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REVIEW

Systematic review and meta-analysis investigating autograft versus allograft cultivated limbal epithelial transplantation in limbal stem cell deficiency

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Abstract

Purpose Currently, regenerative medicine has attracted much attention among researchers investigating new methods to treat ocular surface diseases. Based on this new concept, cultivated limbal epithelial transplantation (CLET), whether in the form of autograft or allograft, has emerged as a promising surgical procedure for treating limbal stem cell deficiency (LSCD). Given that there is no updated comparison between autograft and allograft CLETs, the present review and meta-analysis aims to compare and determine the efficacy of two different CLET techniques, autologous versus allogeneic, based on a literature review of relevant studies.

Methods A comprehensive search of electronic databases, including PubMed, Web of Science, Cochrane Library, Embase and Scopus, for related articles was performed in March 2018 to obtain relevant articles and to conduct a meta-analysis investigating the success rate of ocular surface regeneration and two-line improvement in best-corrected visual acuity (BCVA) using autograft versus allograft transplantations.

Results A total of 30 studies, including 1306 eyes from 1288 patients with LSCD, with a sample size ranging from 6 to 200 and follow-up period of 0.6–156 months, were reviewed. Of 1306 eyes, 982 (75.2%) underwent autograft and 324 (24.8%) received allografts from living or deceased donors. Meta-analysis revealed that there was no significant difference between autograft and allograft CLETs in terms of success rate and two-line BCVA improvement. The prospective studies showed a zero difference between the two groups; only two retrospective studies included in the analysis pulled the autografts up to 1.82 and 1.2 times more than allografts in terms of success rate and two-line BCVA improvement, respectively [pooled OR 1.82 (95% CI 0.80–4.11); pooled OR 1.2 (95% CI 0.54–2.65)]. There was no statistically significant evidence of bias in the meta-analysis in terms of success rates and two-line BCVA improvement.

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Conclusions The present analysis revealed no significant differences in success rates or visual improvement between autograft and allograft surgical techniques.

Keywords CLET · Cultured limbal epithelial transplantation · Limbal stem cell deficiency

Introduction

Limbal epithelial stem cells (LESCs) are important for supporting and maintaining the corneal epithelium and preserving corneal transparency and have attracted much attention in the field of ocular surface regenerative medicine [1]. LESCs have anti-inflammatory and anti-angiogenic roles in supporting the integrity of the entire corneal surface [2]. Limbal stem cell deficiency (LSCD) occurs as a result of thermal and chemical burns, ultraviolet radiation, Stevens–Johnson syndrome, ocular cicatricial pemphigoid, contact lenses, multiple surgical procedures, antimetabolites, microbial infections, aniridia and cryotherapy [3–5]. Based on the extent of the damaged area, LSCD can be divided into partial or total LSCD. It is characterized by chronic inflammation, conjunctivalization, neovascularization of the cornea, epithelial haze, persistent or recurrent epithelial defects, epithelial and stromal inflammations, late fluorescein staining, loss of the limbal palisades of Vogt and fibrous ingrowth, leading to pain, photophobia, low vision and, ultimately, corneal blindness [3, 6–8].

Several cell-based therapeutic techniques have been introduced to treat patients with LSCD. In 1997, for the first time, transplantation of autologous cultivated corneal epithelium was performed as a novel technique for reconstructing and improving ocular surface of a patient with unilateral LSCD [9]. Ex vivo cultivated limbal epithelial transplantation (CLET) is a therapeutic procedure to treat LSCD. In this technique, a small limbal biopsy (2 mm × 2 mm) is harvested from the contralateral healthy eye in cases with unilateral total LSCD, or from a living or cadaveric donor, and is cultivated mostly on human amniotic membrane (HAM) in ex vivo conditions. The cultivated sheet is then transplanted onto the eye affected by LSCD [9, 10]. CLET, with many advantages compared with conventional therapeutic methods, has become very popular in recent years [11]. One of the advantages of this technique is that it requires a smaller piece of donor limbal tissue, which in addition reduces the risk of allogeneic rejection [10] and reduces the risk of LSCD in the donor eye [12]. Several carriers including HAM and fibrin have been used, among which HAM has been more advantageous due to its ability to promote epithelialization, inhibition of fibrosis, anti-inflammatory and anti-angiogenic properties, antimicrobial and antiviral properties and high hydraulic conductivity [13–16] with low or no immunogenicity [17]. Although no differences between the efficacy of autograft and allograft CLET have been reported in terms of success rate and visual acuity (VA) outcomes [18], there has been no updated comparison between these two techniques. In the current meta-analysis, we reviewed the relevant literature to determine whether there is any significant difference between autologous and allogeneic CLET techniques in terms of success rate and visual acuity improvements.

