# Long term ultrastructural changes in human corneas after tattooing with non-metallic substances

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**Abstract**

***Aim*—To investigate the ultrastructural appearance and the deposition pattern of dye particles in long term non-metallic corneal tattooing.**

***Methods*—Two tattooed human corneas were obtained by keratoplasty. One corneal button was fixed in Karnovsky's solution and the other in Trumps' solution. Both corneas were divided and processed for conventional light (LM) and transmission electron microscopy (TEM). Five additional formalin fixed corneas with tattoos were retrieved from paraffin for TEM. The time between tattoo and removal of the corneal button/enucleation ranged from 7 to 61 years. All seven corneas were examined using a Jeol JCXA733 microprobe for wave length dispersive analysis in order to exclude any presence of metallic salts in the tattooed area.**

***Results*—Histologically, clumps of brown-blackish granules were present mainly in the mid stroma, but also in anterior and partially in the posterior half of the stroma. On TEM, numerous round and oval electron dense particles were seen in the cytoplasm of keratocytes arranged as clusters or large islands. The larger particles appeared black, while the smaller particles were grey. In well fixed tissue a unit membrane was observed around these clusters. No granules were detected in the extracellular matrix.**

***Conclusions*—Keratocytes can actively ingest and retain tattooing particles of non-metallic dyes within their cell membrane for very long periods of time.**

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Tattooing of the cornea is an ancient procedure, which has been used to disguise leucomata since the beginning of the first millennium.[1] Owing to progress in keratoplasty and lens fitting, the list of indications for this procedure has dropped significantly. However, in selected cases—for example, in patients with an eccentric corneal scar and contact lens intolerance, complaints of visual disability secondary to light scattering can be managed by this technique. In those instances it is either a good and simple alternative or the only measure to improve visual acuity or cosmesis. In eccentric semitranslucent scars tattoo converts an annoying nebula into an opaque plaque eliminating light scattering and glare and therefore improving visual acuity and/or visual comfort (sometimes an additional optical iridectomy is advisable). Figure 1 demonstrates a recent exemplary case of a patient who developed an eccentric pupil due to iris capture after cataract extraction and complained of monocular diplopia. Because of a long and complicated ocular history another intraocular procedure was considered to be risky. In fact the symptoms of diplopia were resolved by the application of the tattoo.

Two entirely different tattooing methods are known. One method used is chemical dyeing with gold or platinum chloride—a simple technique mainly employed in the West since the pioneering work by Knapp, Krautbauer, Bietty and others.[1] Another method is that of carbon impregnation. Chemical tattooing is easier and quicker than the carbon impregnation method, but it fades more rapidly than non-metallic tattooing.[2] For the latter method India ink, Chinese ink, lamp black, and other organic dyes were employed.[2]

While there are several light microscopic and at least one ultrastructural report on chemical tattooing, long term non-metallic dyeing of human corneas has not yet been ultrastructurally examined. The aim of the present study

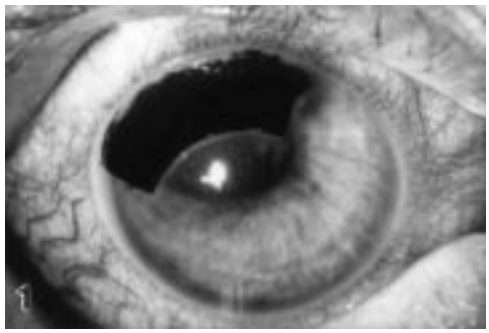

*Figure 1 Example of a peripheral optical tattoo in a patient with an eccentric pupil due to iris capture after cataract extraction. (Courtesy of Dr J Jay, Tennent Institute of Ophthalmology, Glasgow.)*

