MICROSCOPY

Microscopy, 2020, 408–410
doi: 10.1093/jmicro/dfaa037
Advance Access Publication Date: 6 July 2020

Technical Report

TEM observation of inorganic substances distributed in gel materials for medical devices using ultra-thin cryosectioning

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Received 17 March 2020; Revised 27 June 2020; Accepted 6 July 2020

Abstract

In this study, a method was developed for examining the distribution pattern of inorganic substances dispersed in hydrogel-filled medical devices. Transmission electron microscopy (TEM) using ultra-thin cryosectioning (owing to hydrogel's water content) was performed on contact lenses with an iris pattern in which the distribution pattern of inorganic pigments was problematic. We confirmed the depth and distribution pattern of pigments in the hydrogel. The results indicated that ultra-thin cryosectioning with TEM was effective for inspecting the distribution of inorganic substances in hydrogel-filled devices.

Key words: hydrogel, iris lens, inorganic substances, transmission electron microscope, ultra-thin sectioning method, cryosectioning

Medical devices in which various inorganic substances are dispersed in the hydrogel are becoming commonplace. They include a drug delivery system, in which gels are perfused with anticancer drugs, slowly release them [1], an artificial cartilage made of gels with hydroxyapatite combined with gel interface [2] and soft contact lenses with iris patterns dyed with pigments such as iron oxide [3]. For safety and functionality, the distribution of inorganic substances in gel devices is essential. Therefore, we devised a method to inspect the distribution pattern of inorganic substances in hydrogels by attempting to observe the dispersion of pigment particles in such lenses.

In this study, we evaluated soft contact lenses with iris patterns as hydrogel devices in which inorganic substances were distributed. In this soft contact lens, the distribution depth and distribution pattern of pigment used are often closely related to safety from the viewpoint of eye and eyelid damage owing to direct physical stimulation [4] and bacterial adhesion [5]. Because it is a problem whether or not the pigment is present on the surface, it is necessary to accurately determine whether the pigment is exposed on the lens surface or buried inside. Until now, the cross-section of the lens was swollen in a dry state [6] or with an ionic liquid [7–9] and observed by scanning electron microscopy (SEM). However, to more accurately determine the position of pigment, we decided to prepare a sample that was cut vertically relative to the lens surface to create the cross-section of the lens and observe it by transmission electron microscopy (TEM).

TEM is a commonly used method to observe components dispersed in soft matter, particularly those with a depth direction distribution, using samples embedded in resin from which ultra-thin section is prepared. However, hydrogel materials swell in water, which is also a solvent. The combination of epoxy resin and hydrogels with their water content poses challenges because water acts as an inhibitor during the polymerization of epoxy resin. In addition, even if the epoxy resin is polymerized, in the sections of the sample, separation occurs among gel materials that are swollen with water and epoxy components that are not. Consequently, epoxy resins are unsuitable when preparing hydrogel samples for TEM.

Therefore, we attempted to prepare ultra-thin cryosectioning to inspect the embedded inorganic substances by TEM.

First, the observation of distribution pattern was performed on commercial iris-patterned soft contact lenses in the following categories.

