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Name of Journal: *World Journal of Gastrointestinal Pathophysiology*

Manuscript NO: 65807

Manuscript Type: MINIREVIEWS

Application of Electron Microscopy in Gastroenterology

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Abstract

Abstract: Electron microscopy has long been used in research in the fields of life sciences and materials sciences. Transmission and scanning electron microscopy and energy-dispersive X-ray spectrometry (EDX) analyses have also been performed in the field of gastroenterology. Electron microscopy and EDX enables the i) observation of ultrastructural differences in the esophageal epithelial cells in patients with gastroesophageal reflux and eosinophilic esophagitis;, ii) detection of lanthanum deposition in the stomach and duodenum, iii) ultrastructural and elemental analysis of enteroliths and bezoars, iv) detection and characterization of microorganisms in the gastrointestinal tract, v) diagnosis of gastrointestinal tumors with neuroendocrine differentiation, and vi) analysis of gold nanoparticles potentially used in endoscopic photodynamic therapy. This review aims to foster a better understanding of electron microscopy applications by reviewing relevant clinical studies, basic research findings, and the state of current research carried out in gastroenterology science.

Key Words: Transmission electron microscopy; Scanning electron microscopy; Energy-dispersive X-ray spectrometry; gastrointestinal disease, gastro-esophageal reflux disease; pathogens

Iwamuro M, Urata H, Tanaka T, Okada H. Application of Electron Microscopy in Gastroenterology. *World J Gastrointest Pathophysiol* 2021; In press

Core Tip: This review provides an overview of ⁷ transmission electron microscopy, scanning electron microscopy, and energy-dispersive X-ray spectrometry analyses used in the field of gastroenterology. Previously reported articles have been reviewed, focusing on electron microscopy applications. The history and present trends in electron microscopy applications in patients and research associated with digestive system diseases are also summarized.

INTRODUCTION

In light microscopy, visible light is used to obtain magnified views of the object. As the resolution is related to the wavelength of light used to image a specimen, the resolution of an optical microscope is theoretically limited to approximately 200 nm. Thus, it is not possible to observe nanostructures using light microscopy. In contrast, electron beams are used in electron microscopy. As the wavelength of an electron beam is shorter than that of visible light, electron microscopy has extremely high resolution and provides sharp, finely detailed images of the surface or interior of biological and non-biological specimens. In addition, ³ energy-dispersive X-ray spectroscopy (EDX), which is a chemical microanalysis technique used in conjunction with electron microscopy, enables the analysis of elements or chemical characterization of a sample. Since the development of the first prototype in 1931, electron microscopes have been widely used in various fields, such as physics, chemistry, engineering, biology, and medicine ¹. Based on its versatility, electron microscopy analysis has been used in several studies covering various aspects of clinical samples obtained from patients with gastrointestinal diseases. This paper briefly discusses the fundamentals of electron microscopy and reviews the literature concerning the application of electron microscopy in gastroenterology science.

ANALYTICAL METHODS IN ELECTRON MICROSCOPY

Analytical methods in electron microscopy can broadly be categorized into three types: ⁶ transmission electron microscopy, scanning electron microscopy, and energy-dispersive X-ray spectroscopy (EDX). The different types of electron microscopes used in these methods are related and often applied concurrently in the field of biology.

A transmission electron microscope irradiates the specimen with an electron beam. The object must be cut into very thin cross-sections because it is visualized through the spatial distribution of the transmitted electron beam. Although the use of transmission electron microscopy is limited to engineering science at the outset, it has been extensively used in the field of biology since the 1950s. This is largely due to the

improvement of the microtome for ultrathin slice preparation using a diamond knife and the development of staining techniques based on heavy metals, such as osmium.

A scanning electron microscope produces an image using electrons that are reflected or generated from the surface of the specimen. The specimen is placed in a high vacuum state, and the surface is scanned with an electron beam focused by an electric or magnetic field. Scanning electron microscopy (SEM) produces a characteristic three-dimensional appearance that is useful for understanding the surface ultrastructure of a sample.

EDX is an X-ray system used to identify the elemental composition of a material. It has a semiconductor detector to detect the fluorescent X-rays generated when the primary X-ray beam illuminates the sample. The fluorescent X-rays emitted from the material have a spectrum of wavelengths characteristic of the types of atoms present in the specimen. EDX enables both qualitative and semi-quantitative analysis of the elements based on the energy and number of generated electron-hole pairs. EDX is more suited for the analysis of inorganic materials than organic materials.

In the field of gastroenterology, transmission and scanning electron microscopy and EDX analyses have been used to visualize cells (Figure 1) and pathogens, including parasites, bacteria, viruses, biofilms, and elements deposited in the gastrointestinal mucosa. Non-biological materials, such as stents, powders, and bezoars, have also been analyzed at sub-nanometer resolution. In the following sections, we review examples of electron microscopy analyses in association with the pathophysiology of gastrointestinal disorders.