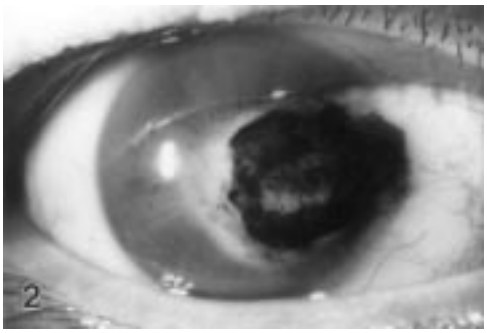

*Figure 2 Case No 1. Preoperative clinical appearance of a 19 year old non-metallic corneal tattoo.*

*Table 1 Summary of clinical data of seven corneal tattoos*

| *Cornea No* | *Sex* | *Age at tattoo (years)* | *Age at surgery (years)* | *Country/ origin* | *Diagnosis* | *Surgery* |
|---|---|---|---|---|---|---|
| 1 | M | 29 | 48 | Iran | twig injury at age of 10 years | triple |
| 2 | F | 16 | 23 | Morocco | stick injury at age of 4 years | PK |
| 3 | F | 20 | 51 | Asian part of USSR | Keratitis in childhood | PK |
| 4* | M | ? | 75 | unknown | perforating corneal injury | enucl |
| 5 | F | 35 | 75 | unknown | twig injury at age of 25 years | enucl |
| 6 | F | 27 | 79 | Asian part of USSR | cat's claw injury at 7 months | enucl |
| 7 | F | 6 and 18 | 79 | Asian part of USSR | post vaccination keratitis at 5 years | PK |

PK= penetrating keratoplasty; triple = simultaneous PK, extracapsular cataract extraction with posterior chamber intraocular lens implantation; enucl= enucleation. *Patient dead.

was to investigate the ultrastructural appearance and the deposition pattern of dye particles in long term non-metallic corneal tattooing of human corneas.

## Material and methods

Two tattooed corneas were obtained by penetrating keratoplasty. One cornea (No 2) was immediately fixed in cold Karnovsky's solution (2.5% glutaraldehyde and 4% paraformaldehyde in phosphate buffer), the second cornea (No 1) (see Fig 2 for clinical appearance) was fixed in cold Trumps' solution (4% formaldehyde and 1% glutaraldehyde in phosphate buffer) and divided by half. One half was processed for conventional transmission electron microscopy (TEM) and the remaining half for standard (paraffin) light microscopy (LM). Five additional formalin fixed corneas with tattoo (No 3–7) were retrieved from paraffin blocks by melting and rehydration in alcohol of decreasing concentration. The specimens were then post fixed in osmium tetroxide, dehydrated, and embedded in epoxy resin. Sufficient clinical data were available in all, but one (No 4) case. In this case the patient had died 15 years ago, and neither a chart nor close relatives could be found. The shortest time interval between tattoo and removal of the corneal button/enucleation was 7 years, the longest period was 61 years. Other relevant clinical details are summarised in Table 1.

In order to be sure that there had been only non-metallic tattooing in the tissue examined, the presence of metallic ions in the tattooed area was excluded in all seven corneas by a Jeol JCXA733 scanning electron microscope with a microprobe for wave length dispersive analysis at the Materials Institute at the University of Erlangen-Nuremberg, Germany. Specimens 1–4 were double checked using an energy dispersive *x* ray analyser fitted onto a Jeol 2000 FX transmission electron microscope at the central electron microscopic laboratory of the Technical University of Aachen, Germany.


*Figure 3 Case No 3. Histologically the highest concentrations of tattoo particles is seen in the mid-stromal layers (haematoxylin and eosin, original magnification ×40).*

## Results

The energy dispersive *x* ray analysis (EDX) showed peaks at carbon (C) in all corneas examined. In specimen No 7, two further peaks showed the presence of phosphorus (P) and calcium (Ca). Histologically this area exhibited band keratopathy and can therefore be regarded as a "built in control" area demonstrating the proper function of EDX. Any other metals, particularly platinum or gold, were absent from all specimens examined.

