

EFFECT OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID) ON THE RABBIT CORNEAL EPITHELIUM STUDIED BY SCANNING ELECTRON MICROSCOPY

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SAMENVATTING:

Het doel van deze studie is het effect van NSAID collyria te evalueren op het cornea-epitheel van de ogen van 12 Nieuw-Zeeland konijnen.

Gedurende 1 week werden 12 Nieuw-Zeeland konijnen 5 maal per dag ingedruppeld met 6 gecommmercialiseerde NSAID collyria. Elk collyrium werd gebruikt in 4 ogen. We onderzochten ook 2 controle cornea's die niet ingedruppeld werden. Het epitheel werd geëvalueerd door een scanning electronen microscoop en de veranderingen werden gegradeerd met een empirisch score-systeem. Niet alleen het actief ingrediënt maar ook de bewaarmiddelen en een lage pH-waarde zijn belangrijke factoren van celbeschadiging.

ABSTRACT:

We investigated the effect of 6 commercially available non-steroidal anti-inflammatory drug (NSAID) eye drops on the normal corneal epithelium of rabbits. Each drug was instilled into both eyes of 2 rabbits, 5 times a day, for 5 consecutive days. Two additional corneas of one rabbit, without any treatment, served as control. After treatment, the corneas were excised and processed for scanning electron microscopic evaluation. The epithelial changes induced by the drugs were graded by an empirical score system. All test compounds caused alterations

in the cell membranes and surface microvilli, or even exfoliation and necrosis of surface cells. The extent of cell damage appeared to be related to the active ingredient in the eye drops, the pH of the solution, and the constituents of the vehicle, especially the type of preservative used.

RESUMÉ:

Le but de cette étude est d'évaluer l'effet des gouttes NSAID sur l'épithélium cornéen des yeux de 12 lapins Néo-Zélandais. Pendant une semaine les 12 lapins Néo-Zélandais ont été instillés 5 fois par jour avec les 6 collyres NSAID commercialisés. Chaque collyre a été utilisé dans 4 yeux. Nous avons examiné également 2 cornées, sans traitement. L'épithélium a été évalué par microscopie électronique à balayage et les changements ont été gradués suivant un système de score empirique. Non seulement l'ingrédient actif mais aussi les produits de conservation et un pH très bas sont des facteurs importants de destruction cellulaire.

ZUSAMENFASSUNG:

Das Ziel der Studie ist der Effekt von NSAID Kollyria zu untersuchen auf das Kornea-Epithel der Augen von 12 Neuseeländer Kaninchen.

Während eine Woche würden diese Kaninchen fünfmal täglich mit den 6 kommerzialisierten NSAID Kollyria getropft. Jeder Kollyrium würde in 4 Augen benutzt. Wir untersuchten auch 2 Kontrolle Cornea die nicht behandelt würden. Das Epithel würde mit einem scanning-electronen-Mikroskop evaluiert und die Änderungen würden abgestuft mit einem empirischem Punktesystem. Nicht nur das aktive Be-

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standteil aber auch die Bewahrmittel und eine niedrige pH-Wert sind wichtige ursächliche Faktoren von Zellschädigung.

KEY WORDS:

NSAID eye drops, corneal epithelium, scanning electron microscope.

MOTS CLÉS:

Collyres NSAID, épithélium cornéen, microscope électronique à balayage.

INTRODUCTION:

Non-steroidal anti-inflammatory drugs (NSAIDs) exert their effect by virtue of cyclo-oxygenase inhibition in the arachidonic acid cascade (11, 22). Of these compounds, diclofenac sodium is also known to interfere with the lipoxegenase pathway (10). Moreover, due to their additional analgesic effect, (1, 5, 21) these drugs are widely used to manage posttraumatic or postoperative pain and pain associated with the musculoskeletal disorders. Since NSAIDs inhibit prostaglandin synthesis, they are equally recommended to treat allergic ocular disorders (12, 23). Several ophthalmic formulations of NSAIDs are marketed for topical use to manage pain and inflammation. Corticosteroids, which exert a profound anti-inflammatory effect, have no direct influence on pain. In contrast to the corticosteroids, NSAIDs do not cause raised ocular tension, secondary cataract formation or reactivation of herpes simplex virus infection upon application to the eye. NSAIDs are especially prescribed to manage pain after refractive laser surgery (2, 3). They are helpful in maintaining mydriasis during cataract surgery (19).

