Purpose: To discuss the unique morphology and origin of epiretinal proliferation associated with macular hole (EPMH) occasionally observed in full-thickness macular hole (FT-MH) or lamellar hole (LH) and to introduce the perifoveal crown phenomenon encountered when removing this unusual proliferative tissue.

Methods: Sixteen patients showing EPMH in spectral domain-optical coherence tomography were selected from 212 patients diagnosed with MH, LH, FT-MH, impending MH, macular pseudohole, or epiretinal membrane between January 2013 and December 2014. Of the 212 patients included for clinical analysis, 33, 23, 11, 7, and 190 exhibited LH, FT-MH, impending MH, macular pseudohole, and epiretinal membrane, respectively. We reviewed visual acuity, macular morphology, and clinical course. Surgical specimens were analyzed histologically.

Results: EPMH presented as an amorphous proliferation starting from the defective inner/outer segment (IS/OS) junction covering the inner macula surface. Among the 16 patients with EPMH, 11 underwent vitrectomy, and all exhibited the intraoperative perifoveal crown phenomenon. EPMH tissue was sampled in three patients, one of whom had more tissue removed than intended and showed delayed recovery in visual acuity. Despite hole closure, IS/OS junction integrity was not successfully restored in four of 11 patients. Five patients were followed-up without surgical intervention. Visual acuity slightly decreased in three patients and did not change in one patient, while the remaining patient was lost during follow-up. Among the three perifoveal crown tissues obtained, two were successfully analyzed histologically. Neither tissue showed positivity to synaptophysin or S-100 protein, but one showed positivity to cytokeratin protein immunohistochemical staining.

Conclusions: EPMH exhibited a distinct but common configuration in spectral domain-optical coherence tomography. An epithelial proliferation origin is plausible based on its configuration and histological analysis. Perifoveal crown phenomenon was observed when removing EPMH during vitrectomy.

Key Words: Epiretinal proliferation, Lamellar hole, Macular hole, Perifoveal crown phenomenon, Retinal pigment epithelium
Recent advances in high-resolution optical coherence tomography (OCT) have helped to reveal the pathogenesis of various vitreomacular pathologies [1-7]. Reassessment of macular diseases such as lamellar hole (LH) or macular pseudohole (MPH) with spectral domain (SD)-OCT led to the discovery of distinct epiretinal proliferations with features different from those of the conventional epiretinal membrane (ERM) [2,8-15]. These structures have been referred to using several unestablished terms such as thick ERM [8], dense non-tractional ERM [9], thicker ERM [11], or LH associated retinal proliferation [13].

This distinct proliferation appears to present properties dissimilar from conventional ERM. Since it was first described by Witkin et al. [8], this phenomenon has been analyzed in several studies. Parolini et al. [9] performed a clinicopathological case series study and conducted a histological comparison between dense ERM and conventional tractional ERM. Pang et al. [13] analyzed 68 of 2,030 SD-OCT images of this proliferation in a retrospective review; the authors presented its distinctive morphological figures and non-tractional properties, proposing the term lamellar hole-associated epiretinal proliferation (LHEP). More recently, Schumann et al. [12] and Compera et al. [15] compared various aspects of this unusual epiretinal proliferation between LH and MPH.

Despite a variety of previous studies on unusual epiretinal proliferation, no consensus has been reached regarding its configuration, intraoperative findings, postoperative prognosis, or origin. The objective of this retrospective review of SD-OCT images was to analyze the incidence, morphology, and clinical prognosis of this unusual proliferation. Based on the potential pathogenesis and origin of this occurrence, we propose the new term “epiretinal proliferation associated with macular hole (EPMH).” In addition, we introduce the “perifoveal crown phenomenon” encountered during vitrectomy when peeling away the EPMH.

