Annals of Anatomy 224 (2019) 1–7

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat

RESEARCH ARTICLE

Ultrastructure of the lacrimal drainage system in health and disease: A major review☆

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ARTICLE INFO

Article history:
Received 29 January 2019
Received in revised form 13 February 2019
Accepted 13 February 2019

Keywords:
Ultrastructure
Scanning electron microscopy
SEM
Lacrimal drainage
Lacrimal sac
Nasolacrimal duct
Transmission electron microscopy

ABSTRACT

Purpose: To provide a systematic review of the literature on the ultrastructural findings of the lacrimal drainage system in healthy state and in few of the disorders studied so far.
Methods: The authors performed a PubMed search of all articles published with reference to electron microscopic features of the lacrimal drainage pathways. Data captured include demographics, study techniques, scanning or transmission electron microscopic features, presumed or confirmed interpretations and their implications. Specific emphasis was laid on addressing the lacunae and potential directions for future research.
Results: Ultrastructural studies have led to better understanding of the lacrimal drainage anatomy-physiology correlations. Cellular interactions between fibroblasts and lymphocytes could form a basis for pathogenesis of punctal stenosis. Ultrastructural characterization of peri-lacrimal cavernous bodies and changes in primary acquired nasolacrimal duct obstruction (PANDO) led to them being partly implicated in its etiopathogenesis. Electron microscopic characterization of the dacryolith core promises insights into their evolution. Ultrastructural tissue effects of mitomycin-C during a DCR surgery has provided potential evidence of its role in cases with high-risk of failure. Lacrimal stent biofilms are common but their clinical implications are currently uncertain.
Conclusion: Ultrastructural exploration of lacrimal drainage system so far has been limited and sparsely explored. The list of unexplored areas is exhaustive. There is a need for the lacrimal Clinician-Scientist to make themselves familiar with techniques and interpretation of electron microscopy to advance the ultrastructural frontier of this science.

1. Introduction

Ultrastructure or fine structure refers to the cellular architecture and study of sub-cellular organelles with the help of an electron microscope (EM). Scanning electron microscopy (SEM) provides high magnification images of the surface topography and contents whereas transmission electron microscopy (TEM) is used to study cellular and sub-cellular morphology. There are few studies that specifically studied the ultrastructural features of lacrimal drainage system in healthy and diseased states (Adenis et al., 1980a, 1980b, 1981; Ali, 2015; Ali et al., 2015a, 2015b, 2015c, 2015d, 2015e, 2016a, 2016b, 2016c, 2017; Ali et al., 2018a, 2018b; Ali, 2019; Balikoglu-Yilmaz et al., 2014; Harada et al., 1983; Murphy et al., 2015; Orhan et al., 1996; Paprizos et al., 2013; Parsa et al., 2010; Paulsen, 2003; Paulsen et al., 1998, 2000a, 2001; Radnot, 1972, 1977; Radnot, 1981; Samimi et al., 2013, 2016; Sugita et al., 2001; Thale et al., 1998; Ugurbas et al., 1997). The earliest major studies were by Radnot followed by Adenis and later by the current authors' group. The current review elucidates electron microscopic features of a normal lacrimal drainage pathway and also of lacrimal disorders where it was studied and attempts to provide potential avenues of future research.

2. Methods

A systematic Medline search was performed on PubMed using the terms 'ultrastructure', 'electron microscopy', 'scanning electron microscopy', 'transmission electron microscopy', 'lacrimal sac', 'punctum', 'canaliculus', 'cellular', 'sub-cellular', 'fine structure', 'sub-microscopic', 'nasolacrimal duct', 'lacrimal', 'disorder', 'disease', 'etiology', and 'pathogenesis'. There was no restriction on the

☆ This paper belongs to the special issue Lacrimal drainage system.

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https://doi.org/10.1016/j.aanat.2019.02.003
0940-9602/

date of publication. All articles published with reference to electron microscopy of the lacrimal drainage system were assessed for the analysis. Relevant cross references from these articles were also considered. Data captured include demographics, study techniques, scanning or transmission electron microscopic features, presumed or confirmed interpretations and their implications. Specific emphasis was laid on addressing the lacunae and potential directions for future research.

