Confocal microscopic evidence of increased Langerhans cell activity after corneal metal foreign body removal

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PURPOSE. *The purpose of the study was to examine the corneal inflammatory reaction and Langerhans cells with confocal microscopy after metal foreign body removal.*
METHODS. *Corneal metal foreign body was removed from 9 eyes of 9 consecutive patients 12.1±13.6 (4 to 72) hours after superficial angle grinder injury. Both eyes were examined with the Heidelberg Retina Tomograph II (HRT II) Rostock Cornea Module. Morphology and density of epithelium, nerves, metal deposits, keratocytes, endothelium, and Langerhans cells were compared to the uninjured fellow eyes (controls).*
RESULTS. *Irregularity and partially missing superficial epithelium was found in all cases. Around the area of injury prolonged basal and wing epithelial cells were found in all eyes. The basal epithelium density is lower than in the control eye (p=0.043). Density of Langerhans cells (68.1±24.2/mm^2) was increased in the epithelium, compared to controls (35.2±21.8/mm^2, p=0.012). Keratocyte and endothelium densities were not different from that of controls. Some keratocytes showed signs of activation and the inhomogeneous background reflectivity revealed extracellular matrix alterations. Inflammatory reaction was observed up to 260 µm depth. The metal foreign body particles had high reflectivity and irregular edge.*
CONCLUSIONS. *In vivo confocal microscopy provided additional information to biomicroscopic signs such as epithelial damage and inflammation. It showed the effects of metal foreign bodies in the cornea: nerve damage and Langerhans cell density increase. Langerhans cells seem to play an important role in the inflammatory response after corneal foreign body injuries. (Eur J Ophthalmol 2008; 18: 703-7)*

KEY WORDS. *Langerhans cell, Confocal microscopy, Cornea, Foreign body, HRT Cornea module, Injury*

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NTRODUCTION

Corneal foreign body is the most common occupational ocular injury, which comprises 35–58% of all ocular trauma (1, 2). In more than 70% of the cases foreign body originates from metal grinding or cutting activities (3). Foreign body removal accounts for about 40% of interventions in outpatient ophthalmology units (4).
In vivo confocal microscopy is a reliable and reproducible method to examine the cellular changes of the cornea (5). Several systematic in vivo studies have assessed normal and pathologic corneas (6-8). Clinically, angle grinder superficial corneal injury does not lead to severe corneal haze indicating keratoplasty, which is why little is known about the microstructural changes after these forms of injuries. Heidelberg Retina Tomograph II (HRT II) and Rostock Cornea Module (RCM) in vivo confocal microscope was introduced by Stave et al in 2002 (9). A recent study (10) on healthy corneas demonstrated the distribution and subtypes of Langerhans cells, which are dendritic leuko-

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cytes residing mainly within stratified squamous epithelia of cornea, skin, and mucosa and participating in antigen presentation (11, 12).

The purpose of our study was the quantitative evaluation of corneas after metal foreign body removal. Investigation was focused on the Langerhans cells, since metal antibodies are supposed to activate the antigen presenting cell-mediated immune response.

METHODS

Foreign body removal

Nine eyes of nine consecutive patients (age: 31.5±12.4 years) with corneal metal foreign bodies were enrolled in the study after giving informed consent. All cases were classified according to the Birmingham Eye Trauma Terminology (13) as closed globe trauma (lamellar laceration in Zone I). According to the anamnestic data, injury was associated with angle grinder activity without using protective device. Radial distance of the foreign body from the limbus was measured using the scale of the slit lamp. Topical anesthetic drops (three times 0.4% oxybuprocaine–Humacain 0.4%, Human Pharmaceuticals, Gödöllö) and 5% povidone iodine were given. Foreign bodies were lifted out with an 18-G needle under the slit lamp 12.1±13.6 (4 to 72) hours after the injury. Patients unable to provide exact data about the time of injury were excluded. Topical antibiotic drops (tobramycin, Tobrex, Alcon Co.) were given three times daily for 5 days. In the study we followed the tenets of the Declaration of Helsinki.