Light microscopy revealed brownish-blackish deposits at different locations. In all but one specimen (No 4) the anterior half of the corneal stroma contained tattoo granules. The highest concentration was usually seen in the deep mid stroma (Fig 3), but in cases 2 and 6 there was an almost equal distribution throughout the involved layers. With regard to particle distribution case No 4 was particularly interesting, as the highest accumulation of tattoo particles was seen in the posterior cornea in pre-Descemet's stromal layers. In case 5, keratocytes within the subepithelial fibrocellular ingrowth also contained tattoo granules.

*Table 2 Summary of light microscopic (LM) and transmission electron microscopic (TEM) findings of seven corneas examined*

| *No* | *LM: location of deposits* | *TEM: location and appearance* | *Size of particles* | *Remarks* |
|---|---|---|---|---|
| 1 | Subepithelial, anterior half of stroma, highest accumulation in the mid stroma | Intracellular, clusters in cytoplasm surrounded by fragments of limiting mebranes | black: 40–68 nm<br>grey: 30–51 nm | Trumps' fixative |
| 2 | Subepithelial and anterior half of stroma | Intracellular, heavily loaded cytoplasm, confluent large clusters situated in vacuoles with surrounding limiting membrane | black: 50–62 nm<br>grey: 34–35 nm | Karnovsky's fixative |
| 3 | Scattered throughout the anterior stroma, highest accumulation in the deep mid stroma | Intracellular, clusters in cytoplasm. Limiting membrane not preserved | black: 35–52 nm<br>grey: 17–35 nm | retrieved from paraffin |
| 4 | Posterior third of stroma | Intracellular, diffusely in the entire cytoplasm. In between dense accumulations in clusters. Limiting membrane not preserved | approx 35 nm | retrieved from paraffin |
| 5 | Scattered throughout the entire stroma and within the fibrovascular subepithelial ingrowth | Intracellular, clusters in cytoplasm. Limiting membrane not preserved | black: 35–89 nm<br>grey: 30–89 nm | band keratopathy, retrieved from paraffin |
| 6 | Paracentrally, just posterior to the epithelial basement membrane | Intracellular, clusters in cytoplasm. Limiting membrane not preserved | black: 61–39 nm<br>grey: 22–61 nm | retrieved from paraffin |
| 7 | Scattered within individual fibroblasts in anterior and mid stroma | Intracellular, clusters in cytoplasm. Limiting membrane not preserved | black and grey: 28–45 nm | band keratopathy, retrieved from paraffin |