3. Results

3.1. Techniques of electron microscopy

The techniques of EM are exhaustive and beyond the scope of this review. In brief, there are two broad categories of electron microscopes — scanning and transmission. SEM scans the sample surfaces with a beam of electron in a raster pattern allowing for acquisition of detailed topographical images. TEM transmits electrons through ultra-thin specimens with resultant higher resolution images than SEM (Ali, 2018). There are few basic principles of sample processing for an electron microscopy and details may vary based on the needs (Ali et al., 2018a). Fresh specimens are fixed, for example, either in 2.5% glutaraldehyde or Ito fixative (2.5% glutaraldehyde + 2.5% paraformaldehyde + 0.3% picric acid dissolved in phosphate-buffered saline, pH 7.3). In addition, other fixation methods are possible. For a commonly used protocol in SEM, the specimens following fixation are impregnated with tannic acid and counterstained with 2% osmium tetroxide followed by dehydration in ethanol in a critical point dryer. The preparation is then coated with gold before SEM examination. The samples for TEM are fixed in Ito fixative and embedded in Epon or a comparable compound before preparing semi-thin sections (1 μm thick) using specific microtomes. The sections are then stained with uranyl acetate and lead citrate before a TEM examination.

3.2. Ultrastructural features of a normal lacrimal drainage pathway

3.2.1. Punctum

The scanning electron microscopic views of the punctum demonstrates its rim or edges to be smooth with a well-defined lumen (Ali et al., 2015a). The surfaces of the lumina were smooth. The junction between the luminal surfaces of the punctum and the proximal vertical canaliculus is usually elevated and well demarcated. Very high magnification of SEM demonstrated the inner punctal surfaces to be smooth and lined by a regular and compact epithelium without any ciliated surface (Ali et al., 2015a). Around the punctal rim, the cut surfaces showed numerous throttle veins and venules. Adenis et al. (1980a, 1980b) studied the TEM of the punctal area and demonstrated the epithelial surfaces to be flat. The epithelial cells demonstrated pavement patterns with tight intercellular junctions and sparse microvilli.

3.2.2. Canaliculus

SEM studies of normal canaliculi demonstrated wider lumina as compared to the punctum but the diameters were variable at different points along its anatomical course (Ali et al., 2015a). The external surfaces of a canaliculus were rough with numerous criss-cross types of collagen arrangement. In comparison, the inner or luminal surfaces were smooth but interrupted by occasional rugae like folds of the mucosa, some being more prominent than others (Ali et al., 2015a). This could be attributed to the possible valvular system of lacrimal drainage. Very high magnification SEM showed the epithelium to be compact and smooth without ciliated surfaces. The external surface of the distal canaliculi showed numerous collagen fibers adhering it to the well-defined muscle bundles of orbicularis oculi and this could play a potential role in tear flow physiology (Ali et al., 2015a). Adenis et al. (1980a, 1980b) reported TEM features of canaliculi and found that the external surfaces were lined by pavement epithelium with numerous microvilli but without cilia and the inner surfaces to be similar but with less or absent microvilli.

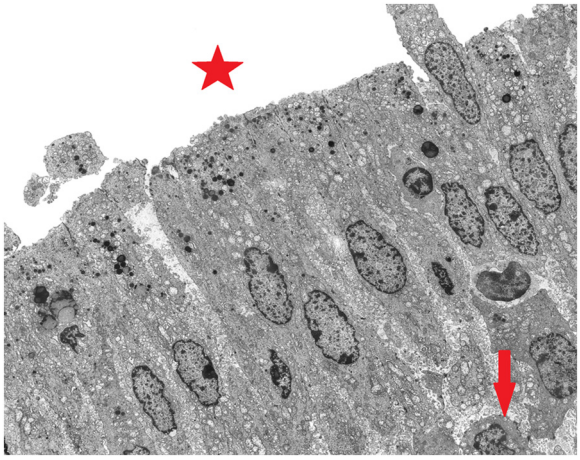

Fig. 1. TEM feature of the lining epithelium of the lacrimal sac and nasolacrimal duct: ultrastructural features showing a superficial layer and a deep layer of basal cells (arrow). Also note the lumen (star) of the lacrimal sac (TEM × 1150).