Chronic oral ingestion of NSAIDs for analgesic, anti-inflammatory, and antithrombotic indications is associated with upper gastrointestinal tract lesions ranging from petechiae and superficial erosions to chronic peptic ulcers, oesophagitis, and gut ulcers which may perforate or cause gastrointestinal bleeding (9). The NSAIDs have been shown to inhibit the proliferation of mucosal cells that normally leads to healing of gastric and duodenal ulcers (13, 14). Although diclofenac does not retard the healing of experimental epithelial erosions (15), nothing is known about the effect of NSAIDs on the normal corneal epithelium. Since the ophthalmic antibiotics and corticosteroid eye drops have been shown to affect corneal epithelium (18), we investigated the effect of NSAID eye drops on the normal corneal epithelium of rabbits *in vivo*.

MATERIALS AND METHODS:

Six commercially available NSAID eye drops, approved for clinical use in Belgium, were test-

Table 1. Details of the active ingredients, preservatives and pH of the NSAID eye drops tested.

Eye Drops	active ingredient	pH	Preservatives
Aculare®	Ketorolac-thromethamine	7.4	Benz.chloride / Sodiumedetate
Indocollyre®	Indomethacin 1mg/ml	6.7 - 7.3	Sodiumedetate/ Methylparahydroxybenzoate
Indoptol®	Indomethacin 10 mg/ml	5	Benz.chloride / Sodiumedetate / Benzylalcohol
Ocuflur®	Sodiumflurbiprofen	6.4	Sodiumedetate / Polyvinylalcohol / Thiomersal
Pranox®	Pranoprofen	7.6	Benz.chloride / Sodiumedetate
Voltaren®	Sodiumdiclofenac	6.8 - 7.2	Thiomersal

ed in the normal rabbit eyes. The eye drops either contained indomethacin, ketorolac thromethamine, flurbiprofen, pranoprofen or sodium diclofenac (Table 1). The pH of ophthalmic solutions varied, and the preservatives and constituents of the vehicle were also different.

Before commencing treatment, the eyes of 13 New Zealand rabbits were examined using fluoresceine sodium 1% ophthalmic solution and blue light of a slitlamp to exclude any surface damage or scarring. Each ophthalmic formulation of the NSAID eye drops was instilled into both eyes of two rabbits. The eye drops were applied 5 times a day for one week. Two eyes of one rabbit were used as untreated control. On day 7, the rabbits were sacrificed and the eyes were flooded with cold buffered glutaraldehyde solution before enucleation. The anterior segments of the eyes were excised and further processed for scanning electron microscopy, according to a technique described in detail elsewhere (17).

The corneal surface damage by scanning electron microscopy was assessed in the peripheral cornea, midperipheral cornea and the central cornea according to the following criteria:

1. Number of dark, grey and bright cells (cell brightness).
2. Number of craters on the cell surface.
3. Change in the hexagonal form of the cells
4. Alterations in the microvilli and microplicae
5. Cell membrane alterations
6. Extent of cell retraction and exfoliation

The damage under each of the above criteria was further graded by an empirical score system from 0 to 5, where grade 0 represented a normal cornea and grade 5 denoted severe damage to the corneal epithelium. Under each criterion the scores from 4 corneas treated with

the same drug were added and compared with the scores of the normal corneas and the corneas treated with other NSAID eye drops.

RESULTS.

The appearance of the epithelium of untreated corneas was similar to that described in the literature (6, 7).

All NSAID eye drops tested caused changes in the epithelial cells. The cumulative empirical scores of cell damage in the peripheral, mid-peripheral and central corneas are shown in Figs.1, 2, and 3 respectively. It is apparent that the severity of damage increases progressively from the peripheral to the central corneas.

The differences of cell damage inflicted under each evaluation criterion by different NSAID eye drops were as follows:

1. Brightness of cells (Figs. 4 and 5):

Normal corneas show a few scattered dark cells. These cells have a markedly decreased number of microvilli on the surface. There are a moderate number of grey cells, whereas the majority of cells are bright cells.

Scanning electron microscopy revealed an increased number of dark cells on the corneal surface in all treated corneas. In Indocollyre® treated corneas the entire corneal surface showed a minimum number of dark cells. Compared to other NSAID eye drops, Pranox® and Voltaren® treated eyes had a higher number of bright cells in the peripheral cornea. Except in the case of Indocollyre®, the extent of change to the dark and grey cells was almost equal in the central corneas treated with Aculare®, Ocuflur®, Pranox®, Indoptol® and Voltaren®.