Materials and methods

Systematic literature search

The literature search of the PubMed, Web of Science, Cochrane Library, Embase and Scopus databases was performed in March 2018. It was limited to English articles without time limitation using the following key words: “limbal stem cell deficiency,” “cultivated limbal epithelial transplantation” and “CLET.” The reference lists of all retrieved articles were scanned to identify additional relevant studies.

Eligibility criteria

Based on the following criteria, eligible studies were included: Participants included all age groups with any type of LSCD; surgical interventions of autologous and/or allogeneic ex vivo CLET. Then, in a comparison meta-analysis, we included the studies that have the information corresponding to the outcomes of the two above-mentioned interventions simultaneously. Full-text articles were reviewed to extract important information. However, review articles, animal or in vitro studies, case reports, conference papers and letters were excluded. In addition to the demographic

data, important variables included preoperative and postoperative information such as source of LESCs, ex vivo cultivation method, cause and extent of LSCD, duration between injury and surgery, types of previous treatments and post-CLET corneal transplantation.

Quality assessment

To determine the quality of the selected studies, a comprehensive and precise coding form consisting of 14 quality questions was used (Supplemental Digital Content 1, http://links.lww.com/ICO/A265) [19]. Prognostic factors in our assessment included age, sex, etiology, lid abnormalities, treatments before CLET, interval between injury and CLET surgery, type of LSCD, source of LESCs and extent of dry eye. Objective outcome measures were success rate and VA improvement after CLET. Success rate was defined as clinical improvement in the corneal surface in the form of complete re-epithelialization of the corneal surface with a stable and transparent corneal epithelium evidenced by clinical examinations and/or impression cytology. VA improvement was defined as achieving at least two Snellen lines of best-corrected VA (BCVA). For the visual acuities of "hand motion (HM)" and "counting fingers (CF)," the change in VA from HM to CF was considered to be a two-line improvement in VA [20]. As for the follow-up period, positive measure of quality was considered for those studies that had a minimum six-month follow-up.

Information extraction

Information including study type, numbers of participants and eyes, extent of LSCD, donor type, duration of follow-up, ex vivo culture method(s) in terms of type of carrier (i.e., HAM or other), use of 3T3 feeder layer and/or animal-free conditions, good manufacturing practice (GMP) grade, post-CLET corneal transplantation and clinical outcomes in terms of complete success rate and two-line VA improvement was recorded.

Statistical analysis

Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were obtained for two-line VA improvement and complete success. Additionally, log OR and its corresponding standard error (SE) were calculated to be used in analyses of effect size in a separate meta-analysis. To obtain the pooled proportion of the VA improvement and complete success and to avoid the exclusion of the studies with observed proportion of zero or 100 percent, we used Freeman–Tukey double arcsine transformation to stabilize the variances. Also, we used exact confidence interval based on binomial distribution for demonstrating the proportion of individual studies in the forest plot. Considering the variability among studies' effects, overall effects were based on the DerSimonian and Laird random-effects model. Subgroup analysis was applied to mitigate possible sources of heterogeneity. Statistical heterogeneity was tested using the *I*-squared (I^2) statistic. A sensitivity analysis was performed by removing studies from the meta-analysis one by one. Publication bias was investigated using Begg's funnel plots, and Egger's and Begg's asymmetry tests. If publication bias was observed, the trim-and-fill method was applied to further evaluate whether the correction of bias would significantly change the results of the meta-analysis. All statistical analyses were performed using STATA, version 14.0 (StataCorp LP, College Station, TX, USA); $P < 0.05$ was considered to be statistically significant.

Results

Study identification

A total of 162 relevant articles were found, among which 47 were included in the present study by a review of abstracts. After excluding five case reports and two reviews, the full texts of the remaining 40 articles were reviewed, and considering duplicate studies, 30 were finally selected to extract important information for the meta-analysis. Two-line improvement in BCVA and complete success rate were analyzed in 24 and 29 studies, respectively.

Study characteristics

All 30 studies were case series (prospective, retrospective and clinical trials) that involved patients with partial or total LSCD between 2000 and 2018 in various countries. In total, 1306 eyes from 1288 patients with LSCD were included. The range in sample size was 6–200, and the follow-up period was