In vivo confocal microscopy

Without further topical anesthesia, the injured area of the cornea was examined with the HRT II RCM (Heidelberg Engineering Inc., Heidelberg, Germany). A drop of carbomer gel (Vidisic; Dr. Mann Pharma, Berlin, Germany) served as coupling medium. Morphology and density of basal epithelium, keratocytes of the anterior and posterior stroma, and endothelium was calculated according to previous studies (5, 14). Image size was 400 x 400 µm. Subbasal nerves, metal deposits, and Langerhans cells were investigated according to Zhivov et al (6). After one drop of the same anesthetic, central cornea of the uninjured fellow eyes was examined (controls). In addition, the removed foreign body particles were examined by immersing them into the carbomer gel.

Statistics

Statistical analysis applying SPSS software (version 13.0 for Windows; SPSS Inc., Chicago, IL, USA) was performed using the Mann-Whitney nonparametric test. Differences were regarded as significant for $p<0.05$ (95% CI).

RESULTS

Confocal microscopy could be performed in all cases; no patient complained about significant discomfort. Results of statistical analysis are summarized in Table I. Corneal foreign bodies were found 4.5±2.8 mm (1.8 to 6.1 mm) radially from the limbus.

Superficial epithelium was partially missing in the area examined. Residual superficial epithelial cells were highly reflective and irregular. Around the area of injury, prolonged basal and wing epithelial cells were found in all eyes. Density of basal epithelial cells was significantly less than in controls ($p=0.043$). Highly reflective cells measuring 1–2 µm detected among the basal epithelial cells were considered as leucocytes (Fig. 1A). Basal cell count could not be performed in three of nine cases in the area of foreign body removal due to missing or damaged basal epithelial cell layer. In these cases the area adjacent to the injury zone was investigated.

Subepithelial nerve plexus were partially missing, nerve fiber density was not exactly measurable. In areas present, nerves were fragmented with irregular structure (Fig. 1B).

Langerhans cells were identified in every injured cornea, and only in three out of nine controls ($p=0.009$, Fisher exact test). The density of Langerhans cells in injured corneas was increased ($p=0.012$) compared to the controls. Langerhans cells were present mostly in the basal epithelial layer (Fig. 1C), but where epithelium was missing, Langerhans cells could be identified in the anterior stroma as well. The exact depth of Langerhans cell localization was not exactly measurable because of epithelial defect.

In the stroma, the reflectivity and density of the keratocytes was not different from that in controls (Tab. I). Some keratocytes showed signs of activation (larger size, round shape, higher reflectivity) and the inhomogeneous back-