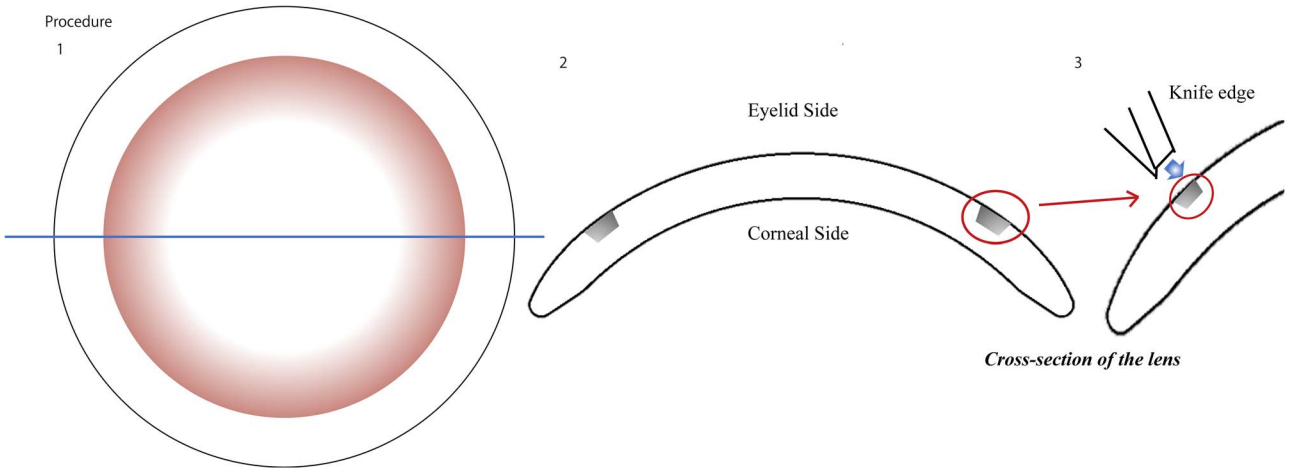

Fig. 1. Preparation of ultra-thin cryosectioning of a soft contact lens with an iris pattern that has a dyed portion on the eyelid side.

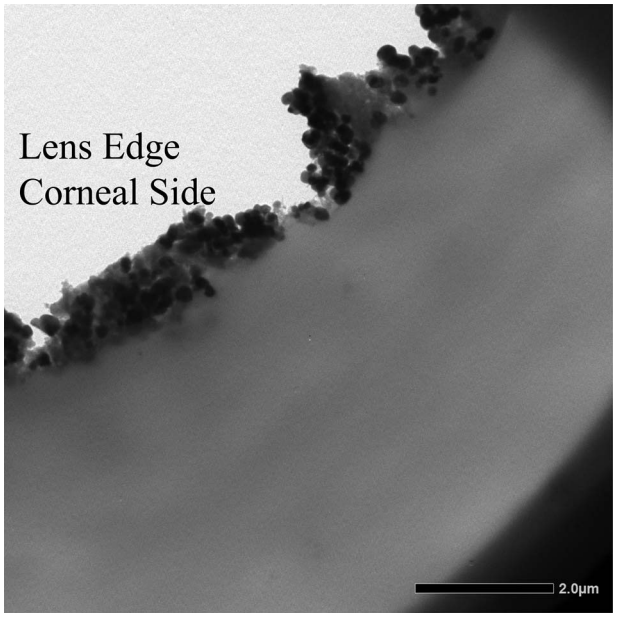

Fig. 2. TEM micrograph of Sample 1; black (iron oxide) particles are located at the lens edge (corneal side); the length of the scale bar represents 2.0 μm.

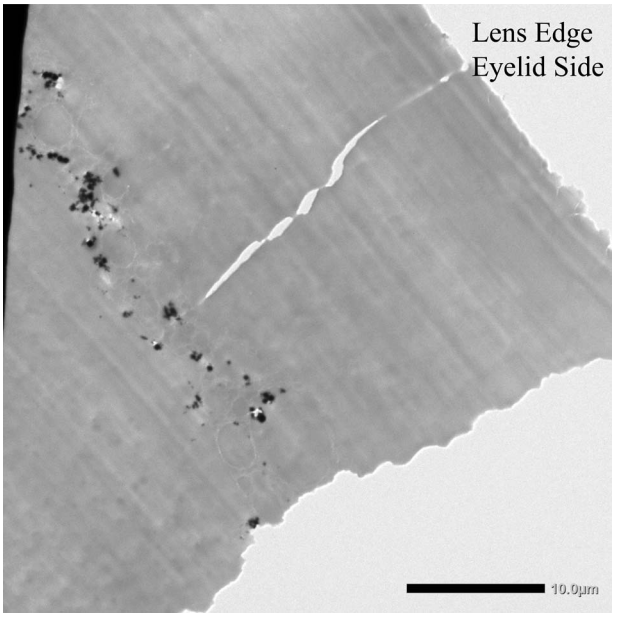

Fig. 3. TEM micrograph of Sample 2; black (iron oxide) particles are located inside the lens edge (eyelid side); the length of the scale bar represents 10.0 μm.