**Materials and Methods**

**Definitions**

1) **Epiretinal proliferation associated with LH or MH**

In this case study, we found amorphous and substantial epiretinal proliferation in 16 patients using SD-OCT. The proliferative tissue showed a thick blanket-like morphology with medium reflectivity, covering the edge of the MH or LH. We named this unusual amorphous epiretinal proliferation EPMH (Fig. 1A-1F).

**Fig. 1.** Preoperative spectral domain-optical coherence tomography images of full-thickness macular hole (A,B), impending macular hole (C,D), and lamellar macular hole (E,F); (A) patient no. 10, (B) patient no. 7, (C) patient no. 8, (D) patient no. 6, (E) patient no. 3, and (F) patient no. 5. Note the thick proliferative tissue mounted at the edge of the hole (bold arrows). Eyes with full-thickness macular hole or impending macular hole were regarded to have inherent disruption of the inner/outer segment junction of the photoreceptor layer. Eyes with lamellar macular hole (E,F) are considered to have defective inner/outer segment junction when an ellipsoidal defect or disconnection is observable (bold triangle).
2) Perifoveal crown phenomenon

We centripetally peeled the yellowish epiretinal tissue on the macula that appeared to present as EPMH in the preoperative SD-OCT scan. After peeling the tissue, we observed a floating crown-like yellowish tissue with its base attached to the edge of the margin. We defined this as the perifoveal crown phenomenon (Fig. 2).

3) Macular hole

For MH diagnosis, we selected patients that fulfilled either one of the two following conditions presented via SD-OCT: (1) full-thickness macular hole (FT-MH) allowing direct connection of the vitreous cavity with bare retinal pigment epithelium (RPE), (2) impending macular hole (I-MH) that showed foveal thinning with evident near-total defect in the outer retina at any point but with no direct connection between the vitreous cavity and bare RPE.

4) Lamellar macular hole

We used previously published SD-OCT criteria [2,8] to diagnose LH: (1) foveal thinning with irregular contour, (2) breaks in the inner layers of the fovea, (3) intraretinal split, and (4) absence of a full-thickness foveal defect.

5) Macular pseudohole

MPH was diagnosed according to the following criteria: (1) steepening or verticalization of the foveal edges, (2) increase in perifoveal thickness, and (3) normal central foveal thickness.

Patients

We retrospectively reviewed the medical records of 259 patients diagnosed with MH, LH, or ERM using SD-OCT performed by one surgeon (JS) between January 1, 2013 and December 31, 2014. Among the 259 patients, 42 were excluded because they had other severe concurrent ocular diseases such as end-stage age-related macular degeneration or proliferative diabetic retinopathy. Five vitrectomized patients were also excluded. The prevalence of each LH, MH (FT-MH and I-MH), MPH, and ERM was analyzed.

Among the 212 patients, the eye morphology of 16 patients with EPMH was analyzed and clinically followed up. Of these 16 patients, 11 with EPMH (three with FT-MH, four with I-MH, and four with LH) underwent standard vitrectomy. Surgical indications comprised one of the following: (1) FT-MH or I-MH, (2) LH with inner/outer segment (IS/OS) junction disruption and reduced visual acuity 0.2 (in logarithm of the minimal angle of resolution [logMAR] ) or worse, and (3) subjective visual discomfort including metamorphopsia. The other five patients (four with LH and one with I-MH) were followed up periodically without surgical intervention because they did not fulfill any of these indications. One patient with I-MH (no. 16) was lost during follow-up.

Surgical procedure

A 23-gauge transconjunctival suture-less vitrectomy was performed by one surgeon (JS). Concurrent ERM was carefully removed using retinal forceps. A yellowish tissue covering the central macula was identified and presented as EPMH in the preoperative SD-OCT. The yellowish tissue was gently peeled in the centripetal direction using a soft-tip cannula or internal limiting membrane (ILM) forceps. When peeling the tissue, a crown-like structure appeared around the fovea. We named this perifoveal crown tissue (Fig. 2).