2. Craters:

Craters are somewhat rounded dark pit-like struc-

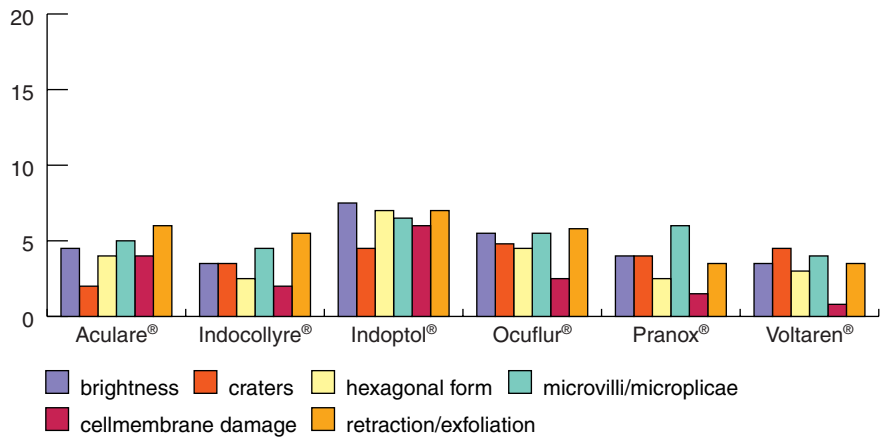


Fig. 1. Severity of cellular damage in the peripheral corneas by different NSAID eye drops. Indocollyre®, Pranox® and Voltaren® cause less damage than other drugs.

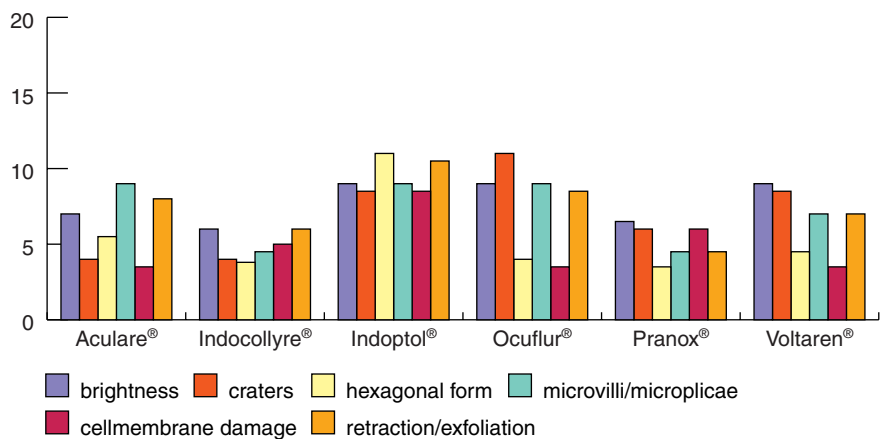


Fig. 2. Severity of damage is more pronounced in the midperipheral area. Indocollyre® and Pranox® seem to be the least toxic.

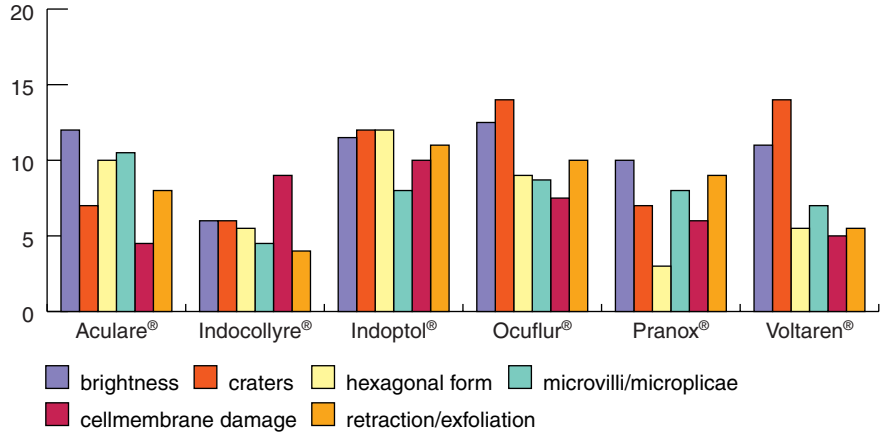


Fig. 3. Cell damage was more severe in the central corneas. Except for an increase in cell membrane alterations, Indocollyre® did not enhance the severity of damage under other evaluation criteria.

tures surrounded by bright elevated margins. They are thought to be produced by bursting of intracellular vesicles on the surface. Normal corneas possess scattered cells with surface craters. Ocuflur[®], Indoptol[®] and Voltaren[®] caused the development of craters in large numbers (Fig. 6).