3.2.3. Lacrimal sac (LS)

Lacrimal sacs are the most well studied portions of the lacrimal drainage system. The external surfaces are rough but the luminal surfaces are the widest with numerous stomach-like rugae. SEM had demonstrated numerous lymphoid follicle areas as focal but well defined elevated mucosal folds (Ali et al., 2015a). These belong to the tear duct-associated lymphoid tissue (TALT) (Paulsen et al., 2000b). The fundus of the LS usually demonstrated intraepithelial glandular tissue with opening into the lumen (Ali et al., 2015a). The lining epithelium is double layered with superficial columnar and deep basal layer (Fig. 1) (Paulsen et al., 1998). The cell borders between the epithelial cells are made up of zonulae occludentes, zonulae adhaerentes, and desmosomes (Paulsen et al., 1998). Occasionally lymphocytes may be seen between epithelial cells but there is a clear distinction between epithelial and goblet cells (Paulsen et al., 2001). The face of the epithelial cells are the numerous microvilli, 60–70 nm in diameter and 400–600 nm in length (Fig. 1) (Paulsen et al., 2001). The microvilli trimming can sometimes be loose and a higher magnification is required to distinguish them from cell process. The cilia are grouped into tufts across the epithelium and on an individual epithelial cell have been found to be arranged in groups of 10–40 (Radnot, 1972, 1977). The goblet cells show columnar respect with cup-like nuclei at the basal pole with numerous mucous droplets filling the entire supranuclear cytoplasm (Fig. 1) (Paulsen et al., 2001). They can be found as single cells or arranged in groups forming intraepithelial glands. A broad basement membrane is where the epithelium rests itself upon with the capillaries and lymphocytes being well appreciated in the sub-epithelial areas (Paulsen et al., 1998).

The wall of the LS is embedded with a robust vascular system consisting of barrier arteries, capacitance veins and throttle veins (Figs. 2 and 3) (Paulsen et al., 2000a). This is comparable to the cavernous system and is connected to the cavernous bodies of the nasal cavity. The regulation of the blood flow through these vessels was found to influence the lacrimal drainage lumina and hence was proposed as potential factors in the etiopathogenesis of PANDO (Paulsen et al., 2000a, 2001). TEM studies of the normal lacrimal sacs have recently demonstrated the presence of cells comparable

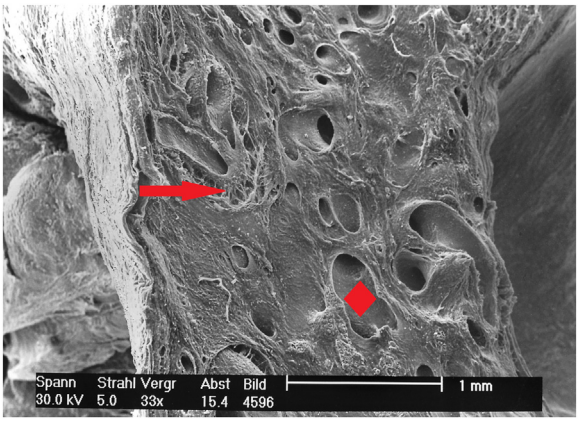

Fig. 2. SEM features of the lacrimal wall vasculature: ultrastructural features showing the cavernous structure of the vessels. The lumina (diamond) of the vessels are separated by the connective tissue fibres (arrow) (SEM, 33×).

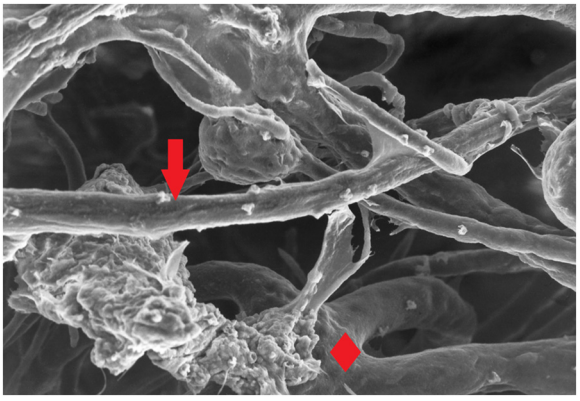

Fig. 3. SEM features of the lacrimal wall vasculature: ultrastructural features of a corrosion vascular cast showing the convoluted veins (diamond) and their vascular connections (arrow) (SEM, 45×).

to cholinergic brush cells (Ali et al., 2018a). These are specialized epithelial cells with a specific tuft of densely packed rigid apical microvilli whose filaments extent into the apical cytoplasm. These are believed to have a chemosensory role and are currently being investigated for their role in lacrimal physiology and pathology.