Table 1 Information on included studies

References	Years	Country	Type of study	Eyes/patients	Extent of LSCD		Type of donor		PK/LK post-CLET transplantation	Immunosuppression	Follow-up (months)
					Total	Partial	Auto	Allo			
Baradaran-Rafii et al. [21]	2010	Iran	CS	8/8	8	0	8	0	4	–	34 (6–48)
Basu et al. [22]	2012	India	RCS	50/50	50	0	50	0	8	–	27.6 (12–90)
Chen et al. [23]	2016	China	CS	41/41	31	28	0	41	5	+	22.13 (10–89)
Cheng et al. [24]	2017	China	RCS	80/80	57	23	0	80	22	+	26.4 (12–60)
Daya et al. [25]	2005	UK	RCS	10/10	10	0	0	10	4	+	28 (12–50)
Di Iorio et al. [26]	2010	Italy	CS	166/166	UN	UN	166	0	33	–	29.9 (6–50)
Fasolo et al. [27]	2016	Italy	RCS	59/59	UN	UN	59	0	19	–	72(12–156)
Ganger et al. [28]	2015	India	RCS	62/62	33	0	54	8	0	+	23 (0.6–45.4)
Gisoldi et al. [29]	2010	Italy	CS	6/6	3	3	6	0	4	–	24 (11–34)
Kawashima et al. [30]	2007	Japan	CS	6/6	UN	UN	2	4	6	+	25.1 (5–41)
Koizumi et al. [31]	2001	Japan	RCS	13/11	13	13	0	13	5	+	11.2 (9–13)
Kolli et al. [32]	2010	UK	PCS	8/8	8	0	8	0	0	–	19 (12–30)
Nakamura et al. [33]	2006	Japan	RCS	9/9	9	0	2	7	2	–	14.6 (6–20)
Parihar et al. [34]	2017	India	PCS	25/20	UN	UN	0	25	0	+	12
Pathak et al. [35]	2013	Norway	RCS	9/9	4	5	9	0	1	–	18.4 (11–28)
Pauklin et al. [36]	2010	Germany	CS	44/38	32	12	30	14	11	+	28.5 (9–73)
Prabhasawat et al. [20]	2012	Thailand	PCS	19/18	11	8	12	7	6	+	26.1 (6–47)
Qi et al. [37]	2013	China	RCS	42/41	42	0	0	42	0	+	17.8 (12–24)
Rama et al. [38]	2010	Italy	CS	113/112	UN	UN	113	0	46	–	35 (12–44)
Ramírez et al. [39]	2015	Spain	PCS	20/19	12	UN	11	9	4	+	12 (6–36)
Sangwan et al. [40]	2011	India	RCS	200/200	200	0	200	0	10	–	36 (12–91.2)
Schwab et al. [41]	2000	USA	CS	14/14	UN	UN	10	4	5	+	13 (6–19)
Sejpal et al. [42]	2013	India	RCS	107/107	92	15	107	0	19	–	41.2 (12–118)
Sharma et al. [43]	2011	India	CS	50/50	41	9	34	16	0	+	11 (1.5–25)
Shimazaki [44]	2007	Japan	RCS	27/27	27	0	7	20	8	+	31.75 (7.25–92.25)
Shortt et al. [45]	2008	UK	PCS	10/10	UN	UN	3	7	0	+	8.7 (6–13)
Shortt et al. [46]	2014	UK	PCS	14/13	14	0	0	14	0	+	36
Tsai et al. [47]	2000	Taiwan	Cs	6/6	2	4	6	0	0	–	15 (12–18)
Vazirani et al. [48]	2014	India	RCS	70/70	0	70	70	0	0	–	17.5 (3.5–31.5)
Zakaria et al. [49]	2014	Belgium	CT (I-II)	18/18	15	3	15	3	7	+	24 (4–48)