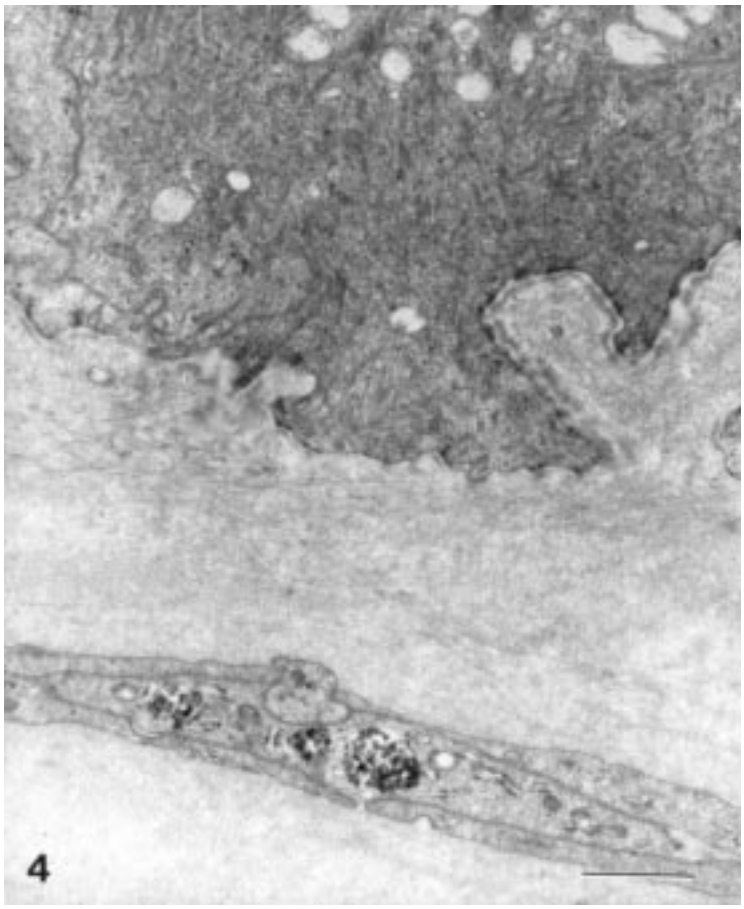


*Figure 4 Case No 2. A subepithelial keratocyte with clusters of tattooing particles. The adjacent extracellular matrix is free of foreign material (TEM, magnification bar = 1 μm).*

Morphological changes associated with the original disease process leading to corneal opacification were quite typical: all corneas showed vascularisation, defects in Bowman's layer, and sometimes also in Descemet's membrane. Only in case 1 was a mild lymphocytic infiltrate observed in the tattooed area. Individual findings are summarised in Table 2.

By transmission electron microscopy numerous round and oval electron dense particles with distinct borders were seen in the cytoplasm of keratocytes arranged as clusters or large islands. The larger particles whose size ranged from 35 to 69 nm appeared black, while the smaller particles were grey, their size ranging from 17 to 61 nm. In case 5, both types of granules showed a wide range in size from 35 up to 89 nm. In case 2, where an excellent fixation for TEM was achieved, the clusters were situated in intracytoplasmic vacuoles delineated by an unit membrane (Figs 4 and 6). In case 1, only fragments of unit membranes were identified (Fig 5). In the tissue which was retrieved from paraffin, unit membranes were not preserved; however, the overall arrangement in the form of clusters was identical to the better preserved specimens (Fig 7). In case 4, tattooing granules were diffusely distributed throughout the cytoplasm of keratocytes; however, even here cluster-like accumulations were present. The extracellular matrix as well as the endothelium were free of tattoo particles in all seven corneas (Figs 4, 6, and 7).

## Discussion

In the past few decades corneal tattooing has significantly lost its popularity as a consequence of progress in keratoplasty techniques and contact lens manufacturing. However, in cases of contact lens intolerance associated with annoying reduction in visual acuity due to light scattering caused by a peripheral corneal scar, a large diameter keratoplasty or a peripheral lamellar keratoplasty are at high risk of rejection.[3] In such a case tattooing of the scarred area can reduce glare and increase visual acuity, because a semitranslucent scar is converted to a total plaque causing an absolute scotoma. The remaining problems of corneal tattooing, however, are still its long term instability as well as imperfect geometrical configuration.[2] A new modification of the tattooing procedure, which might eliminate the problem of irregular staining, was recently published by Anastas *et al*, who used an excimer laser for preparation of an ideal circular and even corneal bed for tattooing.[4] With new generation excimer lasers (for example, flying spot technology) and ablation masks ablations of different profiles can be achieved.[5] Thus, the combination of a new technology and the old technique might increase the popularity of the tattooing procedure in the future. Long term fading resistance, however, remains another important unresolved issue.

Although the metallic chemical tattooing was reported to be not as stable as the non-metallic impregnation method it has some very important advantages: the technique is quick, simple, and gives a better "jet black" stain.[1 2] That is the reason why the chemical method remains the most commonly employed today in the West.[4] Thus, it was not surprising that all tattoos in our series (except for the unknown cases) originated in eastern and/or north African parts of the world (Table 1). But how can we explain that the carbon impregnation is supposedly more durable than the metallic salt method?[2] Are there ultrastructural differences?

