



## Case report

## Effects of corneal epithelial superficial keratectomy in patients with focal limbal stem cell disease

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## ABSTRACT

**Purpose:** Irregular corneal epithelium in limbal stem cell disease can cause visual acuity to deteriorate substantially when it reaches the pupil. In this case series, we assessed the effectiveness of simple corneal epithelial superficial keratectomy in improving visual acuity in patients with irregular corneal epithelium in focal limbal stem cell disease covering the visual axis.

**Observations:** We performed simple corneal epithelial superficial keratectomy in four patients (five eyes) with irregular corneal epithelium covering the visual axis. The main outcome measures were best-corrected visual acuity, slit lamp findings with fluorescein staining, anterior segment optical coherence tomography and histopathology. In all five eyes, slit lamp findings showed uneven fluorescein staining in a spiral pattern, with impaired corneal epithelial smoothness and visual disturbance. We removed the irregular epithelium in all five eyes. Visual acuity in all the eyes was improved immediately after surgery, and good visual acuity and stable epithelium were maintained for the duration of the observation periods. Hematoxylin and eosin staining showed, normal squamous and columnar epithelial cells. Goblet cells were not detected.

**Conclusions and Importance:** Corneal epithelial superficial keratectomy can lead to a pathological diagnosis by examining the removed epithelial tissues, and result in excellent therapeutic outcomes in focal limbal stem cell disease reaching the pupil.

## 1. Introduction

An intact corneal epithelium provides a smooth refractive surface for high quality vision. Several reports have indicated that central corneal epithelial disorder in patients with dry eye is correlated with visual impairment.<sup>1,2</sup> In particular, impaired surface regularity of the corneal epithelium on the visual axis leads to low visual acuity.

Epithelial limbal stem cells (LSCs) provide intact epithelial cells, but can be damaged by many factors, including trauma, adverse immune response, and genetic disorders.<sup>3,4</sup> In patients with LSC disease, the epithelium has an irregular appearance,<sup>5</sup> which sometimes reaches the center of the cornea and reduces visual acuity. Some severe cases with total LSC deficiency may require stem-cell transplantation of autologous<sup>6-9</sup> and cultured sheet<sup>10,11</sup> using limbal epithelium, conjunctivae,<sup>12</sup> and oral mucosal epithelium.<sup>13-16</sup> In partial LSC deficiency corneas, which are covered with vascular and conjunctival

fibroblasts, the combination therapy with superficial keratectomy and amniotic membrane transplantation has been recommended to rebuild the ocular surface conditions.<sup>17-20</sup> In contrast, some mild cases of LSC diseases<sup>5</sup> can be treated simply with medicated eyedrops<sup>5,21-23</sup> or autologous serum eyedrops<sup>24</sup> to rebuild the microenvironment of the improve the dysfunction of the LSCs niche.

Simple epithelial superficial keratectomy is a less invasive therapy than limbal<sup>6-11</sup> and/or amniotic membrane<sup>17-19</sup> transplantation because it does not require grafts, sutures or glue but, to the best of our knowledge there have been no reports of this treatment being used for LSC disease. Here, we report on five eyes in four patients with partial LSC diseases treated by simple epithelial superficial keratectomy with satisfactory clinical courses. In this case series, we include mild LSC disease eyes with irregular epithelium with abnormal fluorescein stain and sometimes mild subepithelial haze, but they do not have neovascularization and opaque conjunctivalization.

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## 2. Findings

We performed simple corneal epithelial keratectomy for irregular corneal epithelium covering the visual axis in the Department of Ophthalmology at the University of Tokyo Hospital. This study is non-comparative interventional case series. The main outcome measures were slit lamp findings with fluorescein staining, best-corrected visual acuity (BCVA), and anterior segment optical coherence tomography (AS-OCT) (SS-1000 or SS-2000, Tomey corporation, Nagoya, Japan) before and after the surgery, as well as histopathological examination of the removed epithelium.

After obtaining informed consent from the patients, we conducted corneal keratectomy with topical 4% xylocaine eyedrops. We examined the removed tissues histopathologically (Papanicolaou staining), using Gill's Hematoxylin Solution to stain cell nuclei. The tissues were counterstained for keratin using Orange G-6, and with Eosin Azure-50. Two percentages of Alcian Blue solution and Kernechtrot solution were also used in Alcian blue staining. All stains and reagents were from Muto Pure Chemicals Co., Tokyo, Japan. This report was approved by the Ethics Committee of the University of Tokyo Hospital. Demographic data and clinical outcomes in 5 eyes of 4 cases are summarized in Table 1.