3.2.4. Nasolacrimal duct (NLD)

The junction between the lacrimal sac and NLD could be appreciated ultrastructurally in end-on views as well as on longitudinal sections (Ali et al., 2015a). The external surfaces of the NLD shows a very dense collagenous network with its wall showing numerous cavernous bodies which are demonstrably much more than that of the lacrimal sac. The lining epithelium and its features are similar to those seen in the lacrimal sac, however the NLD epithelial cells have numerous microvilli, goblet cells and there are few tufts of kinociliae in the lower part of NLD (Paulsen et al., 1998, 2001). SEM features of NLD openings in the inferior meati demonstrated the luminal surfaces to be mostly smooth with well-defined glandular elements (Ali, 2019). Edge mucosal folds were common and profound cavernous bodies were noted even in the vicinity of the opening. The presence of numerous mucosal folds gave an appearance of sub-sulci, whose potential role in tear rheology has been hypothesized.

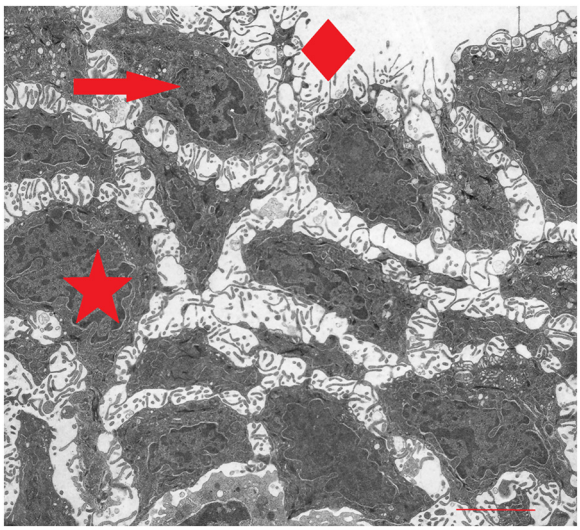

Fig. 4. TEM features of a lacrimal sac in a case of PANDO: ultrastructural features of the epithelium showing squamous metaplasia of the epithelial cells (star). Loose microcilia project into the extra-cellular space (diamond). The cytoplasm appears rarefied with irregular nuclei (arrow) (bar −2.5 μm).

3.3. Ultrastructural features in primary acquired nasolacrimal duct obstruction (PANDO)

PANDO is a clinical syndrome typically characterized by onset of epiphora beyond 40 years of age, female preponderance and subsequent signs of acute or chronic dacryocystitis (Linberg and McCormick, 1986). A vast amount of histopathology data is available for PANDO but the same is not true for the ultrastructural features. Radnot described electron microscopic features in 14 cases of chronic dacryocystitis (Radnot, 1972). The epithelium was double layered and some of these cells had secretory granules near the base of cell and were found to empty their contents into the lumen. The epithelium rested on thin basement membrane. Goblet cells were universal. Desmosomes were found in varying frequency but were found to be disintegrated in cases with profound edema. Ciliated cells were noted in few samples which were either solitary or grouped and demonstrated 9 tubuli in a 9 + 2 pattern (Kinociliae). In most instances the mitochondria were swollen with fragmented crests with inclusion bodies. Bifurcate mitochondria were also observed. Some edematous areas also showed large intercellular spaces and breakdown of cellular boundaries. The sub-epithelial areas were infiltrated by plasmacytes, fibrocytes and mast cells (around the capillaries and in between the collagen fibers). A Japanese study assessed 5 samples of dacryocystitis obtained during a dacryocystorhinostomy and observed that the epithelium of the lacrimal sac had numerous microvilli and the cilia had an average length of 3 μm and diameter of 1.5 μm (Harada et al., 1983).