The main finding of the present study is the fact that all granules in all cases were found within keratocytes, unlike in metallic tattooing. In an ultrastructure study of metallic tattooing of the cornea Olander *et al.* showed both intracellular and extracellular granules 10 years after tattooing with platinum chloride.[2] The observation that not only macrophages, but also fibroblasts are capable of endocytosis was made in several ultrastructural studies of skin tattoos. Dermal tattoos are traditionally based on non-metallic dyes[6–8] similar to our cases. Indeed, the pattern and colour of dermal tattoos remain macroscopically unchanged throughout the lifetime of the carrier.[8] Lea and Pawlowski attributed these features to the prominent network of connective tissue elements surrounding ink particle-containing fibroblasts.[8] Obviously, an identical network is not present in corneal tissue. However, our cases and dermal tattoos have in common the fact that particles were found only within cells and were usually surrounded by a unit membrane.[8] We also saw a unit membrane in

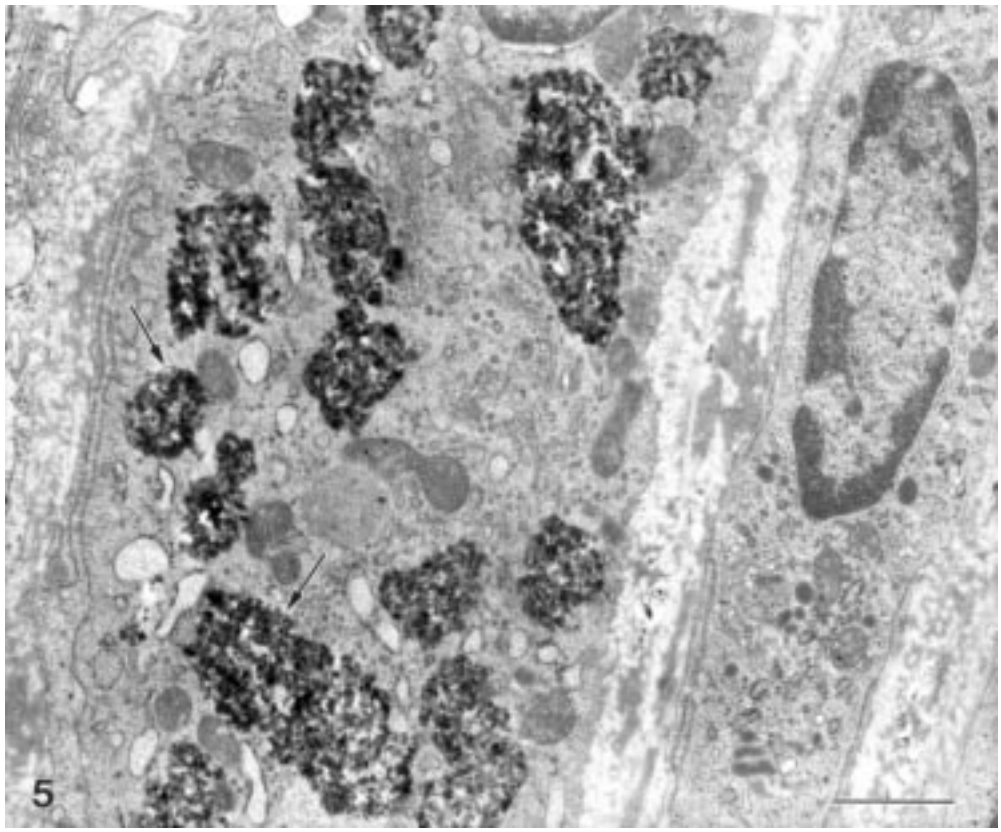


*Figure 5 Case No 1. A keratocyte with several intracellular clusters of tattooing particles. Fragments of limiting unit membranes (arrows) are still preserved. Also note a mixture of more (black) and less (grey) electron dense granules (TEM, magnification bar = 1 μm).*

