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Comparative analysis of long-term results of three epithelial cell transplantation procedures for treating limbal stem cell deficiency

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ABSTRACT

This study compared the long-term outcome of different epithelial transplantation techniques to treat limbal stem cell deficiency (LSCD). We conducted a retrospective 15-year comparative systematic cohort study of patients with LSCD who underwent either cultivated limbal epithelial transplantation (CLET), simple limbal epithelial transplantation (SLET), or cultivated oral mucosal epithelial transplantation (COMET). We reviewed the demographic data, etiology, LSCD severity, best-corrected visual acuity, surgical outcomes, and complications. A total of 103 eyes of 94 patients (mean age, 45.0 ± 16.4 years) with LSCD were enrolled. The most common cause of LSCD was chemical injury (42.7 %). The median follow-up time was 75 months. The success rates of CLET, SLET, and COMET were 45.5 %, 77.8 %, and 57.8 %, respectively. The 7-year survival rates after CLET, SLET, and COMET were 50.0 %, 72.2 %, and 53.2 %, respectively. Steven-Johnson syndrome (SJS) had a significantly lower survival rate than other causes ($p < 0.001$), but SLET had a significantly higher survival rate than CLET ($p = 0.018$) and COMET ($p = 0.047$). Visual improvement of more than four Snellen lines was achieved in 53.1 % of successful cases and 28.2 % of failed cases. SJS, Schirmer I test <5 mm, and the presence of postoperative recurrent epithelial defects were significant risk factors for a failed surgery. All epithelial transplantation techniques had favorable long-term surgical outcomes. More than half of the patients achieved a stable ocular surface and visual acuity improvement up to 7 years postoperatively. SLET tends to have a better surgical outcome than CLET and COMET, especially in patients with SJS.

1. Introduction

Limbal stem cell deficiency (LSCD) results from various severe ocular surface diseases, including chemical or thermal burns, Stevens-Johnson syndrome (SJS), ocular cicatricial pemphigoid, and autoimmune disease [1]. These conditions destroy corneal epithelial stem cells, leading to conjunctivalization, chronic inflammation, ingrowth of fibrous tissue, and stromal scarring [2].

Epithelial cell transplantation is the primary surgery to restore the ocular surface and visual recovery in patients with severe LSCD. Many different surgical techniques have been developed over the past decades. In 1997, Pellegrini et al. [3] introduced cultivated limbal epithelial transplantation (CLET). However, this technique necessitated a costly and availability of a high-standard laboratory for cell expansion [3,4]. Sangwan et al. introduced an efficient single-stage treatment, simple limbal epithelial transplantation (SLET), that does not require a laboratory infrastructure [5]. Nonetheless, in case of bilateral LSCD for which allogenic CLET and SLET were performed, immunosuppressive therapy was required [6,7]. Because of this disadvantage, autologous cultivated oral mucosal epithelial transplantation (COMET) is an alternative surgery that eliminates the risk of allogeneic rejection and the systemic side effects of prolonged use of immunosuppressive agents [8–13].

Previous clinical studies [14–19] have reported the long-term effectiveness and surgical outcomes of each epithelial transplantation technique. However, only a few studies have compared the techniques.

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Shimazaki et al. found no significant difference in success rates between CLET and COMET [20]. Wang et al. reported that CLET had a significantly higher success rate than COMET among patients with total LSCD [21]. No studies have compared the outcomes of the three different epithelial transplantation techniques. The current study compared the long-term surgical outcomes of CLET, SLET, and COMET and determined if the etiology of LSCD impacts the surgical success rates. The study also analyzed the visual improvement and the factors associated with surgical failures.

2. Materials and methods

2.1. Study design and participants

This retrospective systematic study was conducted according to the principles of the Declaration of Helsinki. The Committee for the