Table 2 Assessment of quality items per study

Study	Quality item numbers[a]													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Baradaran-Rafii et al. [21]	Y	N	N	Y	N	Y	Y	U	Y	Y	Y	Y	Y	Y
Basu et al. [22]	Y	Y	N	U	Y	N	Y	U	U	Y	Y	Y	Y	Y
Chen et al. [23]	Y	N	N	Y	N	Y	N	U	U	Y	Y	Y	Y	Y
Cheng et al. [24]	Y	Y	N	U	N	N	Y	U	U	N	Y	Y	U	N
Daya et al. [25]	Y	N	N	U	Y	N	N	U	Y	Y	Y	Y	Y	Y
Di Iorio et al. [26]	Y	U	N	U	Y	U	Y	U	U	Y	N	Y	N	Y
Fasolo et al. [27]	Y	N	N	Y	Y	N	Y	N	Y	Y	Y	Y	Y	Y
Ganger et al. [28]	N	Y	N	Y	N	N	Y	U	Y	Y	Y	Y	Y	Y
Gisoldi et al. [29]	N	N	N	Y	N	Y	U	U	U	Y	Y	Y	Y	Y
Kawashima et al. [30]	Y	Y	N	U	N	Y	Y	U	U	Y	Y	Y	Y	Y
Koizumi et al. [31]	Y	N	N	U	N	N	Y	U	U	Y	Y	Y	Y	Y
Kolli et al. [32]	Y	Y	Y	Y	N	Y	N	U	U	Y	Y	Y	Y	Y
Nakamura et al. [33]	Y	N	N	Y	N	N	Y	U	U	Y	Y	Y	Y	Y
Parihar et al. [34]	N	N	N	U	N	Y	Y	U	U	N	N	y	U	N
Pathak et al. [35]	Y	N	N	U	N	N	Y	U	y	Y	Y	Y	Y	Y
Pauklin et al. [36]	Y	U	N	Y	N	U	Y	U	Y	Y	Y	Y	N	Y
Prabhasawat et al. [20]	Y	Y	N	U	N	Y	N	U	U	Y	Y	Y	N	Y
Qi et al. [37]	Y	N	N	Y	N	N	Y	U	U	N	N	Y	U	N
Rama et al. [38]	Y	Y	N	U	N	U	Y	N	U	Y	Y	Y	Y	Y
Ramírez et al. [39]	Y	U	N	U	N	Y	N	U	Y	Y	Y	Y	Y	Y
Sangwan et al. [40]	N	Y	Y	N	Y	N	Y	U	U	Y	Y	Y	Y	Y
Schwab et al. [41]	N	N	N	U	N	Y	N	U	U	Y	Y	Y	Y	Y
Sejpal et al. [42]	N	Y	N	U	N	N	Y	U	U	Y	Y	Y	N	Y
Sharma et al. [43]	Y	N	N	U	N	Y	N	U	U	Y	Y	Y	Y	Y
Shimazaki et al. [44]	N	N	N	U	N	N	Y	U	U	Y	Y	Y	Y	Y
Shortt et al. [45]	N	N	N	U	N	Y	N	Y	U	Y	Y	Y	Y	Y
Shortt et al. [46]	U	N	N	U	N	Y	N	N	U	Y	N	Y	U	N
Tsai et al. [47]	N	N	N	U	N	Y	N	U	Y	Y	Y	Y	Y	Y
Vazirani et al. [48]	N	Y	N	U	Y	N	Y	N	Y	Y	N	Y	Y	Y
Zakaria et al. [49]	Y	Y	N	Y	N	Y	N	U	U	Y	Y	Y	Y	Y

Y: YES; N: NO; U: UNKNOWN

[a]Based on Supplemental Digital Content 1 (http://links.lww.com/ICO/A265)

0.6–156 months. Of 1306 eyes, 982 (75.2%) underwent autograft transplantation and 324 (24.8%) received allografts from living or deceased donors. In our study, 229 (17.5%) out of 1306 eyes underwent corneal transplantation after CLET (Table 1). All the allograft cases in our meta-analysis had received cyclosporine A for immunosuppression (Table 1).

Quality assessment

Table 2 and Fig. 1 present the results of the quality assessment per study. "YES" refers to studies that had a positive measure of quality, "NO" refers to studies with an unfavorable measure of quality, and "UNKNOWN" describes articles that did not contain the required information to address the level of quality. In one (3.3%) study, participants were at a similar stage of disease, and in ten (33.3%) studies, consecutive selection was performed. Prognostic factors were identified in 27 (90%) studies, and prospective data collection was performed in 14 (46.7%) studies. A clear recruitment period was defined in 18 (60%) studies. One (3.3%) study had masked outcome assessors, and experienced surgeons were reported in nine (30%) investigations. Objective outcome measures were defined in 27 (90%) studies. All important

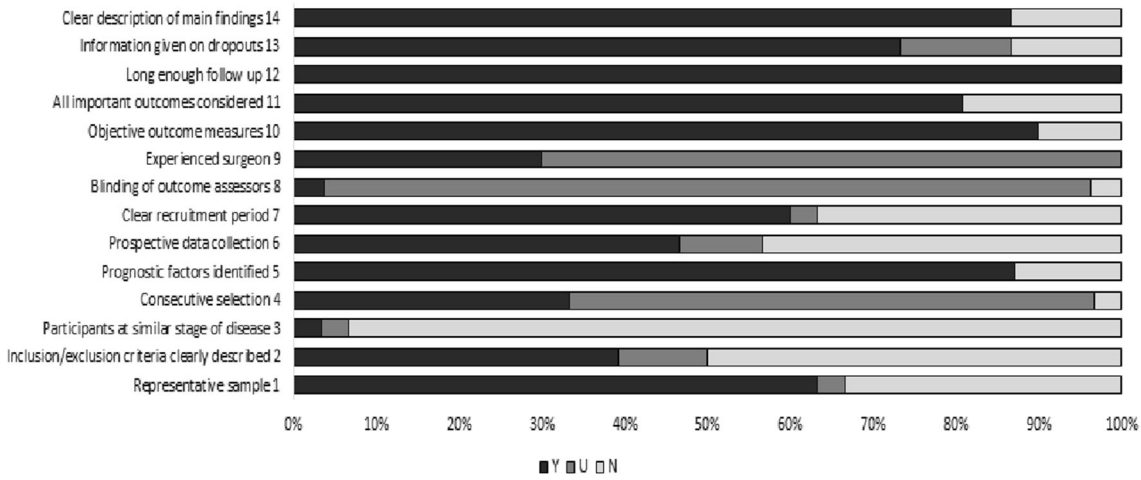

Fig. 1 Bar chart illustrating the proportion of studies per quality objects (Y: YES; N: NO; U: UNKNOWN)

outcomes were considered in 21 (70%) studies, and sufficient follow-up (> 6 months) was reported in 30 (100%) articles. Information pertaining to dropouts was reported in 22 (73.3%) studies. A clear description of the main findings was defined in 26 (86.7%) studies. Representative samples were reported in 19 (63.3%) studies, and inclusion/exclusion criteria were clearly described in 11 (36.7%) studies.