Indocyanine green (ICG)-assisted ILM peeling was performed in all but one patient. ICG was not applied in one
patient (no. 2) due to a well-differentiated ILM. Redundant perifoveal crown tissues were trimmed only when there was dehiscence within the flapping yellowish tissue. In three patients (no. 1, 2, and 3), these torn perifoveal crown tissues were removed with retinal scissors, while the tissue was preserved in the other eight patients. Particular care was taken to minimize unnecessary traction on the fovea. A perfluoropropane gas tamponade was performed in three patients (no. 5, 9, and 10), while an air tamponade was performed in the other eight patients. Surgeries were completed without any complications.

**Visual acuity and macular configuration follow-up**

Visual acuity and macular configuration were recorded throughout the clinical visits of the 16 patients with EPMH. One patient (no. 16) was lost after the initial visit. Visual acuity was measured in logMAR units. Medial opacity was not observed in any of the other 15 patients during follow-up.

Macular configuration was recorded at every visit using SD-OCT (Cirrus, Carl Zeiss Meditec, Dublin, CA, USA; Spectralis, Heidelberg Engineering, Heidelberg, Germany). Volumes of B-scan images were extracted from Heidelberg software (Heidelberg Viewer Module 5.6.4) and saved as JPG files. The saved images were analyzed by two physicians (GS and SL) and confirmed by a senior grader (JS). The largest hole diameter (for FT-MH and LH) and the thinnest foveal thickness (for I-MH and LH) were measured with equipped caliper software, and IS/OS junction defects and hole closures post-surgery were analyzed. Eyes with FT-MH or I-MH were regarded to have inherent IS/OS junction disruption. Eyes with LH were considered to have defective IS/OS junction when ellipsoidal defect or disconnection was observed (bold triangle) (Fig. 1).

**Specimen preparation**

Perifoveal proliferative tissue was removed from the eyes of three patients (no. 1, 2, and 3). Special care was taken to acquire only the yellowish proliferative tissue. Each tissue specimen was fixed in 10% buffered formalin and sent to another affiliation for histological analysis [2]. Each tissue was then embedded in paraffin and stained with hematoxylin and eosin. Automated immunocytochemical staining was performed using the Bond-max system (Leica Microsystems, Bannockburn, IL, USA). Briefly, all steps were performed using the manufacturer’s instructions in the following order: deparaffinization; heat-induced epitope retrieval (antigen unmasking); peroxide block; incubation with synaptophysin (1:400, SY38; Dako, Carpinteria, CA, USA), S-100 protein (1:500, rabbit polyclonal; Dako), and pan-keratin (1:100, AE1/AE3; Novocastra, Newcastle, UK) primary antibodies; color development with 3,3’-diaminobenzidine tetrahydrochloride chromogen; hematoxylin counterstaining; and mounting of the slides. Normal human serum served as a negative control. Samples were considered positive for synaptophysin, S-100 protein, and pan-keratin if they exhibited any degree of cytoplasmic staining. For photodocumentation, we used a digital camera to image the specimens at >200 magnification (E1000; Nikon, Tokyo, Japan). Synaptophysin was used to mark cells of neuronal origin, S-100 protein for Müller cells, and pan-keratin for cells of epithelial origin.

**Ethics statement**

This study was approved by the institutional review board of HanGil Eye Hospital. Informed consent was obtained from all 16 patients with EPMH. This study adhered to the tenets of the Declaration of Helsinki.

**Results**

**Incidence and demographics**

Among the 212 patients, 33 were diagnosed with LH, 34 with MH (23 FT-MH and 11 I-MH), seven with MPH, and 190 with ERM. All patients with LH and MPH exhibited concurrent ERM (100% each), while only eight of the 23 patients with FT-MH (34.78%) and four of the 11 patients with I-MH (36.36%) had ERM. A total of 138 patients had ERM without other concurrent disease. Among the 212 patients, we analyzed 16 with EPMH (Figs. 1 and 3A-3H).