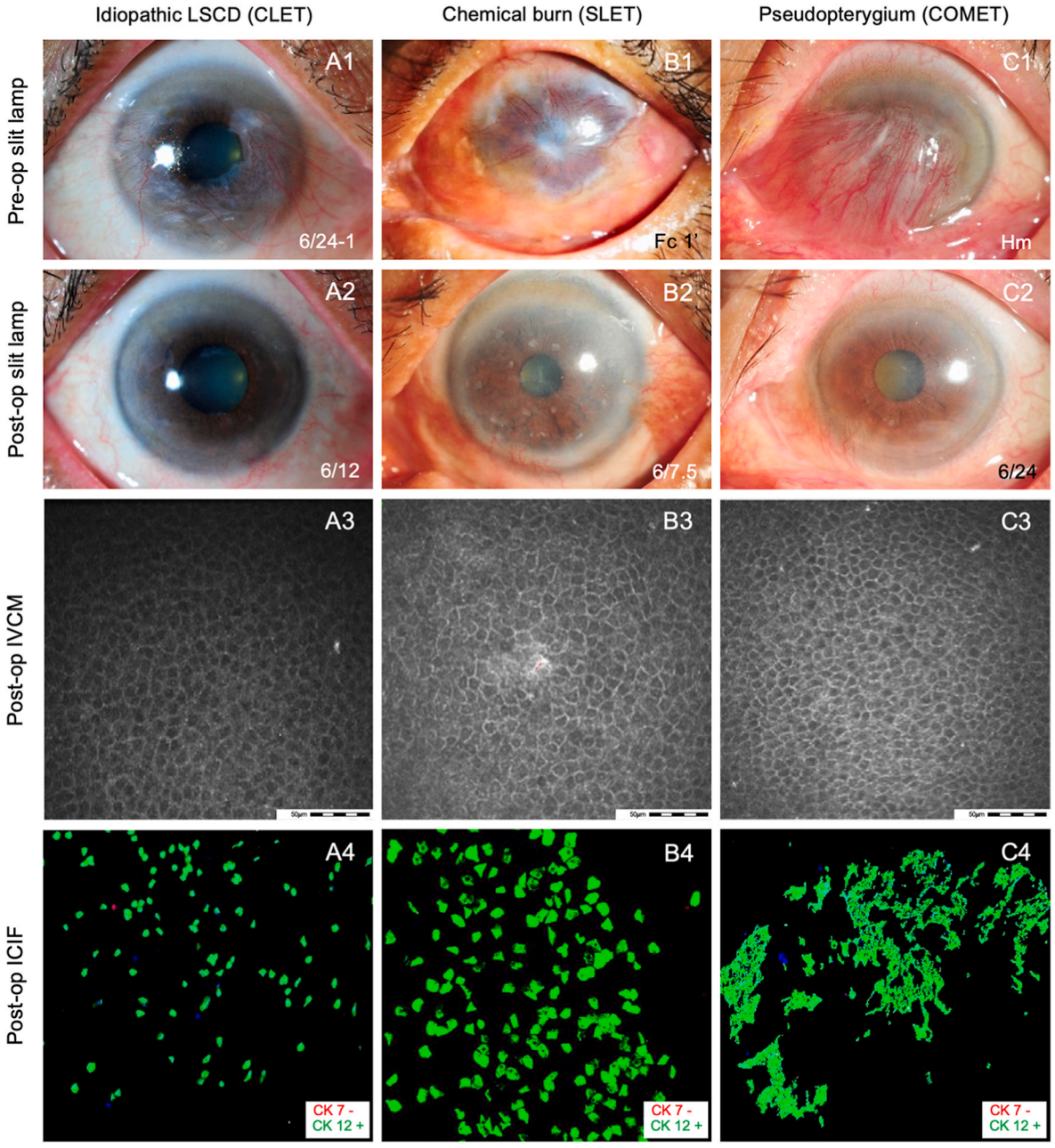

Fig. 1. The clinical success of CLET, SLET, and COMET. (A) An idiopathic case underwent CLET. (A1) A preoperative anterior segment photograph shows corneal conjunctivalization extending from the 1 to the 11 o'clock positions. The VA was 6/24–1. (A2) At 2 years postoperatively, the cornea is clear without conjunctivalization. The VA was 6/12. (A3) Six years after CLET, IVCM shows multilayered corneal epithelium in all areas. (A4) Eight years postoperatively, ICIF shows CK12 positivity (green) and CK7 negativity (red). (B) A chemical burn case underwent SLET. (B1) A preoperative anterior segment photograph, the cornea was opaque with conjunctivalization. The VA was finger count 1 foot (B2) Four years postoperatively, the cornea is clear without conjunctivalization. The VA was 6/7.5. (B3) Four years after SLET, IVCM shows multilayered corneal epithelium in all areas. (B4) Four years 3 months postoperatively, ICIF shows CK12 positivity (green) and CK7 negativity (red). (C) A pseudopterygium case underwent COMET. (C1) A preoperative anterior segment photograph shows the cornea opaque with pseudopterygium extending into the central cornea. The VA was hand motions. (C2) One year 5 months postoperatively, the cornea is clear without conjunctivalization. The VA was 6/24. (C3) Two years 7 months after SLET, IVCM shows multilayered corneal-like epithelium in all areas. (C4) One year 8 months postoperatively, ICIF shows CK12 positivity (green) and CK7 negativity (red).

Protection of Human Participants in Research at the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand [Siriraj ethics committee number 249/2565(IRB3)] and Thai Clinical Trials registry [TCTR20220913002] approved the study. The Research Committee waived the requirement for informed consent.

Fifteen years of medical data and photographs were retrieved from the registry data of epithelial cell transplantation for LSCD [17,22,23]. All patients with LSCD who underwent the epithelial transplantation surgeries between October 2007 and March 2022 were reviewed. International Classification of Diseases (ICD)-10-CM code 18.8 (Other specified disorders of corneal limbal stem cell deficiency), ICD-9-CM code 11.69 (Other corneal transplant), and data from ocular surface clinic records were used for data extraction.

This study included patients with LSCD who had undergone at least one of the three surgeries (CLET, SLET, COMET) at Siriraj hospital performed by four surgeons (P.P., C.C., P.N., S.C.) using the same guidelines and techniques determined based on a comprehensive clinical assessment and investigations. Clinical diagnoses were based on Global LSCD consensus 2019: stage IIB (partial LSCD >180) or stage III (total LSCD). Confirmation of the diagnoses was obtained through impression cytology (ICIF) and in vivo confocal microscopy (IVCM), as described previously [22,23]. The exclusion criteria included individuals under age 18 years, patients with a follow-up period of less than 12 months, or those with insufficient data available for analysis. Demographic data, that included sex, age, etiology, LSCD severity, history of eyelid abnormality and symblepharon, previous eyelid correction and penetrating keratoplasty, history of subsequent ophthalmic surgeries including optical keratoplasty, implantation of a Boston Keratoprosthetic (KPro, Massachusetts Eye and Ear, Boston, MA), and phacoemulsification, were retrieved from the electronic medical records. The clinical and investigative parameter including the visual acuity (VA), ICIF, IVCM, anterior-segment photography, surgical outcomes, follow-up time presence of recurrent epithelial defects, and other postoperative complications were recorded. The postoperative follow-up visits, IVCM, and ICIF were performed every 3 months during the first year and subsequently at 6-month intervals.

2.2. Outcome measures

The primary outcome was the survival rates of different epithelial transplantation techniques. The criteria for determining the success of surgical outcome were based on our previous report [22]. The patients were evaluated by slit-lamp biomicroscopy. Beginning in August 2016, ICIF and IVCM were used as additional investigative tools to identify specific epithelial phenotypes as previous described [23]. In successful cases, corneal or corneal-like epithelial cells were detected on IVCM, and ICIF demonstrated the expression of CK12, a cornea-specific marker (Fig. 1). Failures were characterized by the instability of the ocular surface, which included recurrent/persistent corneal epithelial defects, corneal neovascularization, or invasion of fibrovascular tissue involving the pupillary area. IVCM showed total or predominant conjunctival epithelial cells and detection of CK7, a conjunctival-specific marker, in the central cornea. One interpreter (C.C.) interpreted the ICIF and IVCM performed using the same techniques for the entire study. The survival rate and follow-up duration were calculated from the date of surgery to the date of failure of the epithelial transplantation. The secondary outcomes included visual improvement as measured by the changes in the best-corrected visual acuity (BCVA) between preoperatively and the last follow-up visit and the risk factors associated with failure outcomes.

2.3. Statistical analysis

Statistical analysis was performed using IBM SPSS statistics version 29.0 (IBM Corp, Armonk, NY). Due to the non-normal distribution of the continuous parameters, results were reported as the median and interquartile range (IQR). Categorical data were reported as numbers and percentages (n %). The Kruskal Wallis H test was used to compare numerical data between groups, and Pearson chi-square and Fisher's exact test were applied to compare the categorical data between groups. Survival rates were determined using the Kaplan-Meier method with log-rank testing. A univariable Cox regression analysis was conducted to identify the factors associated with the failure surgical outcome. Independent factors were derived from multivariable cox regression analysis, using the variables identified in the univariable Cox regression analysis with a significant level of $p < 0.15$. The strength of associations was reported as the adjusted hazard ratio with the 95 % confidence interval (CI). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Demographic data

A total of 103 eyes of 94 patients (48 men, 51.1 %; 46 women, 48.9 %; median age at surgery, 45.0 ± 16.4 years) were included in this study. One hundred and twelve surgeries were performed; nine eyes underwent more than one epithelial transplantation due to a previous surgical failure. This study includes the analysis of only the initial surgical intervention. The study cases were categorized into three groups based on the surgical technique: CLET (21 patients, 22 surgeries), which has been performed since 2006; COMET (43 patients, 45 surgeries), performed since 2008; and SLET (32 patients, 36 surgeries) introduced in 2015. The etiologies of LSCD are shown in Table 1.

The median duration of LSCD preoperatively was 36 months (IQR, 15–85 months). Almost one third of patients had severe dry eye (Schirmer l test, <5 mm). The median follow-up duration was 75 months (IQR, 30–138 months). Other clinical data are summarized in Table 1.

3.2. Success and survival rates of epithelial transplantation

The overall success rate of epithelial transplantation was 62.1 %; no significant differences were seen among the three surgical procedures. Among the three surgical outcomes, SLET had the highest success rate (77.8 %) during the median follow-up time of 47 months, followed by COMET (57.8 %) and CLET (45.5 %), that had longer follow-up time of 100 and 117 months, respectively.

Regarding the Kaplan-Meier survival analysis, the total survival rates for all procedures exhibited an exponential decay pattern. During the first year, the survival rate was very high at 83.5 % and dramatically declined to 69.3 % in the second year. Afterwards, the survival rate gradually decreased and remained relatively stable from the 4th year (65.2 %) to the 14th year (55.2 %) (Fig. 2A). Subgroup survival analysis of the three surgeries showed a similar exponential decay pattern. The survival rates across the three surgeries, about 80 %, did not differ during in the first year. SLET had the highest survival rate among the three techniques, maintaining nearly 70 % at 5 years and remaining stable up to 7 years. CLET and COMET had survival rates of about 50 % at 5 years and were stable up to 14 years. However, no statistical significance was found among the three groups ($p = 0.154$) (Fig. 2B).

3.3. Comparison of successful outcomes with different severities of LSCD

Regarding the LSCD severity, the survival rates of the different epithelial transplantation techniques did not differ significantly between the total and partial LSCD cases ($p = 0.383$) (Fig. 3A). Similarly, no significant differences in survival rates among the procedures were observed for total LSCD ($p = 0.378$) (Fig. 3B) and partial LSCD ($p = 0.155$) (Fig. 3C). However, in partial LSCD, the survival rate of SLET remained at 100 % for more than 6 years and was significantly higher than that of COMET ($p = 0.044$) (Fig. 3C).