3. Hexagonal form:

Normal epithelial cells have a hexagonal form. Affected epithelial cells tend to lose the hexagonal pattern. Simultaneously, these cells become darker in appearance (Figs. 4 & 5). Indoptol[®] treated eyes revealed a general tendency of cells to lose their polygonal form. In the central parts of the Aculare[®] and Ocuflur[®] treated eyes the hexagonal form had totally disappeared.

4. Microvilli and microplicae:

The grey and bright cells in the normal corneal epithelium possess abundant microvilli and microplicae on the surface. The brightness of the cells depends on the number and size of the microvilli and microplicae (Figs. 7 and 8). Ocuflur[®], Indoptol[®] and Aculare[®] produced the most alterations of microvilli and microplicae. These alterations included either an increased number of microplicae (Fig 7), or sparsely distributed small globular microvilli or lack of microvilli (Fig.8).

5. Cell membrane alterations:

Changes in the cell membrane included the cell surface roughness (Fig. 9), small and large surface projections, cell wrinkling (Fig. 10), and deformations of the nuclei (Fig.9). Cell membrane damage was most pronounced on the entire corneal surface in the Indoptol[®] treated eyes,

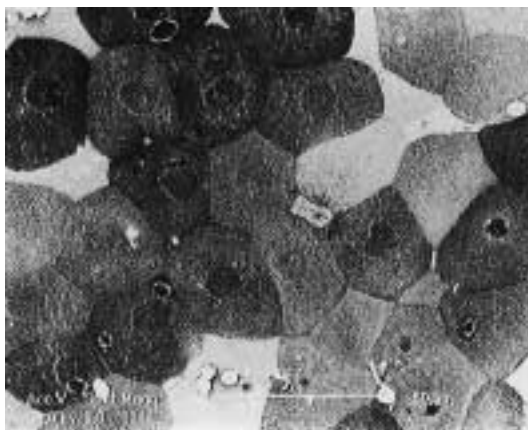


Fig. 4 The number of dark and grey cells has increased by NSAID treatment. However, the cells retain their hexagonal pattern. SEM.

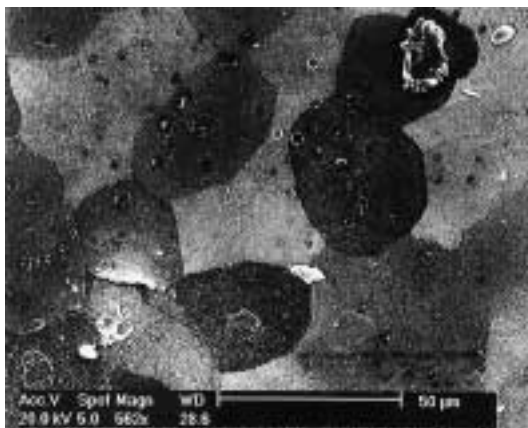


Fig. 5. Globular change in the cell form with increased craters on the surface. NSAID treatment. SEM.

followed in frequency by the Indocollyre[®] and Ocuflur[®] treated eyes. Such alterations are seldom observed in the untreated corneas.

Table 2. Correlation of different damage criteria to the active constituents of the drugs, pH and preservatives. P= peripheral cornea, M = midperipheral cornea, C= central cornea, x= mild to moderate damage, X= moderate to severe damage.

