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Editorial

Scanning electron microscopy (SEM) in ophthalmology: unveiling the ultrastructure of the ocular surface

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Scanning electron microscopy (SEM) has transformed ophthalmological research and diagnostics by offering high-resolution, three-dimensional visualization of ocular microstructures. By surpassing the limitations of light microscopy, SEM provides critical insights into cellular and extracellular architecture, informing both clinical assessment and translational research.[1,2] Its application in dry eye disease (DED), microbial keratoconjunctivitis, and corneal pathology has redefined our understanding of ocular surface disorders.

1. Revealing Microvillar Alterations as Biomarker in Dry Eye Disease (DED)

DED is a multifactorial condition marked by tear film instability, epithelial damage, and inflammation.[3,4] SEM has become a pivotal tool for assessing ultrastructural changes on the conjunctival and corneal surfaces.[5,6] Studies have characterized epithelial microvilli—key elements in tear film adherence and epithelial signaling—demonstrating that DED disrupts their density and organization. Del Prete et al. proposed a grading scale (Grades 0–4) for microvillar damage, ranging from normal architecture to complete loss and epithelial desiccation. This system enables correlation between microstructural damage and clinical severity, offering a reproducible parameter to evaluate treatment outcomes.[7,8]

Recent investigations have extended SEM's relevance in monitoring therapeutic responses. Troisi et al. assessed the ocular surface after treatment with a novel artificial tear formulation (Trimix®) combining cross-linked hyaluronic acid, cationic liposomes, and trehalose. SEM analysis revealed significant recovery of microvilli and epithelial integrity in DED patients, highlighting the technique's utility in linking ultrastructural restoration with clinical improvement.[8-10]

2. SEM as a Novel Frontline Diagnostic Tool in Diagnosis of Microbial Keratoconjunctivitis

Microbial keratoconjunctivitis poses diagnostic challenges, especially when conventional cultures are negative.[11,12] SEM has emerged as a complementary diagnostic method by visualizing pathogens directly on the ocular surface.[13,14] It has identified elusive organisms—such as *Acanthamoeba* cysts, *mycoplasma*, *chlamydia*, and atypical *mycobacteria*—when other methods failed.[15,16] SEM findings have been instrumental in guiding targeted therapy in refractory cases.[17]

Moreover, SEM plays a vital role in biofilm research. Biofilms, consisting of microbial communities within a

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protective matrix, contribute to antibiotic resistance.[18] SEM enables detailed visualization of biofilm architecture on contact lenses and ocular surfaces, providing a foundation for the development of anti-biofilm strategies. [19]

3. Scanning Electron Microscopy in Corneal Pathologies: Insights into Disease Mechanisms, Healing, and Graft Rejection

The cornea, with its layered architecture, is a prime candidate for SEM investigation. SEM has been used to evaluate epithelial and stromal remodeling following injury, surgery, or in disease. In corneal dystrophies, it has visualized abnormal protein deposition patterns, enhancing our understanding of disease progression.[20,21] Following refractive surgery or trauma, SEM assists in evaluating collagen fibril alignment—key to maintaining transparency.[22]

SEM has also been employed to study drug-related surface toxicity. It demonstrated increased corneal and conjunctival epithelial damage in patients using benzalkonium chloride (BAK)-preserved glaucoma medications compared to preservative-free formulations.[23] In collagen cross-linking (CXL), SEM has revealed changes in collagen architecture and depth of stromal remodeling, providing insights into treatment efficacy.[24]

In corneal transplantation, SEM has helped detect early signs of graft rejection through visualization of endothelial disruption or morphological irregularities in donor tissue.[25]

4. Enhancing Diagnostic Precision: SEM Applied to Impression Cytology

The integration of SEM with impression cytology has expanded its utility in diagnosing ocular surface disorders. Impression cytology involves collecting superficial epithelial cells using a specialized filter, which collects a thin surface layer of cells that is then subsequently analyzed under SEM. This approach allows for a detailed examination of epithelial cell morphology, goblet cell density, and inflammatory cell infiltration—parameters essential for diagnosing and monitoring diseases like DED and conjunctival metaplasia.[5,7]

SEM-based impression cytology has been particularly effective in detecting early squamous metaplasia and quantifying goblet cell loss in DED. Such detailed analyses have clinical relevance, as they facilitate early intervention and allow for precise monitoring of therapeutic outcomes.[26] The ability to integrate structural and cellular information makes SEM-impression cytology a powerful diagnostic tool.[5]

5. The Future of SEM in Ophthalmology: Opportunities and Challenges

As SEM technology advances, its applications in ophthalmology are poised to expand. The development of cryo-SEM techniques, which preserve the natural state of hydrated tissues, promises to provide even more accurate depictions of ocular surface ultrastructure. Similarly, combining SEM with other imaging modalities, such as confocal microscopy and optical coherence tomography (OCT), could offer a more comprehensive understanding of the ocular surface and its pathologies.

Challenges remain, particularly in terms of accessibility and the labor-intensive nature of SEM sample preparation, and the examiner expertise required to interpret the results. Efforts to streamline sample collection and processing, along with the advent of automated SEM systems, could make this technology more accessible to a broader range of clinical and research settings. Furthermore, the integration of artificial intelligence in SEM analysis has the potential to enhance diagnostic precision and reduce subjectivity.

6. Conclusion

Scanning electron microscopy has profoundly impacted ophthalmology by providing high-resolution insights into the ultrastructural intricacies of ocular tissues. Its applications in studying dry eye disease, microbial keratoconjunctivitis, and corneal pathologies have enriched our understanding of disease mechanisms and informed the development of innovative therapies. By bridging the gap between cellular-level insights and clinical applications, SEM has cemented its role as a cornerstone of modern ophthalmology, transforming our approach to understanding and treating ocular diseases and paving the way for improved patient outcomes.

As technology evolves, SEM is likely to become even more integral to ophthalmic research and clinical practice, offering new possibilities for diagnosis, monitoring, and treatment of ocular surface diseases.

7. Conflict of Interest

None.

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