Electron microscopic features in 36 samples of PANDO obtained during dacryocystorhinostomy (DCR) were studied and classified as early and late stage findings (Paulsen et al., 2001). Early stages of PANDO are characterized by active inflammation. Although the lacrimal sac and NLD epithelium remained double layered with microciliations of the top layer, there was an edema involving the epithelium and lamina propria (Fig. 4). Bacteria were visible on the surface of the epithelial cells and often infiltrated the cytoplasm and intra-cellular organelles (Fig. 5). It was not very uncommon to note hypersecretory goblet cells and subepithelial sero-mucous glands. The peri-LS and peri-NLD cavernous bodies revealed reactive hyperemia and were more prominent. These changes in cavernous bodies have been partly implicated

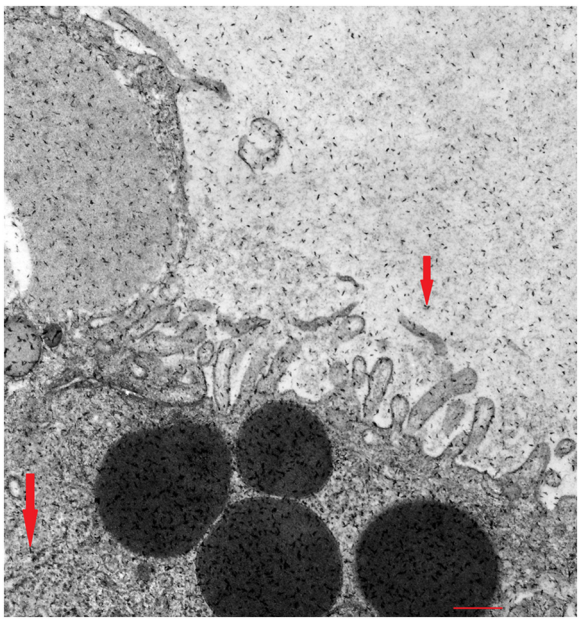

Fig. 5. TEM features of a lacrimal sac in a case of PANDO: ultrastructural features of the epithelium in high magnification shows numerous bacteria (arrows) on the cell surfaces as well as infiltrating the cytoplasm and organelles. (bar −6 μm).

in the etiopathogenesis of PANDO (Paulsen et al., 2001 Ali and Paulsen, 2019). The chronic stages of PANDO showed more pronounced changes. Several degenerated lymphocytes were noted in the epithelial layers. There was a variable loss of differentiated epithelial cells and denudation, and the basal layer showed squamous metaplastic changes. These metaplastic cells revealed loose microciliation on the surface with projections into extracellular spaces. The nuclei were irregular with rarefaction of the cytoplasm and reduction of intracellular organelles like endoplasmic reticulum and Golgi bodies. The cells were attached to each other by desmosomes. The edematous lamina propria progressively showed fibrosis.

3.4. Ultrastructural features in punctal stenosis

Punctal stenosis is the most frequently encountered proximal lacrimal disorder and accounts for 3% of all lacrimal drainage disorders (Das et al., 2018). The fine structures were studied in 6 samples of punctal stenosis and found chronic inflammation and subsequent fibrosis to be the common ultrastructural response (Ali et al., 2015b). The epithelial cells appeared normal with tight junctions but blunted microvilli on the luminal surfaces. The nuclei were elongated with peripheral chromatin condensation. Distinct ultrastructural features were noted in areas with and without extensive fibrosis. Those without extensive fibrosis showed an increase in the regularly arranged collagen fibers. The fibroblasts demonstrated pleomorphic mitochondria and dilated endoplasmic reticulum. Occasional nuclear halo was also noted. Areas with extensive fibrosis demonstrated extensive and irregularly arranged collagen bundles with intervening edema. Mononuclear inflammatory infiltrate was demonstrated within the collagen bundles and in the vicinity of fibroblasts. These electron microscopic findings led to the formulation of a hypothesis that a close interaction between the lymphocytes and fibroblasts could form the basis of the etiopathogenesis. It is possible that fibroblasts are responding to the cytokines from the lymphocytes and not acting on their own and decoding of this cellular talk could eventually pave way in unraveling the mysterious etiopathogenesis of punctal stenosis.

3.5. Ultrastructural features of a DCR ostium cicatrix

Dacryocystorhinostomy (DCR) failures have been reported in up to 10% of patients and cicatricial closure of the ostium is among the common causes (Dave et al., 2016). Ultrastructural features of DCR ostial cicatricial tissue were studied in samples obtained from ten patients during the revision surgery (Ali et al., 2016a). EM of the cicatrix demonstrated abundant and irregular collagen bundles with numerous fibroblasts and a mononuclear inflammatory infiltrate. Active oval to polygonal osteoblasts with escalloped cell surfaces and a clear zone between the cell and surrounding matrix were noted. These osteoblasts showed abundant rough endoplasmic reticulum with large Golgi bodies, vesicular mitochondria and pockets of glycogen. Scattered amorphous bony osteoid with hydroxyapatite deposits in collagen bundles beyond the osteoblasts were seen in all the cicatrix samples. The demonstration of new bone within the DCR ostium cicatrix could propel further insights into the wound healing response following the surgery. Comparing the ultrastructural effects of mitomycin C on the nasal mucosa with that of the ostium cicatrix provides indirect impetus to the use of the drug in cases with high risk of failure.