### 3. Case 1

An 82-year-old woman had a history of cataract surgery and amniotic membrane transplantation (details unknown) 10 years ago in the left eye. Although the BCVA had been 20/20 after cataract surgery, it had deteriorated to 20/66 when she visited our hospital. Purified 0.3% sodium hyaluronate and 0.1% fluorometholone eyedrops had been applied before the first visit. Anterior segment finding with a slit lamp microscope showed that irregular epithelium had reached the visual axis, leading to worsening of visual acuity (Fig. 1A) and fluorescein staining showed irregular epithelium on the upper cornea (Fig. 1B). Irregular epithelium on the upper cornea (From 10 to 3 o'clock) was gently scraped with a spatula, without touching the limbal or lower corneal epithelium. Extended-wear soft contact lens (SCL), 1.5% levofloxacin and 0.1% fluorometholone eyedrops had been applied four times a day post-operatively for one week. One week after the surgery, the scraped upper cornea had recovered its smoothness, and all uneven staining and epithelial surface irregularity had disappeared when examined using fluorescein staining (Fig. 1C). In AS-OCT photographs, epithelial surface irregularity was detected pre-operatively (Fig. 1D), but not post-operatively (Fig. 1E). The postoperative BCVA of the left eye was restored to 20/25. Hematoxylin and eosin staining showed slightly thickened squamous epithelium, and mild nuclear enlargement with anisokaryosis, pathologically diagnosed as mild atypia (Fig. 1F). No recurrence was observed during the 6-months postoperative follow-up.

### 4. Case 2

A 48-year-old man had a small pupil and congenital corectopia in the right (Fig. 1G) and left (Fig. 1H) eyes. In his first visit, BCVA was 20/40

**Table 1**  
Demographic data and clinical outcome.

	Age	Sex	Pre. VA	Post. VA	Pathology or Cytology	Follow-up periods
Case 1	82	F	10/20	16/20	Mild atypia	6 months
Case 2 R	48	M	8/20	16/20	Class1	36 months
Case 2 L			10/20	14/20	No data	36 months
Case 3	48	M	18/20	24/20	No data	36 months
Case 4	82	M	10/20	24/20	Class1	4 months

Abbreviations: R, right; L, left; F, female; M, Male, VA; visual acuity.

in the left eye and 20/50 in the right eye. The irregular epithelium spread from superior and inferior limbus (right eye, Fig. 1I; left eye, Fig. 1J). Epithelial keratectomy of the upper corneas (From 9 to 3 o'clock) was performed in the both eyes. 1.5% levofloxacin and 0.1% fluorometholone eyedrops had been applied twice a day post-operatively for one week on right eye. 1.5% levofloxacin eyedrops twice a day and extended-wear SCL were applied post-operatively for one week on left eye. The corneal epithelial surfaces became smooth in the right (Fig. 1K) and left (Fig. 1L) eyes. Postoperative BCVA was restored to 20/25 in the right eye and 20/30 in the left eye and has been maintained for 3 years postoperatively. Histopathology confirmed a cytological diagnosis of class I-negative columnar epithelial cells.

### 5. Case 3

A 48-year-old man who used SCLs had been treated for irregular epithelium on the ocular surface with vitamin A eyedrops, 0.1% beta-methasone sodium phosphate and fradiomycin sulfate eyedrops, and 0.1% tacrolimus hydrate eyedrops but his visual disturbance had not improved. No abnormality was revealed by impression cytology. The patient's BCVA at the first visit was 20/200. The characteristic irregular epithelium reached the visual axis from the nasal-superior limbus in his left eye. After starting 0.1% fluorometholone eyedrops, the irregular epithelium decreased and his BCVA was improved to 20/20. Upon discontinuation of the eyedrops, the irregular epithelium invasion worsened again within 6 months and BCVA decreased to 20/25 (Fig. 2A and B). Epithelial keratectomy was performed on his left eye (All around) and 1.5% levofloxacin eyedrops were started twice a day and extended-wear SCL were applied post-operatively for one week. One week postoperatively, the area of rough epithelium was decreased but a large portion remained. By 2 months postoperatively, the proportion of irregular epithelium had started to shrink. At 6 months, the smoothness of the central corneal epithelium had improved further, and the irregular epithelium was resolved gradually over 13 months (Fig. 2C). Postoperative BCVA of the patient's left eye was restored to 20/16 and it has been maintained for 3 years.