Culture methods

Table 3 lists the results of the analysis of ex vivo culture methods implemented in each study in terms of type of carrier (HAM, denuded HAM and other substrates), use of 3T3 and/or animal-free conditions and reporting GMP grade. Of 30 studies, 11 (36.7%) and 15 (50%) used denuded HAM and HAM, respectively, three (10%) used fibrin, and one used polymerized fibrin matrix (3.3%). Furthermore, 3T3 fibroblast cells were used in 12 (40%) studies and animal-free culture systems were used in 13 (43.3%) studies. Six of 30 (20%) studies were performed according to GMP.

Clinical results

Clinical outcomes in terms of success rate of ocular surface reconstruction and two-line BCVA improvement are illustrated in Table 4. The mean follow-up period was 26 months (range 0.6–156 months). In the majority of studies (n = 13), the success rate was considered at a mean follow-up of 1 year. Mean follow-up for reported success rates was 2 years in 12 studies and ≥ 3 years in four studies. The success rates in the 29 reviewed studies varied between 28.5 and 100%. Using the random-effects model, the pooled success rate was 71% (95% CI 65–76%; I^2 = 72.8%; P = 0.001) (Fig. 2a) and pooled odds ratio of success was 1.82 (95% CI 0.80–4.11; I^2 = 30.6%; P = 0.174) in autograft compared with allograft (Fig. 2b). Furthermore, stratified by the type of studies (prospective, retrospective and unknown), it was revealed that the odds of success in autografts were not higher than in allografts in prospective studies (pooled OR 0.92, 95% CI 0.24–3.50). Of 1306 eyes, 869 exhibited successful ocular surface reconstruction, among which 679 and 190 underwent autograft and allograft transplantations, respectively. Although the success rate of autologous ex vivo CLET was 1.82 times higher than the allograft, the difference was not statistically significant.

Results obtained from 24 studies indicated that 472 eyes demonstrated a two-line improvement in BCVA. Using the random-effects model, the pooled rate of two-line improvement in BCVA was 54% (95% CI 45–62%; I^2 = 81.88%; P = 0.001) (Fig. 2c). Outcomes of VA in autografts (74 eyes) versus allografts (35 eyes) were reported in nine studies. The pooled odds of two-line improvement in BCVA in autografts were not significantly different than those in allograft (OR 1.2, 95% CI 0.54–2.65; I^2 = 10.7%; P = 0.347) (Fig. 2d). In addition, stratified by type of the studies,

Table 3 Results of culture methods implemented per study in terms of type of carrier, use of 3T3 and/or animal-free condition and possessing GMP grade. HAM: human amniotic membrane

References	Carrier	3T3 s used	Animal-free culturing conditions	GMP
Baradaran-Rafii et al. [21]	HAM (denuded)	−	−	−
Basu et al. [22]	HAM	−	+	−
Chen et al. [23]	HAM	−	−	+
Cheng et al. [24]	HAM	+	−	−
Daya et al. [25]	HAM	+	−	−
Di Iorio et al. [26]	Fibrin	+	−	+
Fasolo et al. [27]	Polymerized fibrin matrix	+	−	−
Ganger et al. [28]	HAM	−	+	−
Gisoldi et al. [29]	Fibrin	+	−	+
Kawashima et al. [30]	HAM (denuded)	+	−	−
Koizumi et al. [31]	HAM (denuded)	+	+	−
Kolli et al. [32]	HAM	−	+	+
Nakamura et al. [33]	HAM (denuded)	+	+	−
Parihar et al. [34]	HAM	−	−	−
Pathak et al. [35]	HAM	−	+	−
Pauklin et al. [36]	HAM	−	+	−
Prabhasawat et al. [20]	HAM (denuded)	−	+	−
Qi et al. [37]	HAM (denuded)	+	−	−
Rama et al. [38]	Fibrin	+	−	−
Ramírez et al. [39]	HAM (denuded)	−	−	+
Sangwan et al. [40]	HAM	−	+	−
Schwab et al. [41]	HAM (denuded)	+	+	−
Sejpal et al. [42]	HAM (denuded)	−	+	−
Sharma et al. [43]	HAM (denuded)	−	−	−
Shimazaki et al. [44]	HAM (denuded)	+		
		−	−	−
Shortt et al. [45]	HAM (denuded)	−	−	+
Shortt et al. [46]	HAM (denuded)	−	−	+
Tsai et al. [47]	HAM (denuded)	−	−	−
Vazirani et al. [48]	HAM (denuded)	−	+	−
Zakaria et al. [49]	HAM (denuded)	−	+	−

the proportion of two-line improvement in BCVA was comparable between autografts and allografts in prospective studies (pooled OR 0.91, 95% CI 0.28–2.98).