3.6. Ultrastructural features of dacryoliths or mucopeptide concretions

Dacryoliths are not very uncommon finding in patients undergoing a DCR and has been reported in up to 18% in them (Ali et al., 2018b). Dacryoliths obtained during a DCR are predominantly composed of mucopeptides and hence are also referred to as mucopeptide concretions (Paulsen et al., 2006). Dacryoliths isolated during an endoscopic DCR are predominantly made up of mucopeptides and hence also referred to as mucopeptide concretions. Only three studies analyzed the ultrastructural features of dacryoliths (Ali et al., 2018b; Orhan et al., 1996; Kominek et al., 2014). Orhan et al. (1996) studied a single sample and found it to be composed of lobes and lobules with an amorphous organic core. An another study also noted similar findings in seven samples but in addition found the surfaces to be rough with ridges and notches (Kominek et al., 2014). Variably sized spaces within the dacryoliths were hypothesized to be gaseous by-products of metabolism. Three of their seven samples showed organic fibers in the central areas. A focused SEM study was performed on multiple dacryoliths obtained from 10 patients undergoing a DCR (Ali et al., 2018b). Mucopeptide concretions were found to take the shape of the lacrimal drainage system based on their bulk and extent. The external surfaces and cut sections were mostly composed of homogenous acellular amorphous deposits. Two distinct types of craters were noticed. One had non-contiguous perforations with blood cells within them and the other was without perforations and blood cells. Multiple areas of vacuoles and fissures were noted and appeared more of cutting or drying artifacts rather than the result of any metabolic process. The core of all concretions was an extensive network of fibrillary tangles filled with granular material and red blood cells (Ali et al., 2018b). There was no evidence across all the samples of any organic fibers, fungal filaments or bacterial biofilms. There were no ultrastructural differences based on age of the patients. Based on these findings, a hypothesis was formulated on the possible evolution of mucopeptide concretion. Focal epithelial breach secondary to mechanical or chemical factors may lead to leakage of blood and this possibly forms the nidus or core of the concretion. The epithelial breach would also trigger a cascade of events leading to increased production of local mucopeptides as well as enhanced expression in the tears, which would get deposited all around the nidus. In addition, washed cellular debris and local debris could also add to the development of mucopeptide concretions. Further studies to char-

acterize the nidus by studying its constituents in the early phases could provide more insights into the etiopathogenesis.

3.7. Ultrastructural features of mitomycin C effects in a DCR

Mitomycin C (MMC) as an anti-fibrotic agent has been used in DCR surgeries to prevent cicatrix formation specially in high risk of failure cases like DCR in revision and post-traumatic cases (Kamal et al., 2014). The minimal effective concentration was found to be 0.02% for 3 min (Ali et al., 2013a Kumar et al., 2015). Circumostial injection of MMC (COS-MMC) is a technique used to potentiate the wound modulatory effects of the drug (Kamal et al., 2014). Only two studies assessed the ultrastructural effects of MMC on nasal mucosa (Ali et al., 2015c Ugurbas et al., 1997). Ugurbas et al. (1997) harvested the nasal mucosa intraoperatively and at multiple time-points post-operatively after subjecting it to 0.5 mg/ml for 2.5 min followed by a wash. They found that attenuated epithelium, intracellular edema and abnormal mitochondria to be the ultrastructural changes in response to MMC. Ali et al. (2015c) compared the ultrastructural effects of topical MMC versus COS-MMC at the standard dose of 0.02% for 3 min. They classified the effects on 4 tissues; epithelial, glandular, vascular and fibro-collagenous.