Evaluation criteria	pH	Benz.chloride	Sod. edetate	Thiomersal	Active ingredient
Hexagonal form	P M C	P M C	P M C	P M C	P M C
Brightness	X X X	x x x		x	X X X
Craters	X X X			X X X	X X
Retraction/ Exfoliation	X X X	x x x		x	
Cell membrane damage	x	x x	X X X		X X
Microvilli	x X x	X x x		x	x X X

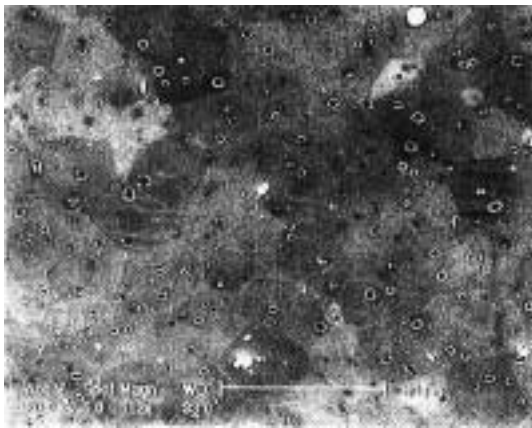


Fig. 6. A marked increase in the number of craters by NSAID application. SEM.

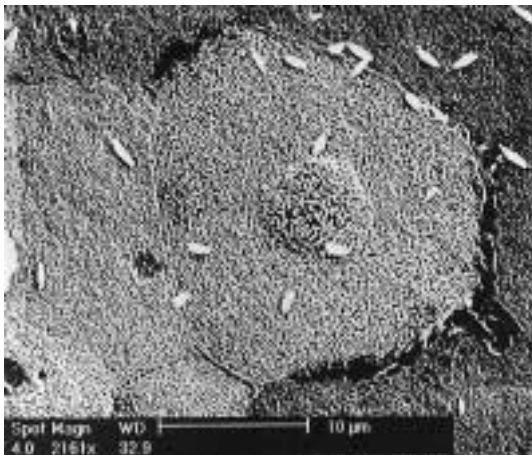


Fig. 7. Abundant microplicae on the cells after NSAID application. SEM.

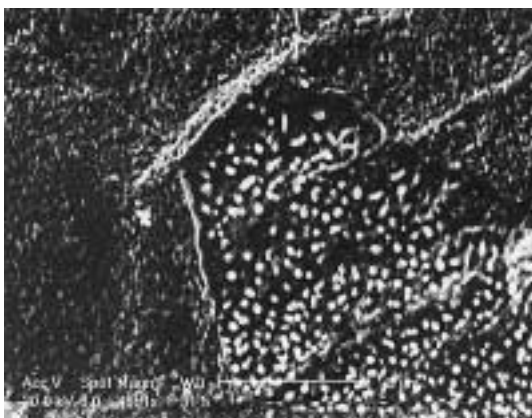


Fig. 8. Sparse globular microvilli are present on a partly visible cell adjacent to dark cell with loss of microvilli. NSAID treatment. SEM.

6. Retraction and exfoliation:

Peripheral parts of all corneas were almost devoid of retracted and exfoliated cells (Fig. 10 and 11). Ocuflur[®], Indoptol[®] and Aculare[®] treated eyes showed retracted and exfoliating cells in the central and midperipheral areas. Indocollyre[®] caused the least retraction or exfoliation. Retraction and exfoliation of the isolated cells may rarely be seen in normal corneas.

The correlation of pH, preservatives, and the active ingredients in the ophthalmic solutions used to the severity of different damage criteria is summarized in Table 2.

A low pH appears to affect the number of microvilli, number of craters, brightness, cell retraction and exfoliation. The active ingredients, in addition, caused major changes in the hexagonal form of cells and the cell membrane damage. Benzalkonium chloride seemed to affect all parameters of damage evaluated. Sodium edetate was probably the least toxic preservative in this study.

DISCUSSION:

Although, systemically administered NSAIDs are well known to cause gastric and intestinal ulcers (9), little is known about the toxicity of locally applied NSAIDs to the eye. Rarely, topically applied NSAIDs may cause systemic symptoms. Sitenga et al (20), described a single case of acute exacerbation of asthma after inadvertent application of ketorolac eye drops in a 44 year old lady with chronic asthma, rhinosinusitis and nasal polyps. They advised not to use NSAIDs eye drops in patients allergic to aspirin and NSAIDs or in combination of asthma and nasal polyps unless the patient is known to tolerate aspirin without any complications

In our study, all NSAID ophthalmic solutions licensed for ophthalmic use, when instilled 5 times a day during one week, caused alterations in the superficial epithelial cells. In all specimens the least damage was observed in the peripheral cornea, and its severity progressively increased towards the central cornea.