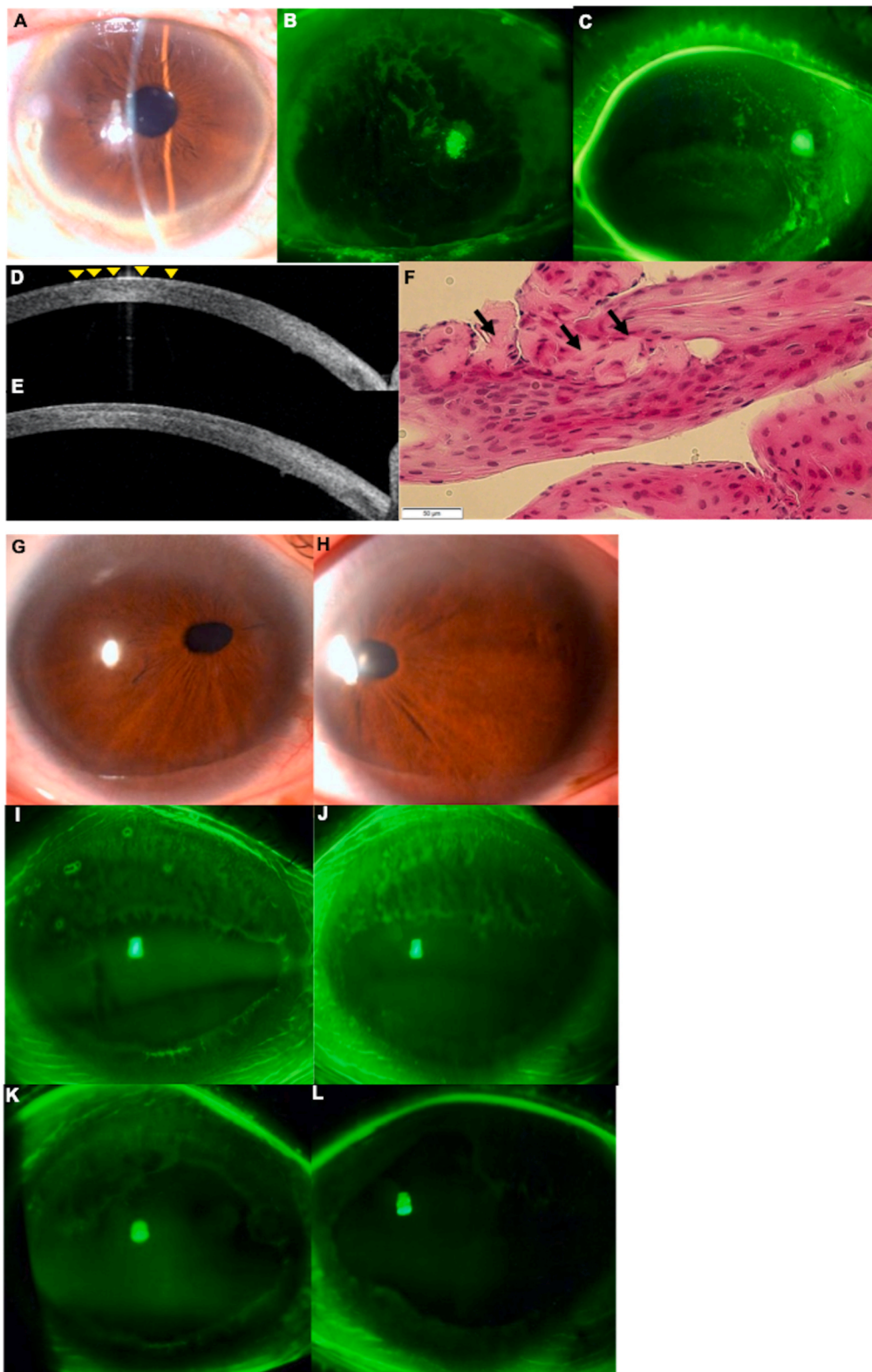
### 6. Case 4

An 82-year-old man noticed visual disturbance during postoperative chemotherapy with Docetaxel, for lung cancer. At his first visit to our hospital, irregular epithelium was infiltrating from the superior limbus in both eyes. The visual axis of his left eye was covered with irregular epithelium (Fig. 2D and E). BCVA in his left eye was 20/40. Schirmer's test results were 2 mm in both eyes. After applying 0.1% purified sodium hyaluronate eyedrops, epithelial keratectomy was performed on his left eye (From 9 to 3 o'clock). 0.3% gatifloxacin eyedrops four times a day and extended-wear SCL were applied post-operatively for one week. The smoothness of the corneal epithelium improved (Fig. 2F) and post-operative BCVA was restored to 24/20, which was maintained for 4 months. The pathological diagnosis was squamous epithelium with scant atypia (Fig. 2G) negative for goblet cells (Alcian blue staining) (Fig. 2H).