Sample 1: Contact lens group classification [10]—Group I, water content 38%.

Sample 2: Contact lens group classification—Group IV, water content 58%.

A lens was removed from its case, and the supplied contact lens solution was replaced with distilled water to remove NaCl and other components in the soaking solution. After exchanging distilled water multiple times, the sample lens was cut in half and then frozen in the ultra-microtome chamber.

According to the sample preparation method shown in Fig. 1, the lens was halved, as shown in 1, and a dyed portion was selected from the lens surface cross-section in 2. The temperature of an ultra-microtome (Leica EM UC7 with FC7) and the inside layer of Cryo Unit (FC7) was adjusted to be between −80 and −100°C. The sample was clamped with a sample holder and cut with a knife at an angle of 90° to the sample cross-section surface. The 100-nm thick cross-sectional slices were prepared with a glass knife, as shown in 3. The sections were placed on copper grids with a carbon substrate.

All sections were analyzed without staining using a TEM instrument (JEOL JEM-1400Plus, JEM-z2500 for obtaining X-rays) with an accelerating voltage of 120 kV.

In Sample 1, a pupil pattern was printed on the corneal side and on the eyelid side in Sample 2. For each type, the dyed lens edge was inspected.

Figures 2 and 3 show the TEM observation results of samples 1 and 2. As shown in Fig. 2, in Sample 1, it is confirmed that black round bodies are aggregated at the edge of the lens. Figure 3 shows the observation result of a different soft contact lens with an iris pattern (Sample 2). For this sample, it was confirmed that pigments were distributed in the depth direction of approximately 20 μm and the pigments were dispersed.

The EDS measurement indicated that the black round bodies at the lens edge observed by TEM analysis in Sample 1 were mainly composed of iron oxide.

Our results suggested that the TEM observation results confirmed that both Samples 1 and 2 had pigment components that were distributed in the hydrogel but the pigments in Sample 1 were aggregated and distributed toward the lens edge. These results confirm that the pigment components were present and aggregated on the lens surface. It was also confirmed that iron was included in the constituent elements of the pigments. Iron oxide is widely used in ink for lenses with an iris pattern. This is consistent with the obtained results. However, it was confirmed that the pigment components in Sample 2 were arranged at a certain depth, and the pigments there were dispersed. This result indicates that pigments were dispersed in the lens.

Until now, lenses using dispersed pigments were typically evaluated by SEM. Currently, for a soft contact lens with an iris pattern, it is reported that the distribution of pigment is observed by SEM using a soft contact lens, which is made of a hydrogel in a dry state or a swollen state with an ionic liquid. In this study, frozen lenses were prepared using a contact lens swollen with water; thus, the original morphology of hydrogel can be observed. Owing to the morphological observation in cryosectioning, the pigment position in the lens was observed more accurately. By including water and freezing it, the hardness was increased, and the production of ultra-thin sections was facilitated. In addition, by half-cutting and cutting out a section at a right angle from the cross-section where the dyed part exists, the depth where the pigment exists can be accurately evaluated by analyzing the lens edge. However, even if TEM is used, the resin embedding method is ineffective because epoxy resins fail to polymerize owing to the water content in the gel.

It was determined that the distribution depth and pattern of pigments dispersed in the soft contact lens swollen by water can be observed more accurately by ultra-thin cryosectioning and using TEM.

Finally, we consider the distribution of pigment in soft contact lenses with the iris pattern as a demanding issue. In this study, the distribution of pigment in hydrogel contact lenses (two different types) with an iris pattern was observed by TEM and an ultra-thin cryosectioning method. The developed method can be utilized to examine the distribution positions of inorganic substances dispersed in hydrogel medical devices. This approach can be applied to lenses and other medical devices that are made of hydrogel.

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