Publication bias

After analyzing ocular surface reconstruction success rates and two-line improvement in BCVA, no publication bias was observed. Statistical Eager test in our meta-analysis showed no significant evidence of bias in terms of success rate ($P = 0.102$) and two-line improvement in BCVA ($P = 0.124$). Funnel plots of publication bias with respect to success rates and two-line improvements in BCVA in autografts and allografts are presented in Fig. 3.

Discussion

Our meta-analysis demonstrated no difference between autograft and allograft CLET techniques in terms of success rate and two-line improvement in BCVA. The results of the current study were

Table 4 Clinical results in terms of success rate of ocular surface reconstruction and two-line BCVA improvement for each study. BCVA: best-corrected visual acuity

References	Success rate			BCVA of at least two-line improvement		
	All %	Autograft	Allograft	All %	Autograft	Allograft
Baradaran-Rafii et al. [21]	(6/8) 75	6/8	–	(6/8)75	6/8	–
Basu et al. [22]	(33/50) 66	33/50	–	(38/50)76	38/50	–
Chen et al. [23]	(32/41) 78	–	32/41	(13/41)32	–	13/41
Cheng et al. [24]	(40/40) 50	–	40/80	(18/80) 22.5	–	18/80
Daya et al. [25]	(7/10) 70	–	7/10	(3/10)30	–	3/10
Di Iorio et al. [26]	(133/166) 80	133/166	–	–	–	–
Fasolo et al. [27]	(24/59) 41	24/59	–	–	–	–
Ganger et al. [28]	(43/62) 69	39/54	4/8	(16/62)25.8	15/54	1/8
Gisoldi et al. [29]	(5/6) 83	5/6	–	(5/6)83	5/6	–
Kawashima et al. [30]	(6/6) 100	2/2	4/4	(4/6)67	2/2	2/4
Koizumi et al. [31]	(10/13) 77	–	10/13	(5/13)38.4	–	5/13
Kolli et al. [32]	(8/8) 100	8/8	–	(5/8)62.5	5/8	–
Nakamura et al. [33]	(9/9) 100	2/2	7/7	(9/9)100	2/2	7/7
Parihar et al. [34]	UN	–	UN	(19/25) 76	–	19/25
Pathak et al. [35]	(5/9) 55.5	5/9	–	(2/9)22	2/9	–
Pauklin et al. [36]	(30/44) 68	23/30	7/14	(30/44)68	21/30	9/14
Prabhasawat et al. [20]	(14/19)74	8/12	6/7	(13/19)68.4	8/12	5/7
Qi et al. [37]	(32/42) 76	–	32/42	UN	–	UN
Rama et al. [38]	(82/113)77	82/113	–	(34/113)30	34/113	–
Ramírez et al. [39]	(16/20)80	10/11	6/9	–	–	–
Sangwan et al. [40]	(142/200)71	142/200	–	(121/200)60.5	121/200	–
Schwab et al. [41]	(10/14)71.4	6/10	4/4	(5/14)36	2/10	3/4
Sejpal et al. [42]	(50/107) 47	50/107	–	(58/107)54	58/107	–
Sharma et al. [43]	(37/50)74	28/34	9/16	(14/50)28	12/34	2/16
Shimazaki et al. [44]	(16/27) 59	6/7	10/20	(13/27)48	–	–
Shortt et al. [45]	(7/10) 70	1/3	6/7	(5/10)50	1/3	4/7
Shortt et al. [46]	(4/14) 28.5	–	4/14	UN	–	UN
Tsai et al. [47]	(6/6) 100	6/6	–	(3/6)50	3/6	–
Vazirani et al. [48]	(50/70) 71.4	50/70	–	–	–	–
Zakaria et al. [49]	(12/18) 67	10/15	2/3	(10/18)55.5	8/15	2/3

consistent with the previous meta-analysis and review studies [18, 50, 51], in which success rates and VA outcomes were not significantly different between the two CLET methods. This may be attributed to the heterogeneity of participants recruited in the studies included in our meta-analysis, in which there was no limitation in terms of age, sex, involved region, type and stage of LSCD, injury to surgical intervention period, culture methods and history of primary versus subsequent CLETs. Further investigations including homogeneous groups of patients with LSCD are required to validate the lack of significant difference between the long-term effects of these two techniques.