EXAMPLES OF ELECTRON MICROSCOPY ANALYSES

Intercellular spaces of the esophageal epithelium

The most typical example of electron microscopy analysis in gastroenterology is the evaluation of the intracellular spaces of esophageal epithelial cells. It is particularly noteworthy that some of the articles on this topic have been published in high-impact journals. Intercellular spaces in the esophageal epithelium are known to be dilated in

patients with non-erosive reflux disease and in patients with esophagitis. Subsequent to several animal studies,² endoscopic esophageal biopsy specimens taken from patients with ($n = 11$) and without ($n = 13$) recurrent heartburn were investigated in 1996 using transmission electron microscopy². A dilated intercellular space diameter was observed in 8 of the 11 patients with heartburn, while none of the asymptomatic individuals exhibited this feature. Dilated intercellular space was also present in the normal-appearing, non-erosive mucosa of patients with symptomatic reflux disease. Other authors have provided further evidence that detached inter-epithelial cell junctions, which are observed as dilated intercellular spaces assessed by electron microscopy, correspond to early esophageal damage induced by acid reflux³⁻⁹. The dilatation of intercellular spaces in the esophageal epithelium is not observed in patients with functional heartburn, suggesting that this microscopic feature is specific to acid reflux¹⁰. Proton pump inhibitor therapy resulted in a complete recovery of dilated intercellular spaces in $> 90\%$ of cases with non-erosive reflux disease and erosive esophagitis, indicating that the electron microscopy features are reversible^{11,12}.

Dilated intracellular spaces arise along the distal and proximal esophagus of patients with non-erosive reflux disease, suggesting that it may be an underlying mechanism accounting for the enhanced perception of proximal acid reflux¹³. It has also been reported that duodenal gastroesophageal reflux causes the dilatation of intercellular spaces in the esophageal epithelium^{3,14,15}. Similarly, in patients with laryngopharyngeal reflux and sore throat, this feature appears at the squamous basal and suprabasal levels in oropharyngeal biopsy specimens^{16,17}. An investigation of patients with bronchial asthma^{12,18} and children with reflux-related cough¹⁹ revealed that the intracellular spaces in the esophageal epithelium are significantly dilated compared with control patients, suggesting a pathophysiological correlation between gastroesophageal reflux and the development of these respiratory tract symptoms.

Although the width of the intracellular spaces can be measured using light microscopy²⁰, the sensitivity of light microscopy was 79.3%, and the specificity was 75.0%²¹. Owing to the inferior specificity of light microscopy analysis, electron

microscopy seems to be more suitable for measuring the intercellular spaces in the esophageal epithelium. Meanwhile, Chu *et al* reported the possible utility of *in vivo* confocal laser endomicroscopy to examine microalterations of the esophagus in patients with non-erosive reflux disease ²².

⁵ *Eosinophilic esophagitis*

Eosinophilic esophagitis is a chronic, allergic inflammatory condition of the esophagus. Dilated intracellular space is seen in the esophageal epithelium of patients with eosinophilic esophagitis, and it is significantly reduced after treatment ^{23,24}. Transmission electron microscopy revealed a significant decrease in the number of desmosomes ²⁵ and increased autophagic vesicle content ²⁶ in active eosinophilic esophagitis, compared with normal individuals and inactive eosinophilic esophagitis patients. Thus, electron microscopy may be useful for investigating the pathophysiology of eosinophilic esophagitis.

Lanthanum deposition

Lanthanum carbonate is a phosphate binder taken orally and is commonly used in patients with chronic kidney disease. Although its tolerability and safety profile have been reported in hemodialysis patients, lanthanum deposition in the gastric and duodenal mucosa of these patients, in the form of lanthanum phosphate, has been reported in the literature ²⁷⁻³². On light microscopy examination of the hematoxylin and eosin-stained specimens, deposited lanthanum is visible as a fine, amorphous, eosinophilic material. Scanning electron microscopy revealed bright areas in the deposited lanthanum (Figure 2A). Images at a higher magnification show deposition as the accumulation of minute particles (Figure 2B). EDX analysis provided evidence directly related to the presence of lanthanum and phosphate (Figure 2C). Elemental mapping by EDX revealed that ¹lanthanum (Figure 2D) and phosphate (Figure 2E) showed an identical location to that of the bright areas in scanning electron microscopy. Although the diagnosis of lanthanum deposition in the gastrointestinal tract can

clinically ¹ be made with conventional light microscopy observation of the fine, amorphous, eosinophilic material, and medication information of the patient's current or past use of lanthanum carbonate, scanning electron microscopy has advantages in the detection of deposited lanthanum, as it is easily identified as bright areas.