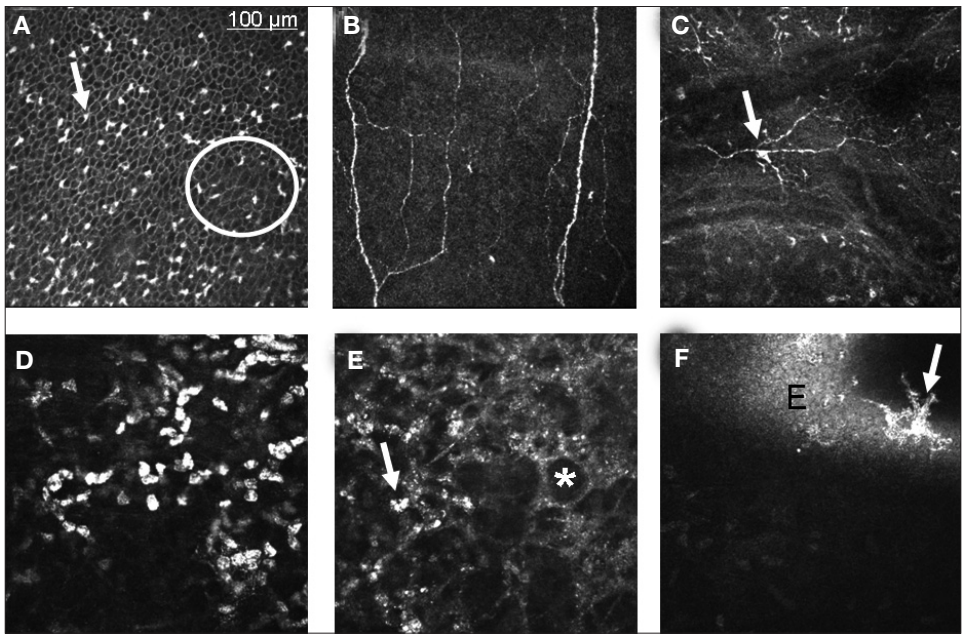

Fig. 1 - *Confocal microscopy images after metal foreign body removal. Bar represents 100 µm.* **(A)** *Basal epithelial cells are elongated in some areas (arrowhead). Among epithelial cells highly reflective cells, probably leukocytes, are observable (arrow).* **(B)** *Subepithelial nerves are fragmented or missing.* **(C)** *Langerhans cells with dendrites in the anterior stroma (arrow), in 58 µm depth from the surface.* **(D)** *Cellular reaction in the anterior stroma 180 µm from the surface: round shaped cells representing activated keratocytes (nuclei) and some lymphocytes.* **(E)** *Corneal edema. Note the round shaped cysts in the stroma (asterisk), and the cellular debris (arrow). The background reflectivity of the stroma is irregular and increased.* **(F)** *Large precipitate (arrow) is adhering to the otherwise intact endothelium (E).*

ground reflectivity revealed extracellular matrix alterations. In the anterior stroma among keratocytes inflammatory cells could be identified (Fig. 1D). Inflammatory reaction comprised up to 260 µm depth. In four out of nine cases confocal microscopy revealed significant stromal edema (Fig. 1E).

Endothelium was not different from controls; however, in one case, when foreign body was removed 72 hours after injury, some precipitates were found on the endothelium (Fig. 1F).

The removed metal foreign body particles had irregular, sharp edges (Fig. 2). The reflectivity was high, and similar to the intracorneal iron deposits. Foreign bodies were surrounded by corneal debris. The inner structure was not visible by this method, because of high superficial reflectivity of the foreign body.

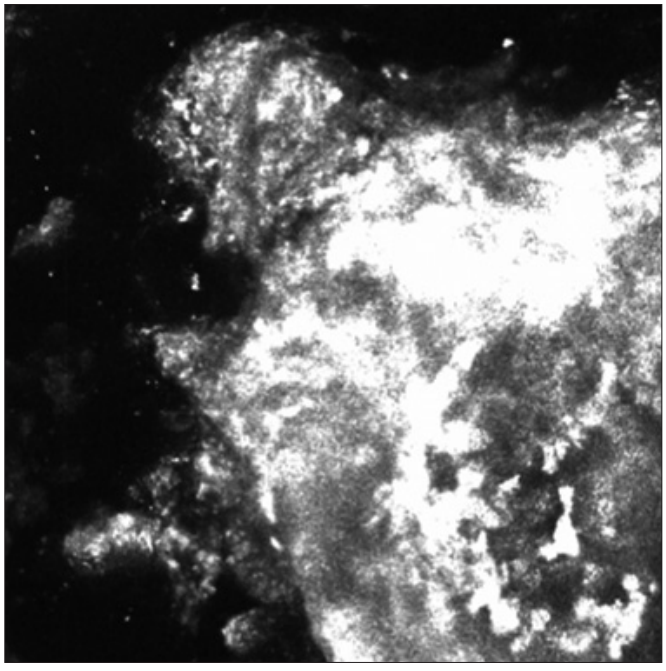

Fig. 2 - *Structure of the removed metal foreign body by confocal microscopy. Iron is highly reflective, similar to the intracorneal iron deposits. Edges of the foreign body are sharp, and surrounded by corneal debris. The inner structure is not visible.*

DISCUSSION

In vivo confocal microscopy was applicable immediately after corneal foreign body removal from the cornea. Our findings showed the effects of metal foreign bodies in the cornea providing additional data compared to slit lamp examination.