Table 1
Patient demographic and baseline data.

	Number (N%)				
	Total (N = 103 eyes)	CLET (N = 22 eyes)	SLET (N = 36 eyes)	COMET (N = 45 eyes)	*P*-value
Number of patients	94	21	32	43	
Unilateral eye transplantation(N)	89	20	28	41	
Autologous transplantation(N)	75	13	17	45	
Age (years); mean ± SD	44.98 ± 16.35	41.50 ± 15.57	45.61 ± 15.32	46.18 ± 17.59	
Etiology of LSCD; N (%)					N/A
Chemical/thermal injury	44 (42.7)	15 (68.2)	16 (44.4)	13 (28.9)	
SJS	27 (26.2)	2 (9.1)	7 (19.5)	18 (40.0)	
Others	32 (31.1)	5 (22.7)	13 (36.1)	14 (31.1)	
Severe allergic conjunctivitis	5	0	4	1	
PUK	7	0	0	7	
Multiple surgeries	4	2	1	1	
Severe MGD	3	2	1	0	
MMC toxicity	4	1	3	0	
Aniridia	3	0	3	0	
Advanced pterygium	2	0	0	2	
Ocular trauma	1	0	1	0	
Idiopathic LSCD	3	0	0	3	
Total LSCD	74 (66.1)	17 (77.3)	28 (77.8)	22 (48.9)	**0.010***
Duration of LSCD(mo)#	36 (15–85)	15 (12–77)	28 (13–68)	37 (24–117)	0.122
Follow-up time (mo)#	75 (30–138)	149 (138–165)	47 (29–60)	110 (22–135)	**<0.001***
Criteria for outcome evaluation					–
Clinical only	28 (27.2)	14 (63.6)	0 (0.0)	14 (31.1)	
Clinical + ICIF and/or IVCM	75 (72.8)	8 (36.4)	36 (100.0)	31 (68.9)	
Preoperative Data					
Schirmer I test <5 mm	29 (28.2)	6 (27.3)	8 (22.2)	15 (33.3)	0.561
Symblepharon	47 (45.6)	12 (54.5)	11 (30.6)	24 (53.3)	0.085
Eyelid abnormality	46 (44.7)	7 (31.8)	14 (38.9)	25 (55.6)	0.142
Prior ophthalmic surgery					
Symblepharon lysis	43 (41.7)	12 (54.5)	7 (19.4)	24 (53.3)	**0.003***
Eyelid correction	27 (26.2)	5 (22.7)	9 (25.0)	13 (28.9)	0.878
PKP	2 (1.9)	1 (4.5)	1 (2.8)	0 (0.0)	0.315
BCVA; logMAR#					
Preoperation	2.3 (0.6–2.9)	2.0 (0.6–3.0)	2.3 (1.5–2.6)	2.0 (0.4–2.6)	0.447
Postoperation	0.9 (0.4–2.6)	0.9 (0.4–2.7)	0.9 (0.4–2.1)	1.4 (0.4–2.6)	0.555
Ophthalmic surgery following LSCT					
Optical penetrating/lamellar keratoplasty	24 (23.3)	8 (36.4)	10 (27.8)	6 (13.3)	0.084
Boston Keratoprosthesis	4 (3.9)	2 (9.1)	0 (0.0)	2 (4.4)	0.197
Phacoemulsification	17 (16.5)	3 (13.6)	8 (22.2)	6 (13.3)	0.517

BCVA = best corrected visual acuity; CLET = Cultivated Limbal Epithelial Transplantation; COMET = Cultivated Oral Mucosal Epithelial Transplantation; ICIF = impression cytology with immunofluorescence; IQR = interquartile range; IVCM = in vivo confocal microscopy; LSCD = limbal stem cell deficiency; LSCT = limbal stem cell transplantation; MGD = meibomian gland dysfunction; MMC = mitomycin C; mo = month; PKP = Peripheral Keratoplasty; PUK = peripheral ulcerative keratitis; SJS = Stevens–Johnson syndrome; SLET = Simple Limbal Epithelial Transplantation; Statistically significant difference when *$p < 0.05$. # median (IQR); N (%): number of eye (%).

3.4. Comparison of successful outcomes among the different LSCD etiologies

The patients were categorized into three groups; burn, SJS, and other causes. The analysis found that the burn and other-cause groups exhibited significantly better outcomes ($p < 0.001$) compared to the SJS group (Fig. 4A). The survival rate for burn patients at 5 years was 72.1 % and remained consistent to 13 years, which was higher than the survival rates for patients with other causes (5 years, 65.9 %; 10 years, 57.6 %); the patients with SJS had a survival rate of only 15.0 % at 5 years and stability up to 10 years (Fig. 4A). Further analysis of each surgical procedure among patients with SJS showed that SLET had a significantly higher survival rate compared to CLET ($p = 0.018$) and COMET ($p = 0.047$) (Fig. 4B). In the burn group, SLET had a significantly higher survival rate than CLET ($p = 0.031$) (Fig. 4C). Conversely, the other-cause groups showed no significant difference among the three procedures ($p = 0.102$), but COMET tended to demonstrate the most favorable outcome (Fig. 4D). Interestingly, if other causes were excluded and only patients with burn and SJS were compared (Table 2, model 2), the success rate of SLET was significantly better than that of CLET ($p = 0.031$) (see Table 3).

3.5. Comparison of successful outcomes among allogenic and autologous transplantations

The autologous graft procedure had the higher survival rate than the allograft procedure performed in CLET and SLET; however, the difference did not reach significance (Fig. 5A and B). The comparison between autoCLET and alloCLET showed no significant difference in survival rates ($p = 0.940$). Similarly, there was no significant difference between autoSLET and alloSLET ($p = 0.533$).

3.6. The overall visual outcomes

A significant improvement in VA was seen after epithelial transplantation; the mean logarithm of the minimum angle of resolution value decreased from 2.3 to 0.9. The improvements in BCVA for each technique are summarized in Table 1. Among the successful cases, 53.1 % showed an improvement of at least 4 lines in BCVA, while 10.9 % had a decline in BCVA largely due to corneal scarring, cataracts, and glaucoma. SLET had a higher proportion of successful cases with more than 4 lines of visual improvement compared to CLET and COMET (Fig. 6). Even in the failure groups, 28.2 % still had at least a 4-line improvement in the BCVA. Following the epithelial transplantation procedures, 24