The mean age of the 16 patients was 60.63 years, five were men, and 11 were women. Eight patients had MH (three with FT-MH and five with I-MH), while eight patients were diagnosed with LH (Table 1). All patients with
**Table 1. Demographics of 16 patients with epiretinal proliferation associated with LH or MH**

| Patient no. | Age (yr) | Sex | DM (yr) | HTN (yr) | SE | Initial VA | Dx | Concurrent pathology | Phakic status | History | Intraocular tamponade | ICG dye use | Prone |
|-------------|----------|-----|---------|----------|----|------------|----|----------------------|--------------|---------|----------------------|-------------|-------|
| 1           | 67       | F   |         | 20       | 8  | -1.63      | I-MH | ERM, DR              | Pseudophakic | None    | Air                  | Yes         | No    |
| 2           | 46       | F   |         | -        | -4.75| 0.4        | LH   | ERM, localized RD    | Phakic       | None    | Air                  | No          | No    |
| 3           | 58       | M   |         | -        | -1.38| 0.2        | LH   | ERM                  | Pseudophakic | None    | Air                  | Yes         | No    |
| 4           | 60       | F   | 6       | 7        | 0.75| 0.2        | I-MH | ERM, DR              | Phakic       | 1 Cycle of panretinal photocoagulation | Air | Yes | No |
| 5           | 73       | F   |         | -        | -1  | 0.10       | LH   | ERM                  | Pseudophakic | None    | Gas (C3F8 6%)        | Yes         | No    |
| 6           | 59       | F   | 20       | -        | -0.75| 0.7        | I-MH | ERM                  | Phakic       | None    | Air                  | Yes         | No    |
| 7           | 56       | M   | 10       | -        | -1.5 | 0.15       | FT-MH | ERM                  | Pseudophakic | None    | Air                  | Yes         | 3 hr  |
| 8           | 65       | F   |         | -        | -4.38| 0.4        | I-MH | ERM                  | Phakic       | None    | Air                  | Yes         | 2 hr  |
| 9           | 58       | F   | 5        | -        | +0.25| 0.5        | FT-MH | ERM                  | Phakic       | None    | Gas (C3F8 8%)        | Yes         | 2 hr  |
| 10          | 67       | F   | 10       | 10       | 0.4 | FT-MH      | ERM  | Pseudophakic         | Barrier laser | Gas (C3F8 10%) | Yes | 2 hr |
| 11          | 62       | M   |         | -        | -2.62| 0.8        | LH   | ERM                  | Pseudophakic | Two previous RD operation | Air | Yes | 3 hr |
| 12          | 74       | F   |         | -        | 0.00 | 0.05       | LH   | ERM                  | Pseudophakic | None    | No surgery*          | No surgery* | No surgery* |
| 13          | 85       | M   | -        | 15       | -0.15| 0.05       | LH   | ERM                  | Pseudophakic | None    | No surgery*          | No surgery* | No surgery* |
| 14          | 50       | F   |         | -        | -2.75| 0.2        | LH   | ERM                  | Pseudophakic | None    | No surgery*          | No surgery* | No surgery* |
| 15          | 50       | F   |         | -        | -2.60| 0.3        | LH   | ERM                  | Pseudophakic | None    | No surgery*          | No surgery* | No surgery* |
| 16          | 56       | M   |         | -        | -0.88| 0.1        | I-MH | ERM                  | Phakic       | None    | No surgery*          | No surgery* | No surgery* |

LH = lamellar hole; MH = macular hole; DM = diabetes mellitus; HTN = hypertension; SE = spherical equivalent; VA = visual acuity in logarithm of the minimal angle of resolution; Dx = diagnosis; ICG = indocyanine green; F = female; I-MH = impending macular hole; ERM = epiretinal membrane; DR = diabetic retinopathy; RD = retinal detachment; M = male; C3F8 = perfluoropropane; FT-MH = full-thickness macular hole.