3.7.1. Topical MMC

The epithelium was attenuated up to the basal layers with loose intercellular junctions, intercellular and intracellular edema, indistinct cytoplasmic organelles, pleomorphic nuclei, indistinct nucleoli with peri-nuclear dilated areas (Ali et al., 2015c). Although the intracellular junctions were distinct in glandular cells, the cytoplasm showed edema with scattered and disorganized secretory granules with disruptive nuclear changes. The microcapillaries were dilated with blood filled lumina, edematous endothelial cells with indistinct nuclei and peripheral chromatin condensation. The most profound effects of topical MMC were noted on the fibro-collagenous tissues (Ali et al., 2015c). The collagen fibers had an edematous aspect. The fibroblast revealed marked intracellular edema, indistinct cytoplasmic organelles, widespread but patchy electron dense granular condensation, pleomorphic and vesicular mitochondria, indistinct nuclei and discontinuous endoplasmic reticulum.

3.7.2. Circumostial injection of MMC (COS-MMC)

The epithelium following COS-MMC showed gross attenuation with loss of microvilli and disruption of the basement membrane. The nuclei were vesicular with irregular chromatin condensation and mitochondria were devoid of its matrix. There was a focal disorganization of the sub-epithelial tissues (Ali et al., 2015c). Similarly, the glandular tissues were disorganized with gross edema, hypoplastic secretory vesicles and granules as well as severely disturbed rough endoplasmic reticulum. Vascular changes were comparable to those of topical MMC group, however the effects of the fibro-collagenous tissues were more profound in the COS-MMC group. Severe swelling of the collagen fibers was noted. All the fibroblasts showed indistinct cytoplasm and nuclei, loss of mitochondria and peripheral chromatin condensation. Few fibroblasts also demonstrated degenerative changes and focal loss of cellular outlines.

Although the effect of MMC was discernible in both the groups, the changes were more pronounced in the COS-MMC group. The effects were limited to the areas treated and possibly point towards a role of MMC in preventing cicatricial closures of the DCR ostia.

3.8. Ultrastructural features of lacrimal stents

Lacrimal stents in the form of punctal plugs, mini-monoka stent and bicanalicular silicone stents are commonly used in the management of severe dry eye disease, punctal stenosis and as an adjuvant to a dacryocystorhinostomy. Electron microscopy of the lacrimal stents are predominantly performed to study the physical deposits, biofilm development and its effect on the hosts.

3.8.1. Punctal plugs

Punctal plugs are often used in severe dry eye disease to retain the tears at the ocular surface. SEM of 15 punctal plugs were studied and 8 of them were found to be covered by bacterial biofilms (Sugita et al., 2001). The debris on the plugs consisted of desquamated epithelial cells and fibrillar proteinaceous material. The deposited material obtained from the inside of one plug was subjected to TEM and it demonstrated numerous rod-shaped and spherical bacteria with an amorphous material in the vicinity, which could represent the polysaccharide extracellular matrix produced by the bacteria themselves. Another study assessed SEM of punctal plugs of 3 patients and found surface defects secondary to plug breakdown as the cause of local irritation of the conjunctiva and inferonasal cornea (Paprizos et al., 2013). Careful monitoring of plugs may be important to either remove the deposited material or replace the plug if needed.

3.8.2. Mini-monoka stents

Mini-monoka stents are often used in the management of canalicular tears and punctal stenosis (Singh et al., 2018). Ultrastructural features of mini-monoka stents extubated at two time points; 6 weeks and 3 months were studied and compared them with sterile stents (Ali et al., 2017). They found that the external surfaces, cut ends and the intraluminal surfaces to harbor bacterial biofilms. The intraluminal surfaces however showed clumps of physical deposits and biofilms with intervening skip areas. The ampullary portion of the monoka stent, owing to its design and tear flow dynamics was the preferred site of most deposits and hence demonstrated heavy load of biofilms. However, none of the patients had a clinical evidence of infection. The biofilms were also extensive in those stents extubated at 3 months as compared to 6 weeks. The ultrastructural findings of this study suggest the need to reduce the duration of intubation to the minimum required and also the need for lumen-less monocanalicular stent to prevent additional surfaces from acting as biofilm nidus.

3.8.3. Lester Jones tubes

Lester Jones tubes are commonly used as a lacrimal bypass tube in cases of complete proximal bicanalicular obstructions (Steele, 2016). Bacterial biofilms were demonstrated on the external and internal surfaces of a Lester Jones tube in a patient who presented with infection but was found to be culture-negative on routine microbiological examination (Parsa et al., 2010). It is not uncommon to find slimy material over the Jones tube. Persistence of irritation in spite of routine care and cleaning may indicate a possible role of biofilms and the need for tube exchange.