The degree of damage induced by different eye drops varied. Indocollyre[®] treated corneas showed a minimum number of dark and grey

cells. Ocuflur[®], Indoptol[®] and Aculare[®] treatment induced a decrease in size, rounding or total disappearance of the microvilli. Since the active ingredients of the formulations are different, it may be assumed that the active ingredients themselves induce this change. It is not known in what respect the combinations of the preservatives, pH, and the active ingredient influence the degree of epithelial damage. In this respect, more craters were present in the Ocuflur[®], Indoptol[®] and Voltaren[®] treated corneas. Since the active ingredients in the three solutions differ, other factors must influence the development of craters. The pH of Ocuflur[®] and Indoptol[®] is low, 6.4 and 5 respectively, and for Voltaren[®] it varies from 6.8 to 7. We did not measure the pH of the Voltaren[®] eye drops used in this study. If the pH of Voltaren[®] eye drops was low, one could assume that the low pH is associated with an increased number of craters. This hypothesis becomes more likely as the three formulations that caused increased number of craters contained different preservatives.

Preservatives are known to cause microscopic damage to the corneal epithelium (4). In this study, benzalkonium chloride appeared to moderately influence all parameters of the cellular damage; thiomersal was mainly related to crater development and sodium edetate inflicted cell membrane alterations.

Hexagonal pattern of the cells disappeared in the central areas of the cornea by Aculare[®] and Ocuflur[®] treatment, whereas Indoptol[®] application caused rounded cell appearance, cell membrane alterations, shrinking and exfoliation of cells. Other compounds noted to cause these changes to a lesser extent were Ocuflur[®] and Aculare[®]. Although Indocollyre[®] induced some cell membrane changes, it caused least cell exfoliation.

The capacity of the NSAID eye drops to inflict microscopic cellular damage should be considered when prescribing these drugs, especially after corneal injury. In our short-term study, Indocollyre[®] and Pranox[®] appear to be the least toxic to the corneal epithelium. Here again, Indocollyre[®] may have an edge over Pranox[®] as the latter induced more dark and grey cells than the former. When judged by the same criteria, Indoptol[®] and Ocuflur[®] were the most toxic to the corneal epithelium.

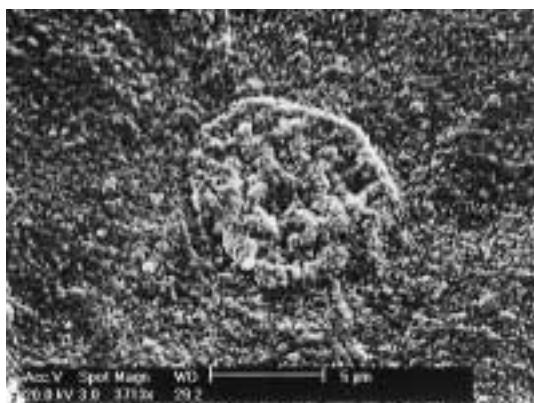


Fig. 9. Loss of microvilli on a rough cell surface. The nuclear chromatin appears to be clumped. NSAID treatment. SEM.

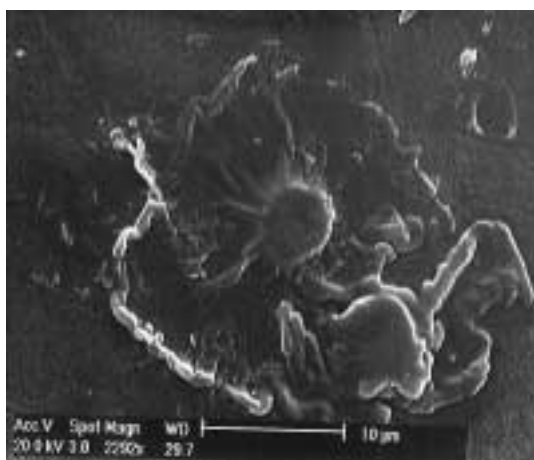


Fig. 10. An exfoliating cell in the NSAID treated cornea. SEM.



Fig. 11. A shrunken epithelial cell in the process of exfoliation. The underlying cell possesses a large number of microplacae. NSAID treatment. SEM.

The use of NSAID eye drops has distinct advantages over the corticosteroids. Corticosteroid treatment caused weaker corneal wound scars than did the NSAIDs (16). NSAIDs prevent surgically induced miosis during cataract extraction (19), and may have a role in prophylaxis of aphakic cystoid macular edema (8). Lack of secondary glaucoma or reactivation of viral ocular disease with NSAID use makes them an important tool in the management of ocular inflammation and pain.

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