*Patients no. 12 to 16 were followed-up periodically without surgical intervention.
EPMH had concurrent ERM (100%). Six patients exhibited phakic eyes, while the remaining 10 had pseudophakic eyes. In two patients (no. 1 and 4), signs of mild non-proliferative diabetic retinopathy, such as microaneurysm or cotton wool spot, were observed but were not complicated with diabetic macular edema. Peripheral localized retinal detachment was accompanied in one patient (no. 2), although the lesion was on the inferior retinal periphery.

**Morphological analysis**

The largest hole diameter and the thinnest foveal thickness were measured on the initial visit using caliper software. The thinnest foveal thickness was not measurable in eyes with FT-MH, and the largest hole diameter was not measurable in eyes with I-MH. Each value was compared according to the presence of EPMH within each group using the Mann-Whitney U-test (Table 2). In the LH group, the largest hole diameter (± standard deviation) was smaller in eyes with EPMH than in eyes without EPMH, but the difference was not statistically significant (952.27 ± 433.76 vs. 1,300.19 ± 713.48 µm, \( p = 0.103 \)). The thinnest foveal thickness was significantly thinner in LH eyes with EPMH than in LH eyes without EPMH (110.36 ± 75.45 vs. 152.15 ± 47.36 µm, \( p = 0.003 \)). In eyes with FT-MH, there was no

| Table 2. Results of initial spectral domain-optical coherence tomography morphological analysis |
|---------------------------------|------------------|------------------|------------------|------------------|------------------|
| Group                          | Measurement      | With EPMH (µm)   | Without EPMH (µm)| \( p\)-value     |
| LH (8 vs. 25) \( ^1 \)         | Largest hole diameter | 952.27 ± 433.76  | 1,300.19 ± 713.48| 0.103            |
|                                | Thinnest foveal thickness | 110.36 ± 75.45   | 152.15 ± 47.36   | 0.003            |
| FT-MH (4 vs. 23) \( ^1 \)      | Largest hole diameter | 727.50 ± 132.88  | 797.37 ± 269.97  | 0.655            |
| I-MH (4 vs. 7) \( ^1 \)        | Thinnest foveal thickness | 23.00 ± 4.71     | 30.50 ± 11.02    | 0.298            |

Values are presented as mean ± standard deviation.

EPMH = epiretinal proliferation associated with macular hole; LH = lamellar hole; FT-MH = full-thickness macular hole; I-MH = impending macular hole.

Mann-Whitney U-test was performed to compare mean values; \(^1\) Data in parentheses indicate number of eyes with or without EPMH.

| Table 3. Surgical outcomes in relation to visual acuity and macular configuration in patients no. 1 to 11 |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Patient no.                                     | Perifoveal crown tissue removal | Final visit (mon) | Visual acuity (in logMAR) | IS/OS junction (ellipsoidal defect) | Macular configuration | Hole closure |
|                                                |                          |                 | Preop | POM no. 1 | POM no. 3 | POM no. 6 | Final | Change | Preop | Final | |
| 1 \(^1\)                                       | Yes                        | 18               | 0.2   | 0.3       | 0.5       | 0.5       | 0.1    | Gain   | Defective | Defective | Closed |
| 2 \(^1\)                                       | Yes                        | 14               | 0.4   | 0.5       | 0.4       | 0.2       | 0.2    | Gain   | Defective | Restored  | Closed |
| 3 \(^1\)                                       | Yes                        | 6                | 0.2   | 0.15      | 0.15      | 0.15      | 0.15   | Gain   | Defective | Defective | Closed |
| 4                                              | No                         | 13               | 0.2   | 0.3       | 0.3       | 0.2       | 0.15   | Gain   | Defective | Restored  | Closed |
| 5                                              | No                         | 7                | 1.1   | 0.5       | 0.5       | 0.5       | 0.5    | Gain   | Defective | Defective | Closed |
| 6                                              | No                         | 6                | 0.7   | 0.3       | 0.3       | 0.3       | 0.3    | Gain   | Defective | Restored  | Closed |
| 7                                              | No                         | 6                | 0.15  | 0.3       | 0.15      | 0.0       | 0.0    | Gain   | Defective | Restored  | Closed |
| 8                                              | No                         | 6                | 0.4   | 0.05      | 0.05      | 0.05      | 0.05   | Gain   | Defective | Defective | Closed |
| 9                                              | No                         | 6                | 0.5   | 0.3       | 0.1       | 0.1       | 0.1    | Gain   | Defective | Restored  | Closed |
| 10                                             | No                         | 1                | 0.4   | 0.3       | 0.3       | 0.3       | 0.3    | Gain   | Defective | No data\(^2\) | Closed |
| 11                                             | No                         | 1                | 0.8   | 0.0       | 0.0       | 0.0       | 0.0    | Gain   | Defective | No data\(^2\) | Closed |