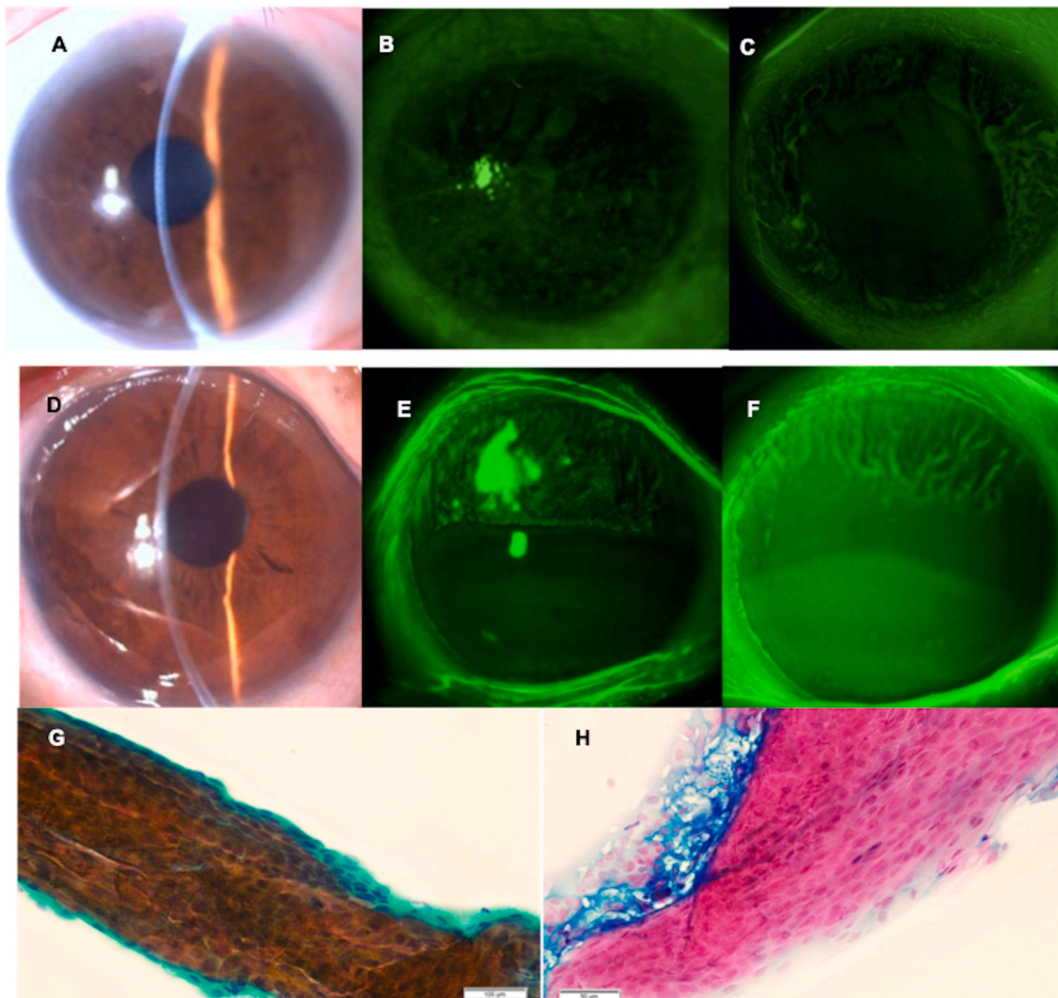
## 7. Discussion

We performed simple corneal epithelium keratectomy using a spatula on five eyes with focal LSC disease whose visual axis was covered with irregular epithelium. Visual disturbance and irregularity of the corneal epithelium were repaired immediately after surgery and smooth epithelial surfaces were maintained for periods of 4 months to 3 years. The irregular epithelium completely disappeared from the pupil after epithelial keratectomy and was localized in the peripheral cornea. Our findings indicate that simple corneal epithelium keratectomy using a spatula is effective for focal LSC disease due to irregular epithelium.