In the current study, the pooled success rate was 71%, which was comparable with the 70% overall success rate previously reported for CLET [11]. However, despite this rate of successful transplantation, the pooled rate of two-line improvement in BCVA in our analysis was 54%. This suboptimal increase in VA might be partially due to the lack of post-CLET corneal transplantation in the majority of cases reviewed in the current study. Only 17.5% of

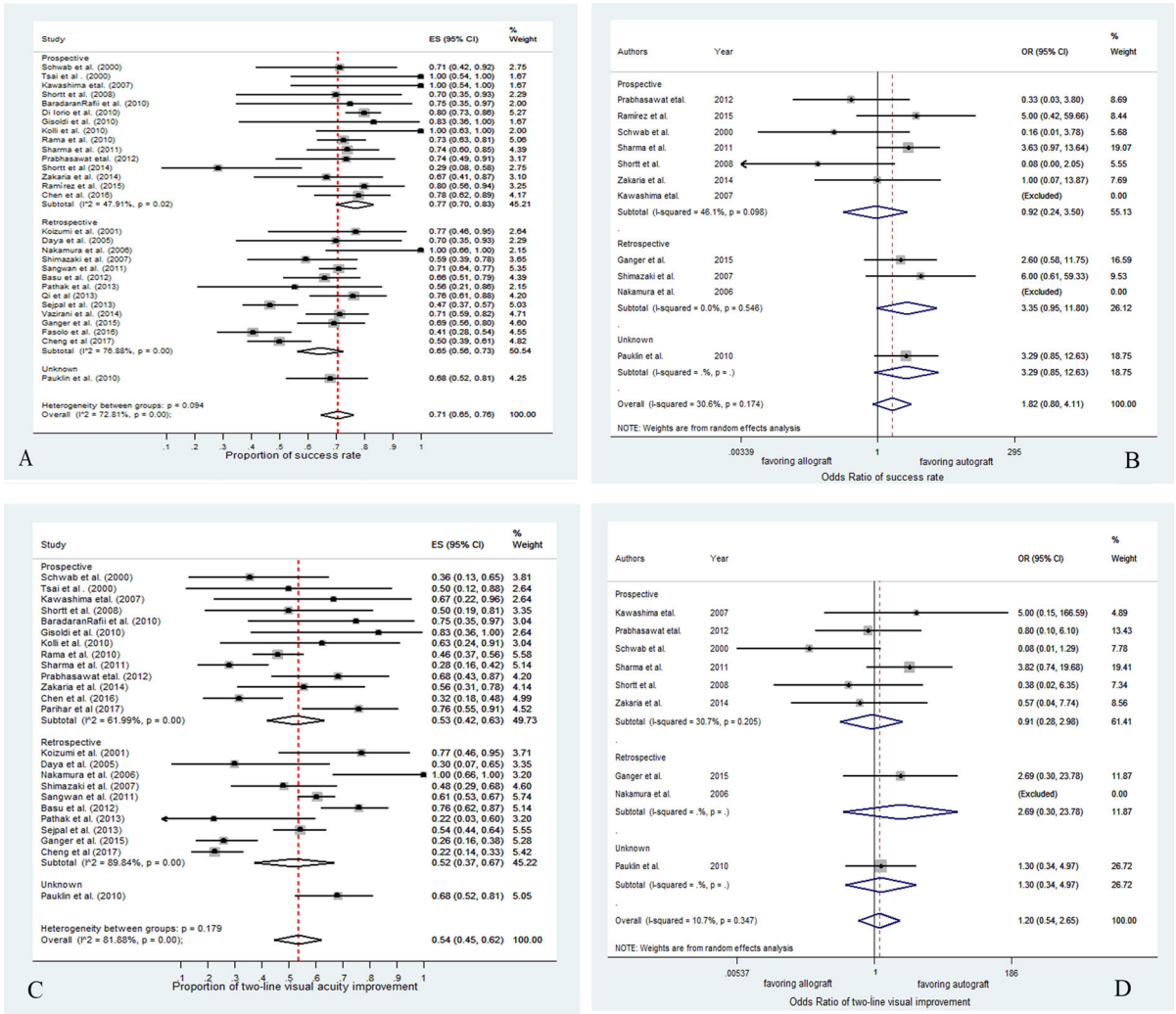

Fig. 2 Forest plots of meta-analysis outcomes. **a** Forest plot for odds ratio of success in CLET autograft and allograft transplantations and its demonstration by type of study. **b** Forest plot for odds ratio of two-line improvement in BCVA in CLET in autograft and allograft transplantations and its demonstration by type of study. **c** Proportion of success rates of CLET. **d** Proportion of two-line improvements in CLET. *CLET* cultivated limbal epithelial transplantation

cases in our meta-analysis, due to the occurrence of corneal stromal opacities, received corneal graft after CLET. Whether increased rate of post-CLET corneal transplantation increases the rate of two-line BCVA improvement following autograft and allograft CLETs, needs long-term investigations.