logMAR = logarithm of the minimal angle of resolution; Preop = preoperative; POM = postoperative month; IS/OS = inner/outer segment.

\(^1\) In patients no. 1 to 3 (shaded rows), the perifoveal crown tissue was removed during the operation; \(^2\) Patient no. 7 had relatively favorable initial visual acuity despite full thickness macular hole indicated by spectral domain-optical coherence tomography; \(^3\) Follow-up spectral domain-optical coherence tomography image was not available in two patients (no. 10 and 11).
significant difference in largest hole diameter between eyes with EPMH and eyes without EPMH (727.50 ± 132.88 vs. 797.37 ± 269.97 μm, \( p = 0.655 \)). In eyes with I-MH, there was no significant difference in thinnest foveal thickness between eyes with EPMH and eyes without EPMH (23.00 ± 4.71 vs. 30.50 ± 11.02 μm, \( p = 0.298 \)).

**Perifoveal crown phenomenon**

We encountered the perifoveal crown phenomenon during the operations of 11 patients who had undergone vitrectomy. This phenomenon appeared when we gently swept the yellowish epiretinal proliferation tissue centripetally toward the central fovea after removing the ERM. The perifoveal crown had a yellowish cylindrical crown-like shape with its base attached to the edge of the hole (Fig. 2). The ILM stained with ICG dye stopped abruptly at the end of the hole margin under the perifoveal crown tissue.

**Visual acuity and macular configuration**

We followed up the visual acuity and macular configuration of 11 patients who underwent surgical intervention during their clinical course (Table 3). All 11 patients showed improved visual acuity. A section of EPMH tissue was removed in three patients (no. 1, 2, and 3); one of whom (no. 1) had more EPMH tissue removed than intended and presented delayed recovery in visual acuity. Eight patients whose perifoveal crown tissue was preserved showed an improvement in visual acuity during the follow-up visits.

The LH and MH of the 11 patients were closed successfully; however, the defective IS/OS junction was only restored in five patients (no. 2, 4, 6, 7, and 9). Visual acuity

![Fig. 3. Consecutive sections of preoperative macular configuration at a single instance in two patients; patient no. 5 (A,B,C,D) and patient no. 12 (E,F,G,H). Successive sections enable us to identify anatomical continuity of epiretinal proliferation associated with macular hole to the defective base of the lamellar macular hole (outlined manually by the translucent white line). Note that epiretinal proliferation associated with macular hole tissue appears to creep out of a defective outer retina and proliferate beyond the edge of the hole.](image)

| Patient no. | Final visit (mon) | Visual acuity (in logMAR) | Macular configuration | Hole closure |
|-------------|------------------|---------------------------|----------------------|-------------|
|             |                  | First visit | 1 mon FU | 3 mon FU | 6 mon FU | Final FU | Change | IS/OS junction | Initial | Final |              |
| 12          | 12               | 0.05        | 0.1      | 0.15     | 0.2      | Decreased | Defective | Defective | Remain open |
| 13          | 12               | 0.0         | 0.05     | 0.05     | 0.05     | 0.05      | Decreased | Defective | No data*  | Remain open |
| 14          | 18               | 0.2         | 0.2      | 0.2      | 0.2      | No change | Defective | Defective | Remain open |
| 15          | 18               | 0.15        | 0.15     | 0.3      | 0.3      | Decreased | Defective | Defective | Remain open |
| 16          | -                | 0.1         | FU loss† | -        | -        | -         | -        | -          | -          |

logMAR = logarithm of the minimal angle of resolution; FU = follow-up; IS/OS = inner/outer segment.