3.8.4. Bicanalicular lacrimal stents following DCR

Numerous variants of bicanalicular silicone stents like the Crawford, O'Donoghue, and Bika are used as adjuvants in a DCR surgery. Microbiological cultures of extubated stents were performed with controversial outcomes with regards to its role in causing tissue infections (Kim et al., 2012; Ali et al., 2013b). Parsa et al. (2010) first studied SEM of lacrimal silicone stents and demonstrated dense well established as well as recent biofilms on all the surfaces of a single stent extubated from a patient, who presented with an infection, 6 months after a DCR. Although the culture was negative by routine microbiological examination, SEM demonstrated mixed-species biofilms on the silicone stents. Samimi et al. (2013) isolated atypical mycobacteria in 90% of their infected lacrimal stents and demonstrated biofilms on 4 of the stents they studied using SEM.

The same group later compared non-infected post-DCR extubated stents with those having clinical infection (Samimi et al., 2016). Culture positivity was noted for non-tuberculous mycobacteria in 90% of the infected stents versus none in the non-infected group. SEM studies confirmed culture results with demonstration of similar bacteria in planktonic forms and biofilms on the stents. There was also a statistically significant higher amounts of biofilms on the infected stents as compared to the non-infected ones. Biofilms were also studied on extubated lacrimal stents following either an external, endoscopic or transcanalicular approach DCR and did not find any significant differences in the biofilms based on the type of surgical procedure (Balikoglu-Yilmaz et al., 2014).

Murphy et al. (2015) first demonstrated quantification of biomass on the lacrimal stents. The mean biomass was 0.9385 $\mu m^3/\mu m^2$ (range: 0.3901–1.9511 $\mu m^3/\mu m^2$). They demonstrated that presence of biofilms on the lacrimal stents was common but this does not necessarily lead to a clinical infection. Polymicrobial biofilms were demonstrated on the Crawford lacrimal stents and it was found that their ocular segments have thicker and focal biofilms as compared to the thinner and diffuse ones on the nasal segments (Ali et al., 2015e). The same group demonstrated that lumina of stents uniformly harbor biofilms and advocated the need for possible lumen-less stents to prevent additional surfaces from acting as biofilm nidus (Ali et al., 2016c). Ultrastructural features of stents retained for prolong durations (minimum of 12 months) following a DCR were also studied (Ali et al., 2016b). They found that as the duration progressed, the polymicrobial biofilms got thicker, denser and more integrated with physical deposits in the vicinity. The study indirectly indicated that the lesser the duration, the better, in terms of biofilms, although there were no instances of a clinical infection.

Further research is needed on stent mechanics and stent-tissue interactions for a meaningful clinical translation of these ultrastructural studies. The development of biofilm resistant lacrimal stents would be an example of translational value of such studies.

4. Conclusions

Ultrastructural exploration of Lacrimal drainage system so far has been limited and sparsely explored. The list of unexplored areas is exhaustive. There is a need for the lacrimal clinician-scientist to make themselves familiar with techniques and interpretation of electron microscopy to advance the ultrastructural frontier of this science.

Financial disclosure

Mohammad Javed Ali received support from the Alexander von Humboldt Foundation for his research and he also receives royalties from Springer for the 2nd edition of the textbook “Principles and Practice of Lacrimal Surgery’ and treatise ‘Atlas of Lacrimal Drainage Disorders’. Friedrich Paulsen was supported by Deutsche Forschungsgemeinschaft (DFG) grants PA738/1-1 to 1–5 as well as PA738/2-1. He receives royalties from Elsevier for the 24th Ed. Of the anatomy atlas “Sobotta” and the ‘Sobotta Textbook of Anatomy’.

Ethics statement

Originality and plagiarism: The authors ensure that they have written entirely original works, and if the authors have used the work and/or words of others, that this has been appropriately cited or quoted.

Multiple, redundant or concurrent publication: This article has not been submitted for publication nor has it been published in whole elsewhere.

CRediT authorship contribution statement

Mohammad Javed Ali: Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft. **Friedrich Paulsen:** Resources, Supervision, Writing - review & editing.

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