Enteroliths and bezoars

Enteroliths are calculus that occur in the intestines. There are two types of enteroliths, "true" and "false" enteroliths ³³. True enteroliths, for example, cholic acid and calcium stones, are made from the sediments of substances found in enteric contents. False enteroliths, such as bezoars, gallstones, and foreign objects, are formed from indigestible substances stuck in the alimentary tract. Infrared spectroscopy is generally used for the identification of chemical substances that constitute enteroliths removed from patients. Electron microscopy and EDX have the advantages of imaging the microstructure and analyzing the elements, leading to the clarification of the nature of enteroliths.

Figure 3 shows examples of enteroliths and bezoars that we previously investigated. One patient had an enterolith in the stomach composed of bilirubin calcium, calcium carbonate, and fatty acid calcium ³⁴ (Figure 3A, 3B). Another patient had a rare pharmacobezoar in the stomach, which was made of magnesium oxide (Figure 3C–3F) ³⁵. We also investigated the ultrastructure of the persimmon phytobezoar in the stomach (Figure 3G–3I) ³⁶. Thus, electron microscopy and EDX analyses offer insights into the microstructure and elemental composition of enteroliths.

Pathogens including bacteria, parasites, and viruses in the gastrointestinal tract

Electron microscopy has been widely used in microbiology to elucidate the number, distribution, and adherence of microorganisms in clinical samples. One of the typical applications of electron microscopy for pathogens in gastroenterology is the detection of *Helicobacter* species, such as *Helicobacter pylori* ^{37–41} and *Helicobacter heilmannii* ⁴². These bacteria have a spiral form, which is a distinct difference from other

bacteria. Another example is *Tropheryma whipplei* ⁴³⁻⁴⁶, which causes a rare systemic infectious disorder Whipple's disease. Electron microscopy revealed that *Tropheryma whipplei* shows a characteristic trilamellar plasma membrane. Other rare pathogens identified by electron microscopy include Anisakiasis ⁴⁷, Amoebiasis ⁴⁸, Intestinal spirochetosis ⁴⁹, *Sutterella wadsworthensis* ⁵⁰, *Giardia Intestinalis* ⁵¹, and *Brachyspira aalborgi* ⁵².

A biofilm is a thick layer formed by microorganisms attached to the surface of a solid material or liquid. Scanning electron microscopy has been used to visualize the shape and localization of biofilms and the steps of the biofilm formation process. For instance, several authors have investigated the efficiency of the cleaning, disinfection, and sterilization processes of biofilm-contaminated endoscopes ^{53,54}.

Gastrointestinal tumor with neuroendocrine differentiation

Neuroendocrine and mixed neuroendocrine neoplasms can arise in most of the epithelial organs of the body and are not rare in the gastrointestinal tract. Transmission electron microscopy revealed that neuroendocrine tumor cells in the gastrointestinal tract contained numerous dense-core secretory granules of variable sizes and shapes in the cytoplasm. Because these neurosecretory granules are characteristic of neuroendocrine tumors, electron microscopy analysis has been used to support its diagnosis. For instance, neuroendocrine differentiation was assessed using electron microscopy images in cases of malignant peripheral nerve sheath tumor of the esophagus ⁵⁵, gangliocytic paraganglioma in the duodenum ⁵⁶, mixed acinar-endocrine carcinoma arising in the ampulla of Vater ⁵⁷, combined adenocarcinoma and neuroendocrine tumor in the stomach ⁵⁸, neuroendocrine carcinoma in the stomach ⁵⁹, mixed acinar-endocrine neoplasm in the stomach ⁶⁰, and large cell neuroendocrine carcinoma in the esophagogastric junction ⁶¹.

Gold nanoparticle potentially used in endoscopic photodynamic therapy

Based on the properties of absorption and scattering of electromagnetic radiation, gold nanoparticles are emerging as promising agents and are of particular interest for applications in photothermal therapy, in addition to efficient drug carriers and diagnostic agents. For instance, endoscopic fluorescence-guided near-infrared photothermal therapy using gold nanoparticles is in development for the treatment of gastrointestinal tumors ⁶². The size, morphology, and composition of synthesized gold nanoparticles and their location within the tissue can be assessed using transmission electron microscopy and EDX analysis ⁶³.

CONCLUSION

Electron microscopy enables the i) observation of ultrastructural differences in esophageal epithelial cells in patients with gastroesophageal reflux and eosinophilic esophagitis, ii) detection of lanthanum deposition in the stomach and duodenum, iii) ultrastructural and elemental analysis of enteroliths and bezoars, iv) detection and characterization of microorganisms in the gastrointestinal tract, v) diagnosis of gastrointestinal tumors with neuroendocrine differentiation, and vi) analysis of gold nanoparticles potentially used in endoscopic photodynamic therapy. Therefore, electron microscopy has had a profound impact on our knowledge and understanding of various digestive tract diseases. We hope that this article will help gastroenterologists to widely utilize electron microscopy analysis for clinical diagnosis and basic research.

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