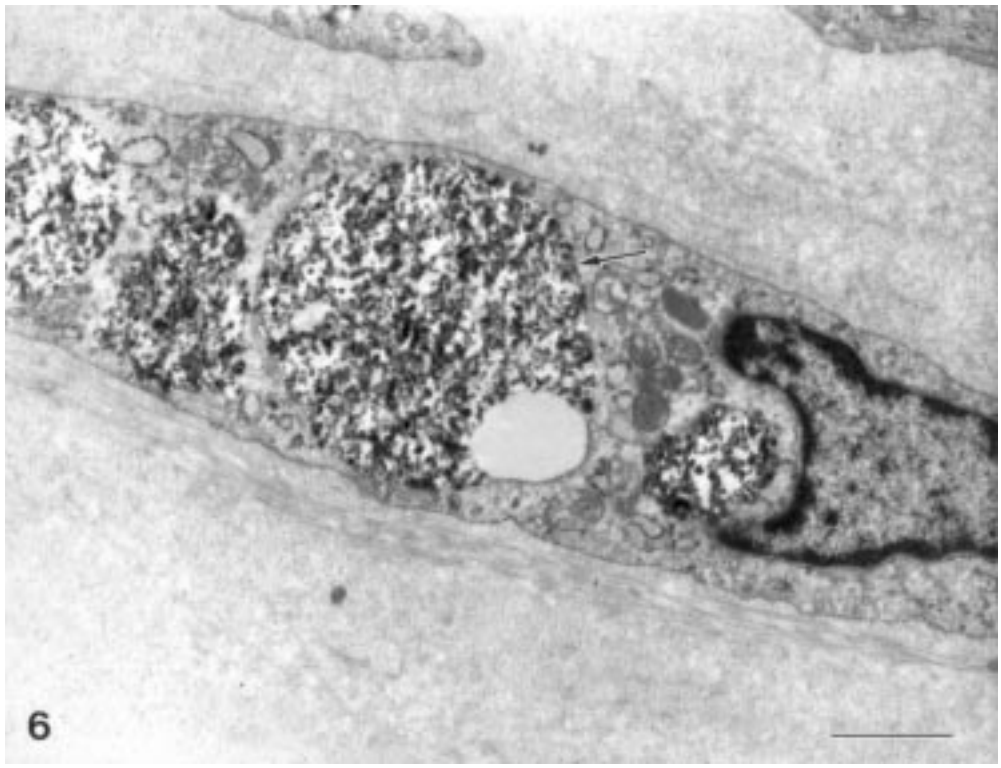


*Figure 6 Case No 2. High power of a mid stromal keratocyte with tattoo granules of different electron density. Individual clusters are surrounded by an unit membrane (arrow) (TEM, magnification bar = 1 μm).*