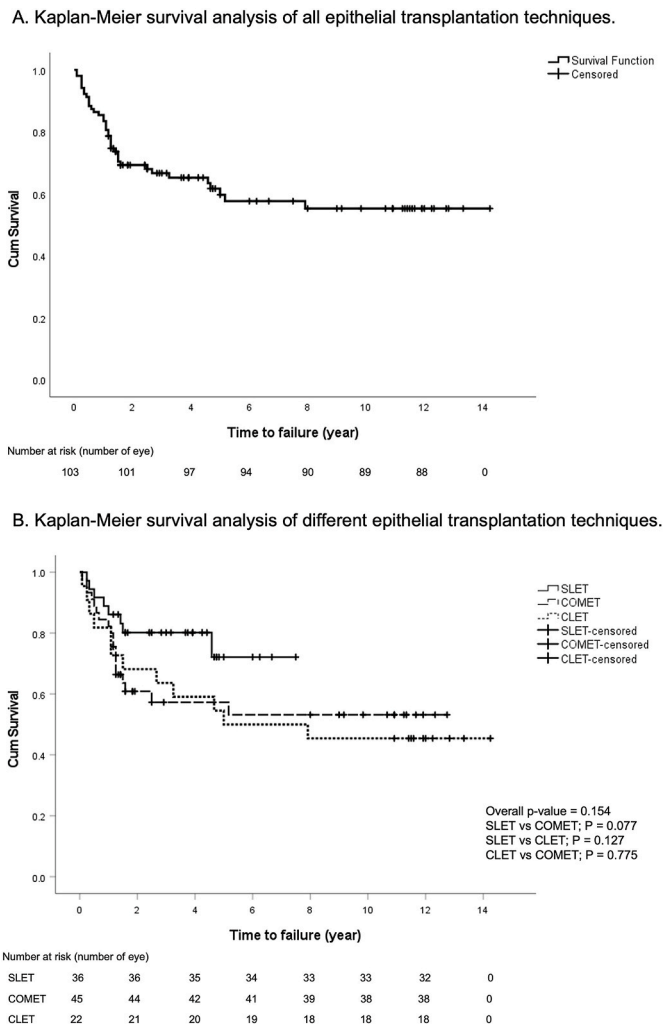

Fig. 2. Kaplan-Meier survival analysis of epithelial transplantation techniques. (A) Kaplan-Meier survival analysis of all epithelial transplantation techniques. (B) Kaplan-Meier survival analysis of different epithelial transplantation techniques.

surgeries (23.3 %) were performed to improve the BCVA as shown in Table 1.

3.7. Risk factors for failed surgical outcomes

There was a higher proportion of cases needed management of symblepharon prior to COMET (53 %) and CLET (54 %) as compared to SLET (19 %). However, the multivariable analysis has shown that prior ophthalmic surgery was not a significant risk factor associated with failure of epithelial transplantation ($p = 0.443$; HR1.53 95 % CI, 0.52–4.54) (Table 2). Regarding the etiology of LSCD, SJS ($p = 0.003$; HR 4.27 95 % CI, 1.64–11.12), Schirmer I test <5 mm ($p = 0.034$; HR 2.16; 95 % CI 1.06–4.42), and the presence of a postoperative recurrent epithelial defect ($p < 0.001$; HR 3.60; 95 % CI, 66–7.77) were risk factors for surgical failure.

3.8. Complications

No intraoperative complications occurred in any study group. A small number of postoperative complications were identified, ie, one eye in the CLET group and three eyes in the COMET group with SJS. These complications manifested as infectious keratitis attributed to incomplete epithelialization. Secondary ocular hypertension developed in four eyes and resolved after steroid tapering. No patient developed glaucoma postoperatively.

4. Discussion

Epithelial cell transplantation is one of the most challenging procedures when treating ocular surface disorders. Several surgical techniques for cell transplantation have been developed and improved to restore limbal stem cell functions [3,5,9]. However, comparative research on the outcomes of different types of epithelial transplantation technique in the same patient population is lacking. In this study, we analyzed data from long follow-up periods after CLET, SLET, or COMET. The median follow-up time was approximately 70 months, with the longest follow-up reaching up to 14 years in CLET. The survival analysis showed a favorable overall survival rate for all types of epithelial cell transplantations, with an initial rate of nearly 80 % during the first year postoperatively. The survival rate gradually declined to about 50 % in CLET and COMET at 7 years and remained stable up to 14 years, which is consistent with previous reports [16,17,19,20,22,24–28]. Interestingly, the overall survival rate and those for each epithelial transplantation technique showed a similar pattern characterized by rapid decline during the first quartile followed by a slower decline and maintenance. This observation can be credited to the resilience of epithelial stem cells, which enables them to thrive in their new environment and undergo gradual growth and differentiation. The long-term success of cell transplantation relies heavily on the cell's ability to sustain itself over time, with a conducive niche playing a crucial role in this aspect [29–31]. It is worth noting that even though different techniques and procedures for preparing the original stem cells were used, all can provide stem cells. P63-bright cells were positive in CLET cultures and clinical results [32]. Likewise, in SLET, p63, a marker of stem cells, was positively expressed in the basal epithelial layers of the entire corneal surface [22]. Moreover, in allo-limbal cells transplanted, up to 67 % of the genotypic results changed to the recipient genotype [33]. Similarly, in COMET, in which the transplanted cells were not derived from limbal stem cells, the transplanted cells survived and maintained long-term survival [17]. This phenomenon can be explained by the survival and differentiation of oral mucosal epithelial cells into corneal-like epithelium [34]. Overall, this study provides valuable insights into the long-term outcomes of different types of epithelial transplantation techniques. The findings highlight the favorable survival rates and shed light on the cellular mechanisms underlying the success of these procedures.

Among three surgical techniques performed, no significant differences were seen in the overall survival rate. However, SLET tended to have a superior survival rate compared to COMET and CLET, even though the severity of patients was unequal among these groups as the majority of SLET (78 %) and CLET (77 %) cases were total LSCD, while most of COMET patients (51 %) were partial LSCD. Further analysis for partial LSCD outcomes revealed the same results. SLET had better outcomes compared to CLET ($p = 0.195$) and COMET ($p = 0.044$*). In addition, there were no significant differences seen between partial and total LSCD or allo- and auto-limbal transplantation (Fig. 5), which are similar to previous studies [20,22,35,36]. Despite that some previous studies reported a better outcome with auto-limbal transplantation than allo-limbal transplantation [27,37], the use of long-term postoperative immunosuppressive agents contributed to favorable outcomes in allo-limbal transplantation [33]. Although the standard guideline of systemic immunosuppressive agents for limbal stem cell transplantation has never been established, the regimen we used in most of our cases were mycophenolate mofetil and cyclosporin. Nevertheless, in partial LSCD, the survival rate after SLET remained at 100 % for 6 years postoperatively, which was significantly better than in COMET. The plausible reason might be that in COMET, the transplanted cells originated from the oral mucosa, which might not be as good as cells originating from the limbus. Thus, SLET may be a good choice for treating partial