Our keratectomy technique for irregular epithelium has some



**Fig. 1.** Pre- and postoperative anterior segment photographs and pathological examinations of the removed tissues in Cases 1 and 2 (A–F) Case 1. Anterior segment photograph (A). Fluorescein staining shows irregular epithelium on the upper cornea (B). No sign of irregular epithelial surface on the visual axis upon fluorescein staining (C). In anterior segment optical coherence tomography photographs, irregular epithelial surface (arrowheads point to rough epithelial surface) is detected before corneal epithelial superficial keratectomy (D) but not after surgery (between arrowheads) (E). Hematoxylin and eosin staining shows mildly thickened squamous epithelium and mild nuclear enlargement with anisokaryosis., and some dyskeratosis (arrows)(F). (G–L) Case 2. Anterior segment photograph of right eye (G) and left eye (H) shows a small pupil and congenital corectopia. The irregular epithelium spreads from superior and inferior limbus in the right (I) and left (J) eyes. After corneal epithelial superficial keratectomy, corneal epithelial surfaces become regular in the right (K) and left (L) eyes.



**Fig. 2.** Pre- and postoperative anterior segment photographs and pathological examinations of the removed tissues in Cases 3 and 4. (A–C) Case 3. Anterior segment photograph (A) and fluorescein staining showing diffuse irregular epithelium before abrasion (B). Thirteen months after surgery, irregular epithelium has been resolved (C). (D–H) Case 4. Anterior segment photograph (D). Fluorescein staining shows the visual axis partially covered by irregular epithelium (E). After superficial keratectomy, smoothness of visual axis is totally recovered (F). Papanicolaou staining shows squamous epithelium with scant atypia (G). Alcian blue staining shows no goblet cells (H). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

practical merits from a clinical standpoint. We do not have to use amniotic membrane and/or corneal limbal tissue from the other eye. The treatment can be performed easily in many clinics as it requires no special devices or medication. It is relatively safe because only differentiated epithelium on the clear cornea is removed, without touching the LSCs. Furthermore, the removed tissues are available for cytological or histopathological examination. These advantages suggest that the keratectomy for irregular epithelium can be used clinically, with good outcomes.

Since LSC disease is reversible without stem-cell transplantation, it has been speculated that the cause is not LSCs loss, but LSCs dysfunction.<sup>5</sup> It is not known whether the irregular epithelium originates from corneal LSCs or conjunctival stem cells, and the exact mechanism by which the keratectomy works in focal LSC disease remains unclear. Cells from the irregular epithelium are periodic acid-Schiff-negative (Fig. 1F), suggesting that these cells are derived from corneal LSCs rather than conjunctival stem cells. Considering that irregular epithelium slowly expands in the clear corneal area, the proliferative capacity of irregular epithelium-producing cells is inferior to that of normal LSCs. This low proliferative capacity of irregular epithelium-producing cells may explain the clear and stable ocular surface covered by normal epithelium without recurrence after epithelial keratectomy.

This study had some limitations relating to the epithelial superficial keratectomy. We chose only partial and mild irregular epithelium covering the pupil as an indication for superficial keratectomy, because we think the existence of highly proliferative normal LSCs is essential for successful clinical results. Corneas with insufficient numbers of LSCs require auto- or allo-cell grafts involving stem cells such as LSCs,<sup>6–11</sup> conjunctival epithelium,<sup>12</sup> and oral mucosal epithelium<sup>13,14,15</sup> These severe cases are a contra-indication for our technique. According to the principle of starting with the least invasive treatment, topical eye drops such as mitomycin C<sup>25</sup> should have been performed first, but we did not try mitomycin C prior to keratectomy because there was a report that its use can, in fact, induce LSC disorder.<sup>26</sup> A further limitation is that long-term survival has not been assured, as the longest follow-up period was 3 years (Case 2).

In summary, corneal epithelial keratectomy using a spatula recovered visual impairment caused by irregular epithelium covering the visual axis, by replacing it with normal epithelium. We recommend simple epithelial superficial keratectomy as an effective treatment for patients with focal LSC disease.

## Patient consent

Written consent to publish this case has not been obtained. This report does not contain any personal identifying information.

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## Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

## CRedit authorship contribution statement

**Aya Inamochi:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. **Takashi Miyai:** Writing – review & editing, Supervision. **Tomohiko Usui:** Supervision. **Makoto Aihara:** Supervision. **Satoru Yamagami:** Writing – review & editing, Supervision.

## Declaration of competing interest

The following authors have no financial disclosures: A.I., T.M., T.U., M.A., S.Y.

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