*There was no available final visit spectral domain-optical coherence tomography data for patient no. 13; †Patient no. 16 was lost after the initial visit.
increased to some extent despite the defective IS/OS junction. In patient no. 1, inadvertent traction was applied to the perifoveal crown tissue during tissue biopsy with retinal scissors, and more tissue was removed than intended. Although the hole itself was closed successfully, the IS/OS junction remained defective. The visual acuity worsened from 0.2 in the preoperative period to 0.5 6 months postoperatively; however, it recovered to 0.1 18 month postoperatively.

Four patients were followed up for longer than 12 months without surgical intervention (Table 4). One patient (no. 16) was lost after the initial diagnosis. Three patients (no. 12, 13, and 15) showed a slight reduction in visual acuity, while one patient (no. 14) maintained visual acuity through the final visit. None of the patients showed significant change in macular configuration.

**Histological assessment**

Of three patients (no. 1, 2, and 3) for which histological analysis was attempted, only two patients’ (no. 1 and 3) tissue samples were suitable for histological analysis (Table 5). In hematoxylin and eosin staining, both tissues showed aggregates of lymphocytes and scattered cells with medium-density nuclei. In immunohistochemical staining, we first attempted synaptophysin and S-100 staining on tissue from patient no. 1; this did not yield any positive results. We added pan-keratin antibody staining to tissue from patient no. 3 and obtained a strong positive reaction (Fig. 4A-4D).

| Table 5. H&E and immunohistochemical staining of tissues from patients no. 1 and 3 |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Staining                        | H&E             | Synaptophysin   | S-100           | Pan-keratin     |
| Target                          | Nucleus & cytoplasm | Neuron          | Muller cell     | Epithelium      |
| No. 1                           | +               | -               | -               | None            |
| No. 3                           | +               | -               | -               | +               |

H&E = hematoxylin and eosin.

![Fig. 4. Histological analysis of perifoveal crown tissue acquired from patient no. 1 (A) and patient no. 3 (B-D). (A) Hematoxylin and eosin stain staining of paraffin-embedded perifoveal crown tissue from patient no. 1. Aggregation of lymphocytes and cells with medium-density nucleus are scattered. (B) Hematoxylin and eosin staining of paraffin-embedded perifoveal crown tissue from patient no. 3. (C) Synaptophysin staining of perifoveal crown tissue from patient no. 3. No specific staining is observed. (D) Pan-keratin staining of perifoveal crown tissue from patient no. 3. Note cells with brownish-stained cytoplasm scattered throughout the specimen.](image-url)
Discussion

The unusual epiretinal proliferation found in MH or LH is now accepted as a distinct clinical entity from conventional ERMs [13]. Recent studies have observed some common morphological configurations of this unusual proliferation [12,14,15]. It is most frequently observed in eyes with LH [8,11,13,16] and is associated with a large hole diameter and a thin fovea [14]. It does not appear to occur in eyes with an intact fovea [9]. There have been various attempts to explain the pathogenesis of this unusual epiretinal proliferation. Parolini et al. [9] suggested premacular vitreous remodeling as a potential causative mechanism and presented positive staining results of “dense membrane” for glial fibrillary acidic protein (GFAP) and hyalocyte markers (CD45 and CD64) as histologic evidence. Although that group reported only a few positive staining results of dense membrane for cellular retinaldehyde binding protein, which targets both glial cells and RPE cells, they suspected hyalocytes as the causative agent instead of RPE cells. Shiraga et al. [16] speculated that a portion of the retinal tissue would migrate along the posterior hyaloid face, forming ERM with macular pigments. Pang et al. [13] hypothesized the possibility of Müller cell proliferation with SD-OCT based anatomical assessment of LHEP. Recently, Compera et al. [15] presented a similar opinion to that of Parolini et al. [9], that LHEP appears to occur due to vitreous reaction.