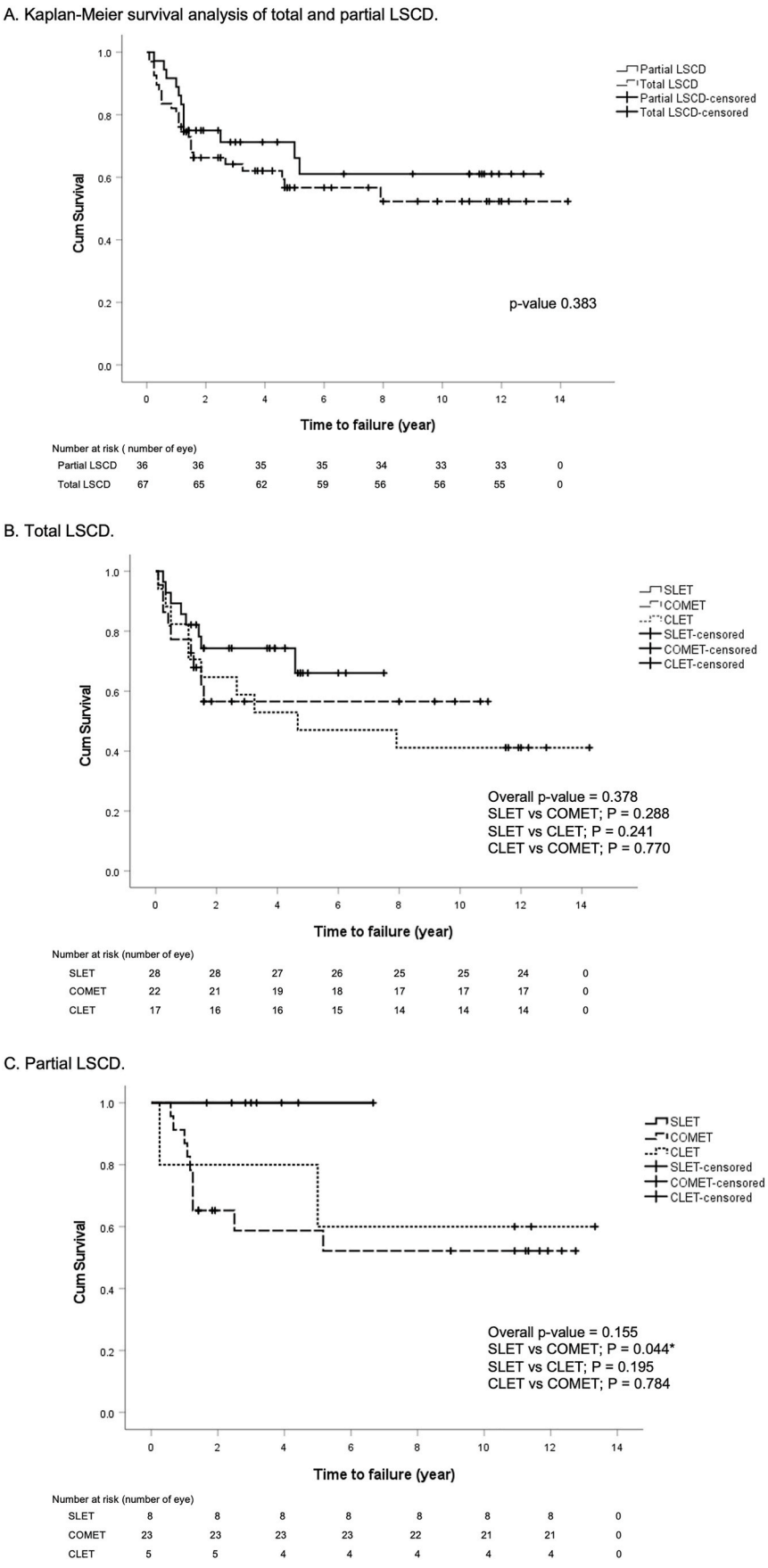

Fig. 3. (A) Kaplan-Meier survival analysis of total and partial LSCD. (B) Total LSCD. (C) Partial LSCD.

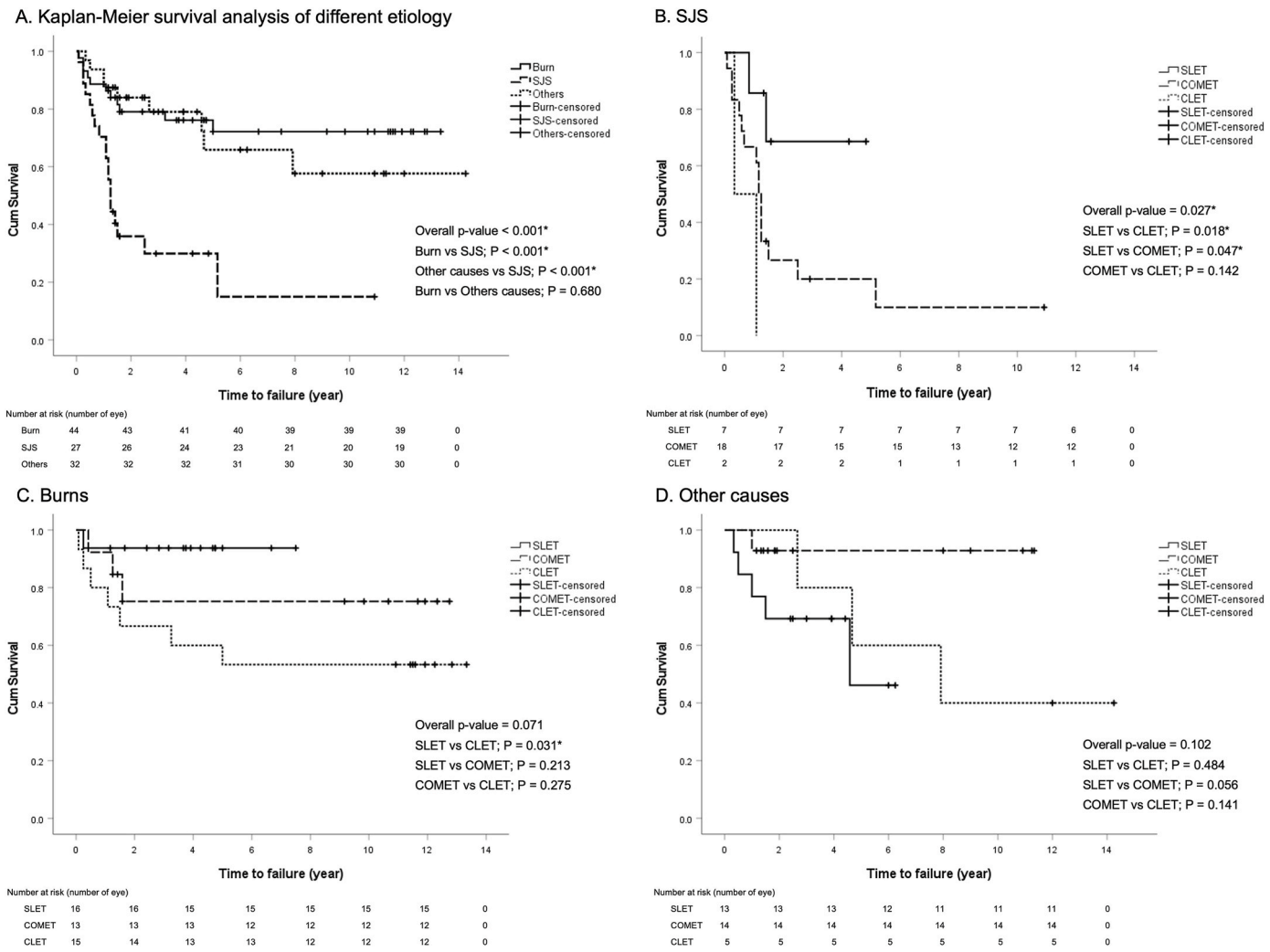

Fig. 4. (A) Kaplan-Meier survival analysis of different etiology. (B) SJS. (C) Burns. (D) Other causes.

Table 2
Factor associated with failure of epithelial transplantation.

Characteristic	Success N (%)	Failure N (%)	Univariate Analysis		Multivariable Analysis	
			HR (95 % CI)	p-value	HR (95 % CI)	p-value
Age (years); mean ± SD	46.00 ± 17.26	43.30 ± 14.87	0.99 (0.97, 1.02)	0.351	–	–
Etiology						
Chemical/Thermal injury	33 (75.0)	11 (25.0)	1		1	
SJS	8 (29.6)	19 (70.4)	4.78 (2.23, 10.27)	**<0.001***	4.26 (1.62–11.21)	**0.003***
Others	23 (71.9)	9 (28.1)	1.19 (0.49, 2.88)	0.695	1.61 (0.62–4.18)	0.331
Total LSCD	40 (59.7)	27 (40.3)	0.86 (0.61, 1.21)	0.388	–	–
Duration of LSCD(mo)	34.50 (15.25–72.00)	36.00 (12.00–116.00)	1.00 (0.99, 1.01)	0.188	–	–
Preoperative data						
Schirmer I test <5 mm.	11 (37.9)	18 (62.1)	2.89 (1.52, 5.47)	**0.001***	2.18 (1.06–4.46)	**0.034***
Symblepharon	22 (46.8)	25 (53.2)	2.46 (1.28, 4.73)	**0.007***	0.76 (0.26–2.18)	0.604
Eyelid abnormality	25 (54.3)	21 (45.7)	1.72 (0.91, 3.23)	**0.094**	1.23 (0.54–2.81)	0.630
Prior ophthalmic surgery	28 (49.1)	29 (50.9)	2.69 (1.31, 5.53)	**0.007***	1.53 (0.52–4.54)	0.443
Preoperative BCVA (logMAR) ≥ 1.00	41 (56.2)	32 (43.8)	2.27 (1.00, 5.15)	**0.050***	1.15 (0.45–2.92)	0.773
Limbal Stem Cell Transplantation						
SLET	28 (77.8)	8 (22.2)	1		1	
CLET	10 (45.5)	12 (54.5)	2.24 (0.91, 5.53)	**0.081**	2.14 (0.77–5.92)	0.145
COMET	26 (57.8)	19 (42.2)	2.02 (0.88, 4.63)	**0.098**	1.07 (0.41–2.76)	0.897
Postoperative recurrent epithelial defect	12 (33.3)	24 (66.7)	4.58 (2.39, 8.76)	**<0.001***	3.76 (1.77–7.97)	**<0.001***