According to our results we can state that such an injury induces epithelial damage with signs of regeneration, and inflammatory reaction displaying leukocyte and Langerhans cell density increase. Traditionally, visualization of Langerhans cells required the use of electron microscopy for identifying the unique trilaminar cytoplasmic organelles (the Langerhans cell granules [15] or Birbeck

TABLE I - SUMMARY OF CELL DENSITIES (MEAN±SD) IN THE INJURED (STUDY) EYE COMPARED TO THE FELLOW EYE

Cell density (cells/mm^2)	Injured (study) eye	Fellow (control) eye	p (Mann-Whitney test)
Basal epithelium	3625 ± 458	3852 ± 249	0.043
Keratocyte–anterior stroma	492 ± 54	524 ± 64	0.089
Keratocyte–posterior stroma	521 ± 49	452 ± 95	0.235
Endothelium	2514 ± 321	2609 ± 197	0.341
Langerhans cell	68.1 ± 24.2	35.2 ± 21.8*	0.012

n=9; *n=3. 95% CI

granules). Later, ATPase histochemistry and the expression of major histocompatibility complex class II molecules (11) made it possible to identify them.

Recently, Zhivov et al (6)—with confocal corneal microscopy—differentiated several types of Langerhans cells in normal corneas such as a) individual cell bodies without processes, b) cells bearing dendrites, and c) cells arranged in a network via long interdigitating dendrites. In our cases most of the Langerhans cells were of type b and c. The possible explanation of increased Langerhans density is the large amount of corneal metal deposition and oxidation. Foreign bodies from the angle grinder have high temperature, and oxidation in the tear film and cornea starts immediately after contact. The Langerhans cells seem to react fast to the iron antigens and activate the immune response. Healthy corneas also contain Langerhans cells, but after foreign body injury, the density increases. Langerhans cell density increase can also be explained by migration activity, described by Auran et al (16). Zhivov et al (10) demonstrated that chronic mechanical irritation of the cornea such as contact lens wear can change Langerhans cell density. Langerhans cells were found both in the center and the periphery of the cornea without difference in distribution between healthy volunteers and contact lens wearers. However, contact lens wearers revealed almost twofold higher Langerhans cell densities in both locations. A corneal foreign body is suspected to cause similar mechanical irritation, but in a smaller area.

Nerve regeneration is essential in the stability of the epithelium, as presented in keratoconus (17). Müller et al provided a detailed description of the corneal nerves' morphology, function, and distribution (18). We postulate that in metal foreign body injury not only the mechanical trauma but the locally elevated iron concentration may lead to the observed irregular and fragmented condition of the corneal nerves, such as the biochemical neurotoxic effect of commercial pepper spray (19). The reduced density of basal epithelial cells also could be explained by toxic reaction.

Our study has limitations, i.e., the exact effects of foreign body injury and removal are not clearly distinguishable, and the impact of topographic difference of Langerhans cell distribution in the cornea was not taken into account. However, confocal microscopy in the presence of intracorneal foreign body is not applicable because of the total reflectivity of the foreign body, and also is ethically questionable. Furthermore, the current pilot study does not provide information about the follow-up of the cases; a prospective study is planned based on the observations obtained in this study.

We conclude that by confocal microscopy additional information could be obtained to the conventional slit lamp examination. Beyond biomicroscopic examination, the nerve damage and Langerhans cell activation could be detected. Nerve damage can explain the delayed epithelial healing observed in some patients after corneal foreign body removal. Langerhans cells seem to play an important role in the inflammatory response after intracorneal foreign body injuries.

None of the authors has financial or other interest in any of the materials or instruments used in the study.

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