Some authors have suggested gliosis as the potential causative mechanism of the formation of EPMH. Positive GFAP staining against EPMH tissue was observed in sev-
eral previous studies [9,15]. However, the authors did not appear to distinguish EPMH from ILM or ERM and instead performed a combined fixation process. ILM specimens are composed of a variety of cells including hyalocytes, glial cells, RPE, fibrocytes, and myofibrocytes [17-19]. Therefore, their positive staining results do not appear to be limited only to EPMH. In our study, we harvested only a portion of the yellowish pigment-rich tissue when we noted dehiscence within the fluttering perifoveal crown tissue. Since the tissue acquisition was performed after ICG-assisted ILM peeling, there should have been a lower risk of target tissue contamination with ILM components. Therefore, we believe that more accurate histological analysis was possible with our harvesting technique. Furthermore, in immunohistochemical staining, none of our tissue specimens presented a positive reaction to S-100 protein, a calcium-binding protein that labels the cytoplasm of Müller cells [20], the principal glial cells of the retina. The negative results for S-100 do not support the hypothesis of Müller cell gliosis as the causative mechanism of EPMH.

Removal of EPMH is not recommended. In our study, three of four patients with LH, whose EPMH was preserved, presented a stable natural clinical course throughout the 1-year follow-up period. In contrast, one patient in whom a greater amount of EPMH was removed than intended presented initial reduction and delayed recovery in visual acuity. In previous studies, EPMH showed morphological stability in more than 90% of eyes without surgical intervention [11,13], and removing EPMH may have led to development of a FT-MH [8,9,13]. Pang et al. [13] hypothesized that removing this tissue would draw the central plug out of the structurally compromised macula that was stabilized by the tissue. In our experiences, trimming a portion of the perifoveal crown tissues did not appear to decrease visual acuity or structural recovery. However, considering the potential complications, preserving the tissue or at least leaving the stump may comprise a better strategy when performing vitrectomy.

There are a few limitations to this study. First, it was a retrospective review of 212 patients at a single institute; this sample size was insufficient to establish statistical significance. Additional cases are required to elucidate detailed characteristics and the potential origins of this unusual proliferative tissue. Second, the positive staining result of pan-keratin was noted in only a single case. The scarcity of histological analyses in this study is due to our speculation that preserving the EPMH would be better for visual acuity and macula stabilization. We did not suspect RPE as a potential origin of proliferation when we harvested the first specimen. As this study progressed, analyses of SD-OCT images led us to consider RPE as a possible origin and to add pan-keratin antibody to the second immunocytochemical staining. Third, using S-100 instead of GFAP could be another limitation in this histological analysis. The upregulation of GFAP is the most sensitive non-specific response to retinal disease [21], and GFAP has been used as universal marker of Müller cell gliosis [22,23]. However, S-100 protein has also been used as a Müller cell marker [24-26], and two consecutive negative staining results of S-100 should not be disregarded. Further histological analysis is required to provide further information on the potential causes of EPMH formation.

In conclusion, we suggest that EPMH could be the result of RPE proliferation that migrated through IS/OS junction defects. EPMH appears to occur mainly in eyes with LH; however, it can also be present in eyes with other vitreo-macular pathologies such as FT-MH or I-MH. Perifoveal crown phenomenon can be regarded as an intraoperative characteristic. Additional histological analysis utilizing more accurate harvesting techniques is necessary to improve our understanding of this phenomenon.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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