BCVA = best corrected visual acuity; CI = Confidence Interval; CLET = Cultivated Limbal Epithelial Transplantation; COMET = Cultivated Oral Mucosal Epithelial Transplantation percentile range; LSCD = limbal stem cell deficiency; mo = month; OR = Odd Ratio; SJS = Stevens–Johnson syndrome; SLET = Simple Limbal Epithelial Transplantation; Statistically significant difference when *p < 0.05.
HR =Hazard Ratio.

Table 3
Summary of factors associated with failure of epithelial transplantation in LSCD patients.

Characteristic	Univariable analysis		Multivariable analysis			
			Model 1		Model 2	
			N = 103		N = 71	
	HR (95 % CI)	p-value	aHR (95 % CI)	p-value	aHR (95 % CI)	p-value
Total LSCD	0.86 (0.61, 1.21)	0.388	0.90 (0.61–1.33)	0.601	0.878 (0.572–1.350)	0.554
Preoperative data						
Schirmer I test <5 mm.	2.89 (1.52, 5.47)	**0.001***	2.16 (1.06–4.42)	**0.034***	2.38 (1.00–5.65)	**0.050***
Symblepharon	2.46 (1.28, 4.73)	**0.007***	0.75 (0.26–2.16)	0.594	0.378 (0.092–1.544)	0.175
Eyelid abnormality	1.72 (0.91, 3.23)	0.094	1.18 (0.51–2.76)	0.70	1.44 (0.548–3.788)	0.46
Prior ophthalmic surgery	2.69 (1.31, 5.53)	**0.007***	1.55 (0.52–4.62)	0.428	3.419 (0.577–20.279)	0.176
Preoperative BCVA (logMAR) ≥ 1.00	2.27 (1.00, 5.15)	**0.050***	1.15 (0.45–2.93)	0.773	0.781 (0.255–2.392)	0.665
Etiology						
Chemical/Thermal injury	1		1		1	
SJS	4.78 (2.23, 10.27)	**<0.001***	4.27 (1.64–11.12)	**0.003***	4.758 (1.651–13.713)	**0.004***
Others	1.19 (0.49, 2.88)	0.695	1.538 (0.59–4.03)	0.382	–	–
Limbal Stem Cell Transplantation						
SLET	1		1		1	
CLET	2.24 (0.91, 5.53)	0.081	2.15 (0.77–5.98)	0.143	6.36 (1.18–34.19)	**0.031***
COMET	2.02 (0.88, 4.63)	0.098	1.07 (0.41–2.76)	0.765	2.43 (0.57–10.36)	0.229
Postoperative recurrent epithelial defect	4.58 (2.39, 8.76)	**<0.001***	3.60 (1.66–7.77)	**<0.001***	3.44 (1.20–9.85)	**0.021***

CI = Confidence Interval; CLET = Cultivated Limbal Epithelial Transplantation; COMET = Cultivated Oral Mucosal Epithelial Transplantation; aHR = Adjusted Hazard Ratio; LSCD = limbal stem cell deficiency; mm = millimeter; SJS = Stevens–Johnson syndrome; SLET = Simple Limbal Epithelial Transplantation; *Statistically significant difference when $p < 0.05$.
Model 1: All causes of LSCD.
Model 2: LSCD derived from chemical injury and SJS. Other causes of LSCD, which included severe allergic conjunctivitis, PUK, multiple surgeries, severe MGD, MMC toxicity, aniridia, advanced pterygium, ocular trauma, and idiopathic etiology were excluded.

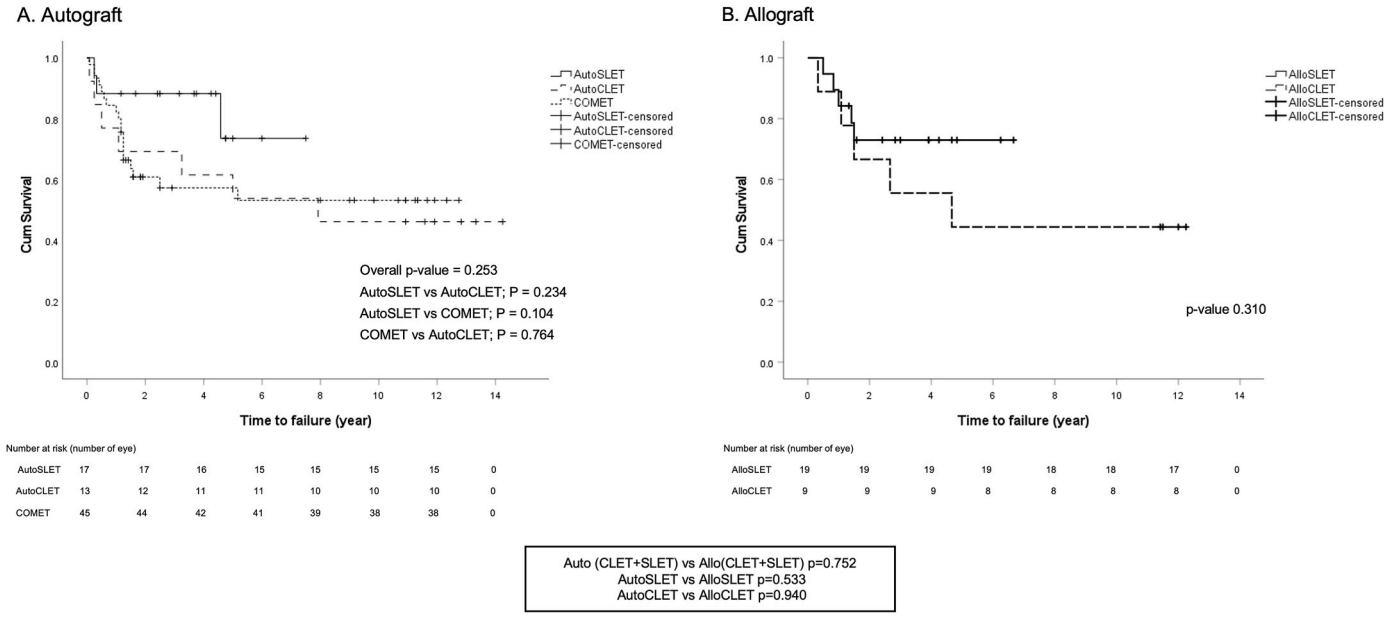

Fig. 5. Kaplan-Meier survival analysis of different donor sources. (A) Autograft. (**B**) Allograft.

LSCD due to its good outcomes, single-step surgery, and no need for stem cell laboratory culture.

