examiners were interested in comparing the substructure of the keratoconus, the artificially aged cornea and a fresh correctly prepared human cornea.

Materials and methods

A fresh human cornea, an artificially aged cornea (aging performed by keeping the cornea for 4 days in TC-199 medium at 20–25 °C) and a

* The work was carried out at the Centre for Medical Electron Microscopy.
keratoconus cornea were carefully washed in a 0.1 M sodium cacodylate buffer prior to fixation by immersion in a 2% glutardialdehyde solution in the same buffer solution for 24 hrs (pH 7.4, 20 °C). The specimens were then post-fixed in a 1% OsO₄ solution in the same buffer for 16 hrs (pH 7.4, 4 °C) and subsequently washed in buffer solution for 24 hrs (pH 7.4, 20 °C). The specimens were then post-fixed in a 1% OsO₄ solution in the same buffer for 16 hrs (pH 7.4, 4 °C) and subsequently washed in buffer solution and distilled water. After dehydration in a graded ethanol series up to 100% ethanol, the samples were critical-point dried in liq. CO₂ and mounted on rings in order to be able to examine both surfaces. Finally the samples were sputtercoated with Au (appr. 15 nm) on both sides and examined with a JEOL SEM, type 35C, operated at 25 kV.

Results

Epithelial surface (Figures 1–8)

a. Normal cornea. The epithelial surface of a normal healthy cornea consists of flat polygonal cells with numerous microvilli; the number of microvilli varies from cell to cell and is the cause of the contrast differences in the SEM-image (Figure 1a). At a higher magnification the variety in microvilli populations is clearly visible (Figure 1b); note the small holes or depressions in the cell surface, which probably are associated with cell renewal.

In the living state the cells are covered with a mucus layer, which can be seen in Figure 2. This layer is easily washed away in the preparation procedure for the SEM, when an inappropriate fixation technique is used. The mucus layer appears quite dark because of osmification.

b. Artificially aged cornea. At low magnification the epithelial surface is covered with a large number of more or less rounded cells (Figure 3a). At a higher magnification the depressions left by the detached cells are observable (Figure 3b); the cell borders look somewhat disrupted.

The process of detachment of the cells and the changing of their shape can be more clearly seen in Figure 4. Some of the partly detached cells have pseudopodia-like extensions.

c. Keratoconus cornea. At low magnification the epithelial surface shows a protruding centre bordered by depressions (Figure 5).

In these depressions the normal polygonal cell pattern seems quite disrupted (Figures 6a and 6b). Rounded cells, single flat cells and groups of polygonal cells are found (Figure 6b).

At higher magnification the variation in cell types is more pronounced (Figure 7). Somewhat rounded cells are found close to cells with many cilia and somewhat elongated epithelial cells.