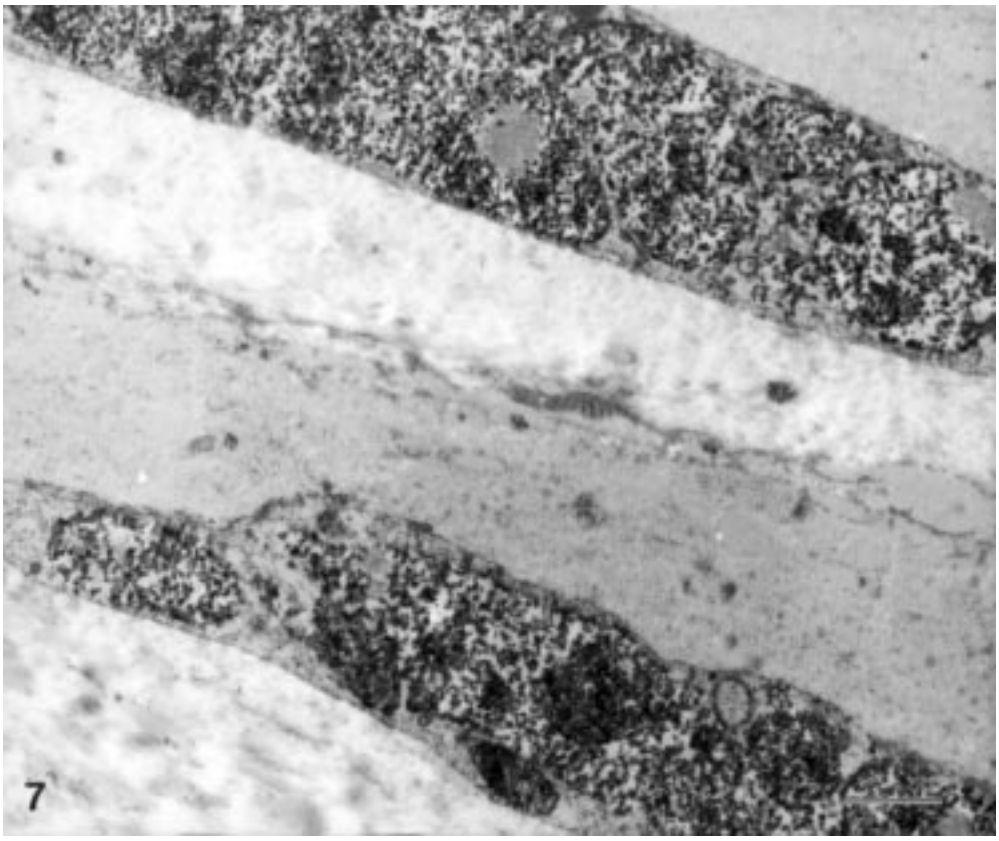


*Figure 7 Case No 7. Two keratocytes with numerous intracytoplasmic clusters. Unit membranes are not preserved. The extracellular space is free of tattooing granules (TEM, magnification bar = 1 μm).*

our cases 1 and 2. Such a membrane was not detected in cases 3–7, but we believe that inappropriate fixation and retrieval from paraffin destroyed most of the unit membranes present originally, and that the arrangement in clusters still indicates the previous existence of such a membrane. In contrast, even under appropriate fixation conditions, platinum tattooed cornea was reported to have no unit membrane around the accumulations of tattoo particles.[2] Apart from location, a further distinct difference between metallic and non-metallic tattoo granules was their ultrastructural appearance. In all our cases dark (black) and light (grey) granules with sharp angulated borders were detected. In contrast, Olander *et al* showed black round granules with partially vapid borders.[2]

The absence of extracellular tattooing particles in our seven specimens suggests that endocytosis of organic substances by human corneal fibroblasts is more permanent and stable than endocytosis of metallic materials, where the extracellular location was easily detected.[2] This stability was particularly evident in case 5 where tattoo granules containing keratocytes were observed in the space between Bowman's layer and the epithelium as a so called subepithelial fibrocellular ingrowth. Although we do not know whether these cells have migrated from the stroma as a result of a long standing bullous kerathopathy or from the limbus as a consequence of the previous corneal disease, the interesting fact is that keratocytes can retain endocytosed particles while migrating within the tissue. Experimentally, this issue was adressed by Fujita *et al* who showed that corneal fibroblasts in rabbits can endocytose injected india ink particles within 3–4 days and keep them for at least 6 months.[9] Our study confirms these results in humans for a significantly longer period of time (up to 61 years). To explain this phenomenon, Fujita *et al* suggested that phagocytosis by corneal fibroblasts is a reaction that protects the cornea from the injury and harm by non-toxic foreign materials.[9] Assuming that metallic salts (also at a cellular level) are more toxic than organic substances used, one would expect a higher cellular breakdown and subsequently more cellular debris in the corneal extracellular matrix. Thus, it is not surprising that the probability of observing extracellular granules by TEM is higher in metallic than in non-metallic tattoos.

In summary, at the ultrastructural level non-metallic tattoo of the cornea differs from metallic dyeing by a more variable appearance of the tattoo particles and by an exclusively intracellular location even after many decades. The keratocytes can clean up and control the extracellular matrix by ingestion and retention of organic material for a long period of time.

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