The etiology significantly affects both the prognosis and survival rate. The survival rates of ocular surface reconstructions were excellent in patients with burns and worst in those with SJS. This finding is consistent with previous studies, which identified SJS as a significant risk factor for unsuccessful procedures [17,22,38]. The poorer outcomes observed in patients with SJS can be attributed to the severe ocular involvement often present in these cases, including dry eye, squamous metaplasia of the conjunctival epithelium, and especially eyelid abnormalities [40]. These factors can cause further ocular surface damage through mechanical trauma and lead to a higher incidence rate of postoperative complications than burns and other causes despite similar preoperative conditions [39,41]. Despite the poor prognosis in SJS cases, living related-alloSLET had a significantly higher survival rate than CLET and COMET, possibly attributed to the use of fresh tissue without laboratory manipulation and the higher number of limbal stem cells transplanted in SLET, which could have been twice as many as in CLET. In patients with burns, SLET had a significantly higher survival rate than CLET. The results from this study shed light on the selection of the appropriate surgical method for each patient based on the etiology of the LSCD.

Regarding the visual outcomes, epithelial transplantation achieved excellent visual recovery in more than half of the successful cases. Notably, even in failed cases, a quarter of the cases also had a BCVA exceeding four lines postoperatively. Furthermore, nearly half of all

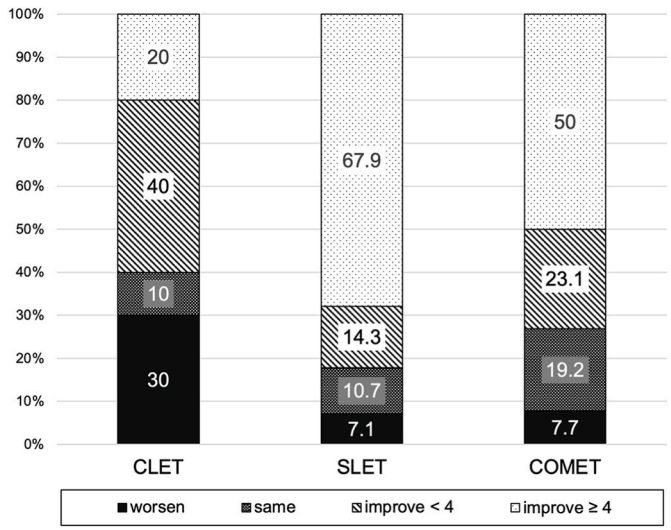

Fig. 6. Comparison of the improvement of Snellen BCVA between different epithelial transplantation techniques in successful cases.

patients can successfully undergo supplementary surgical procedures to enhance the visual outcome after epithelial transplantation. These findings emphasize that epithelial transplantation is a reasonable option for treating patients with LSCD, as it has the potential to improve visual outcomes even in cases of surgical failure. Moreover, the procedure creates opportunities to facilitate the performance of other surgical interventions, thereby providing additional avenues for enhancing the VA in patients with LSCD with a lower incidence of complications.

A comprehensive analysis was conducted to identify the risk factors associated with the failure of epithelial cell transplantation. Multivariable analysis showed that SJS, preoperative Schirmer I test <5 mm, and recurrent postoperative epithelial defects were the key factors contributing to the failed surgeries. The findings strongly indicate that SJS and preoperative severe dryness should be given high consideration for all types of epithelial cell transplantations. The persistence of epithelial defects postoperatively indicates deterioration of stem cell function and populations, an unsuitable ocular surface environment, and the dysfunction and destruction of the limbal stem cell niche. These factors are crucial in determining the survival capacity of transplanted cells [29]. Furthermore, no significant correlations were observed with other factors.

The advantage of this study was its long follow-up time, which was crucial for assessing the surgical efficacy, outcomes, and long-term clinical stability of the different epithelial transplantation techniques for LSCD. Confirmation of the preoperative diagnoses and postoperative outcomes were obtained through impression cytology (ICIF) and in vivo confocal microscopy (IVCM), in addition to clinical examination. The congruence of these objective measures supported the validity of our results. To the best of our knowledge, no previous research has directly compared the results across different types of epithelial transplantation techniques in the same population while following similar guidelines for management and postoperative care. This study provides novel insights into the comparison of long-term outcomes among different epithelial transplantation techniques for treating LSCD in a similar environment.

The current study had some limitations. First, this study was retrospective in nature, which is associated with limitations regarding controlling bias, confounding factors, and missing data. In addition, unequal numbers of patients in each group and varying follow-up times can introduce variations, although statistical techniques were used to adjust for potential bias. Despite these limitations, our data were recorded in a registry and together with photographs, all patients followed the same guideline treatment and follow-up as in previous prospective reports. In difficult and complicated cases such as LSCD, the treatment choice must consider ethical concerns for the best results for each patient, so it is difficult to design a randomized clinical trial. Second, the results of CLET and COMET in this study represent the outcome of our media, which were free of 3T3 and serum, which might not directly represent the outcome of different media in other studies. The retrospective nature of the study provided an opportunity to investigate many cases within the defined setting. Conducting a prospective study with a large sample size may help address some of these limitations and provide more robust evidence.

In conclusion, this study showed that all epithelial transplantation techniques provide favorable long-term surgical and visual outcomes for patients with LSCD, with more than half of the patients achieving stable ocular surface and VA improvement up to 7 years postoperatively. Importantly, the LSCD etiology emerged as a crucial factor in determining the most suitable surgical approach. Tailoring the choice of technique based on the underlying cause of LSCD can optimize treatment outcomes and enhance patient satisfaction. Overall, epithelial cell transplantation is a valuable option for treating LSCD and has the potential to significantly improve the quality of life and visual function of affected individuals. Moreover, this study contributes to the identification of prognostic factors that can aid clinicians in predicting outcomes and informing treatment decisions for patients with LSCD.

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Proof English reading letter

Cintia S. de Paiva, MD, PhD.
Editor-in-chief, Ocular Surface.
December 4, 2023.

Dear Dr. de Paiya, This message is to certify that I edited the English in Dr. Sathiya Kengpunpanich's article before submission to your journal.

My company, Medical International, has assisted doctors who have English as a second language for the past 35 years.

I hope this information is helpful to you.
Best regards, Lynda Charters.
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CRediT authorship contribution statement

Sathiya Kengpunpanich: Conceptualization, Formal analysis, Methodology, Validation, Writing – original draft, Writing – review & editing. **Chareenun Chirapapaisan:** Validation, Visualization, Writing – review & editing. **Panotsom Ngowyutagon:** Data curation, Formal analysis, Methodology. **Suksri Chotikavanich:** Data curation, Investigation, Methodology. **Rosanun Sikarinkul:** Conceptualization, Formal analysis. **Nuttacha Taetrongchit:** Conceptualization, Data curation, Formal analysis. **Simaporn Setthawong:** Formal analysis, Investigation, Methodology, Resources, Writing – original draft. **Pinnita Prabhasawat:** Conceptualization, Resources, Supervision, Validation, Visualization.

Declaration of competing interest

No conflicting relationship exists for any author.

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References

[1] Haagdorens M, Van Acker SI, Van Gerwen V, et al. Limbal stem cell deficiency: current treatment options and emerging therapies. Stem Cell Int 2016;2016:9798374.
[2] Deng SX, Borderie V, Chan CC, et al. Global consensus on definition, classification, diagnosis, and staging of limbal stem cell deficiency. Cornea 2019;38(3):364–75.
[3] Pellegrini G, Traverso CE, Franzi AT, Zingirian M, Cancedda R, De Luca M. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. Lancet 1997;349(9057):990–3.
[4] Tsai RJ, Li LM, Chen JK. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. N Engl J Med 2000;343(2):86–93.
[5] Sangwan VS, Basu S, MacNeil S, Balasubramanian D. Simple limbal epithelial transplantation (SLET): a novel surgical technique for the treatment of unilateral limbal stem cell deficiency. Br J Ophthalmol 2012;96(7):931–4.
[6] Amescua G, Atallah M, Nikpoor N, Galor A, Perez VL. Modified simple limbal epithelial transplantation using cryopreserved amniotic membrane for unilateral limbal stem cell deficiency. Am J Ophthalmol 2014;158(3):469. 75.e2.
[7] Mittal V, Jain R, Mittal R. Ocular surface epithelialization pattern after simple limbal epithelial transplantation: an in vivo observational study. Cornea 2015;34(10):1227–32.
[8] Koizumi N, Inatomi T, Suzuki T, Sotozono C, Kinoshita S. Cultivated corneal epithelial stem cell transplantation in ocular surface disorders. Ophthalmology 2001;108(9):1569–74.
[9] Nakamura T, Inatomi T, Sotozono C, Amemiya T, Kanamura N, Kinoshita S. Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. Br J Ophthalmol 2004;88(10):1280–4.
[10] Inatomi T, Nakamura T, Koizumi N, Sotozono C, Yokoi N, Kinoshita S. Midterm results on ocular surface reconstruction using cultivated autologous oral mucosal epithelial transplantation. Am J Ophthalmol 2006;141(2):267–75.
[11] Nishida K, Yamato M, Hayashida Y, et al. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. N Engl J Med 2004;351(12):1187–96.
[12] Ang LP, Nakamura T, Inatomi T, et al. Autologous serum-derived cultivated oral epithelial transplants for severe ocular surface disease. Arch Ophthalmol 2006;124(11):1543–51.
[13] Nakamura T, Inatomi T, Cooper LJ, Rigby H, Fullwood NJ, Kinoshita S. Phenotypic investigation of human eyes with transplanted autologous cultivated oral mucosal epithelial sheets for severe ocular surface diseases. Ophthalmology 2007;114(6):1080–8.
[14] Ganger A, Vanathi M, Mohanty S, Tandon R. Long-term outcomes of cultivated limbal epithelial transplantation: evaluation and comparison of results in children and adults. BioMed Res Int 2015;2015:480983.
[15] Basu S, Sureka SP, Shanbhag SS, Kethiri AR, Singh V, Sangwan VS. Simple limbal epithelial transplantation: long-term clinical outcomes in 125 cases of unilateral chronic ocular surface burns. Ophthalmology 2016;123(5):1000–10.
[16] Satake Y, Higa K, Tsubota K, Shimazaki J. Long-term outcome of cultivated oral mucosal epithelial sheet transplantation in treatment of total limbal stem cell deficiency. Ophthalmology 2011;118(8):1524–30.
[17] Prabhasawat P, Ekpo P, Uiprasertkul M, et al. Long-term result of autologous cultivated oral mucosal epithelial transplantation for severe ocular surface disease. Cell Tissue Bank 2016;17(3):491–503.
[18] Shanbhag SS, Nikpoor N, Rao Donthineni P, Singh V, Chodosh J, Basu S. Autologous limbal stem cell transplantation: a systematic review of clinical outcomes with different surgical techniques. Br J Ophthalmol 2020;104(2):247–53.
[19] Fasolo A, Pedrotti E, Passilongo M, et al. Safety outcomes and long-term effectiveness of ex vivo autologous cultured limbal epithelial transplantation for limbal stem cell deficiency. Br J Ophthalmol 2017;101(5):640–9.
[20] Shimazaki J, Satake Y, Higa K, Yamaguchi T, Noma H, Tsubota K. Long-term outcomes of cultivated cell sheet transplantation for treating total limbal stem cell deficiency. Ocul Surf 2020;18(4):663–71.
[21] Wang J, Qi X, Dong Y, et al. Comparison of the efficacy of different cell sources for transplantation in total limbal stem cell deficiency. Graefes Arch Clin Exp Ophthalmol 2019;257(6):1253–63.
[22] Prabhasawat P, Chirapapaisan C, Ngowyutagon P, et al. Efficacy and outcome of simple limbal epithelial transplantation for limbal stem cell deficiency verified by epithelial phenotypes integrated with clinical evaluation. Ocul Surf 2021;22:27–37.
[23] Prabhasawat P, Ekpo P, Uiprasertkul M, Chotikavanich S, Tesavibul N. Efficacy of cultivated corneal epithelial stem cells for ocular surface reconstruction. Clin Ophthalmol 2012;6:1483–92.
[24] Basu S, Mohan S, Bhalekar S, Singh V, Sangwan V. Simple limbal epithelial transplantation (SLET) in failed cultivated limbal epithelial transplantation (CLET) for unilateral chronic ocular burns. Br J Ophthalmol 2018;102(12):1640–5.
[25] Sangwan VS, Basu S, Vemuganti GK, et al. Clinical outcomes of xeno-free autologous cultivated limbal epithelial transplantation: a 10-year study. Br J Ophthalmol 2011;95(11):1525–9.
[26] Vazirani J, Ali MH, Sharma N, et al. Autologous simple limbal epithelial transplantation for unilateral limbal stem cell deficiency: multicentre results. Br J Ophthalmol 2016;100(10):1416–20.
[27] Wang Y, Hu X, Yang K, et al. Clinical outcomes of modified simple limbal epithelial transplantation for limbal stem cell deficiency in Chinese population: a retrospective case series. Stem Cell Res Ther 2021;12(1):259.
[28] Nakamura T, Takeda K, Inatomi T, Sotozono C, Kinoshita S. Long-term results of autologous cultivated oral mucosal epithelial transplantation in the scar phase of severe ocular surface disorders. Br J Ophthalmol 2011;95(7):942–6.
[29] Bonnet C, Roberts JS, Deng SX. Limbal stem cell diseases. Exp Eye Res 2021;205:108437.
[30] Tseng SC. Concept and application of limbal stem cells. Eye 1989;3(Pt 2):141–57.
[31] Nubile M, Curcio C, Dua HS, et al. Pathological changes of the anatomical structure and markers of the limbal stem cell niche due to inflammation. Mol Vis 2013;19:516–25.
[32] Rama P, Matuska S, Paganoni G, Spinelli A, De Luca M, Pellegrini G. Limbal stem-cell therapy and long-term corneal regeneration. N Engl J Med 2010;363(2):147–55.
[33] Chotikavanich S, Poriswanish N, Luangaram A, et al. Genetic analysis of allogenic donor cells after successful allo-limbal epithelial transplantation in simple and cultivated limbal epithelial transplantation procedures. Sci Rep 2023;13(1):4290.
[34] Prabhasawat P, Chirapapaisan C, Jiravarnsirikul A, et al. Phenotypic characterization of corneal epithelium in long-term follow-up of patients post-autologous cultivated oral mucosal epithelial transplantation. Cornea 2021;40(7):842–50.
[35] Zhao Y, Ma L. Systematic review and meta-analysis on transplantation of ex vivo cultivated limbal epithelial stem cell on amniotic membrane in limbal stem cell deficiency. Cornea 2015;34(5):592–600.
[36] Sacchetti M, Rama P, Bruscolini A, Lambiase A. Limbal Stem cell transplantation: clinical results, limits, and perspectives. Stem Cell Int 2018;2018:8086269.
[37] Le Q, Chauhan T, Yung M, Tseng CH, Deng SX. Outcomes of limbal stem cell transplant: a meta-analysis. JAMA Ophthalmol 2020;138(6):660–70.
[38] Kang MH. Ocular manifestations of Stevens-Johnson syndrome and toxic epidermal necrolysis. Hanyang Med Rev 2016;36(3).
[39] Ramachandran C, Basu S, Sangwan VS, Balasubramanian D. Concise review: the coming of age of stem cell treatment for corneal surface damage. Stem Cell Transl Med 2014;3(10):1160–8.
[40] Jain R, Sharma N, Basu S, et al. Stevens-Johnson syndrome: the role of an ophthalmologist. Surv Ophthalmol 2016;61(4):369–99.
[41] Yoshikawa Y, Ueta M, Fukuoka H, et al. Long-term progression of ocular surface disease in Stevens-Johnson syndrome and toxic epidermal necrolysis. Cornea 2020;39(6):745–53.