In our analysis, success rates for CLET were reported in the studies that had a 26-month average follow-up period. In a study by Ramírez et al., the overall success rate was 80% at 1–2 years and 75% at 3 years [39]. It appears that when eyes treated with transplantation of expanded limbal epithelial cells achieved successful results after 1 year, the improved ocular surface would remain stable over time [38, 40, 52]. Among multiple factors that compromise the outcomes of both autologous and allogeneic CLETs, concurrent adnexal abnormalities such as trichiasis, symblepharon, lagophthalmos, eyelid malposition and fornix shortening are of great importance [53] and their correction is mandatory before any type of CLET [54] to support the posttransplantation ocular surface improvement. As the heterogeneity of the findings was high and we used random effect, it should be emphasized that the superiority of one method over

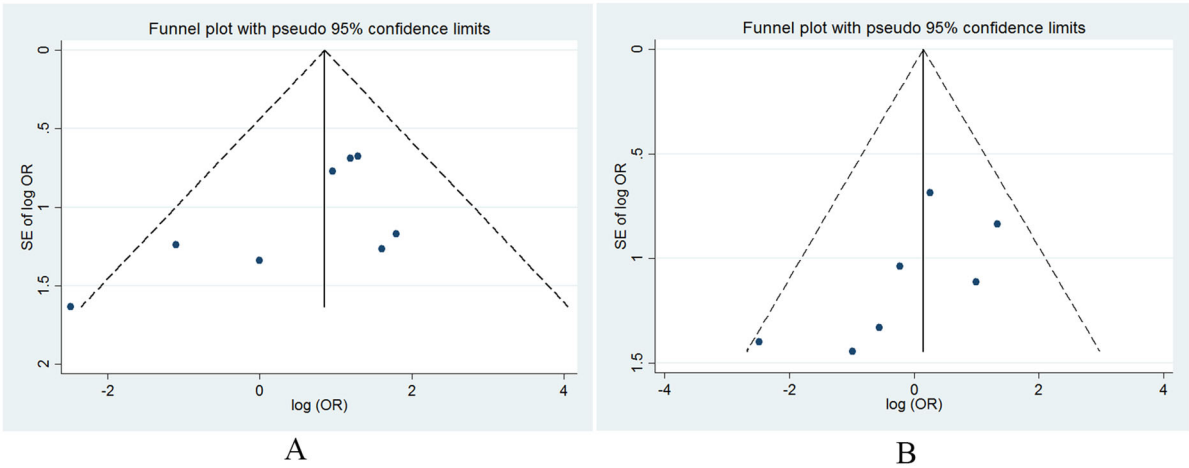

Fig. 3 Funnel plots of publication bias. **a** Meta-funnel of OR success rate. **b** Meta-funnel of OR two-line improvement in BCVA. The horizontal axis presents the log of odds ratio, and vertical axis represents standard error of corresponded log odds ratio. *BCVA* best-corrected visual acuity

the other could be variable from a population (or condition) to another.

The majority of studies (81.7%) in our meta-analysis used HAM as a suitable substrate for limbal epithelial stem cell cultivation and a carrier of cultivated cells for CLET. In a comparative study, decellularized HAM was superior to intact HAM for cultivation of LESCs [31]. However, in a series of studies, fibrin has been used as a highly manageable and quickly degradable natural carrier for limbal stem cells when transplanted [26, 27, 29, 38]. Additionally, the use of human autologous serum as a xeno-free culture medium supplement when cultivating LESCs on HAM can induce a stable ocular surface after CLET [20, 22, 29, 32, 33, 36, 40, 44, 49].

Of the 30 studies included in our meta-analysis, only six were performed according to GMP standards and conditions [23, 26, 29, 32, 39, 45]. One of the crucial aspects of ocular regenerative medicine and cell-based therapies is the implementation of GMP guidelines. To achieve a higher rate of success in future CLET trials, possessing a GMP grade for ex vivo LESC cultures and HAM preparations may be necessary.

The percentage of p63-positive cells in the heterogeneous population of cultured limbal epithelial cells has been shown to be linked to the clinical success of CLET; populations with $< 3\%$ p63-positive cells were associated with a low success rate [38]. Hence, cultures containing $> 3\%$ ΔNp63α have been considered to be suitable for CLET techniques [27].

In unilateral LSCD, autologous CLET is advantageous over allogeneic CLET as the autografts have the minimal risk of immunoreactivity and require no systemic immunosuppression. However, the use of autologous cells is not possible in bilateral LSCD and the cell resources for the CLET procedure are limited to cadaveric, living-relative or living-nonrelative donors [55]. It is noteworthy to mention that antigen-presenting macrophages do not stay alive during the ex vivo cultivation; hence, the risk of rejection may not be as high as what proposed for allograft CLETs [10]. Furthermore, in autograft CLETs and allograft CLETs from living-relative or living-nonrelative donors, the cultivated cell sheets on HAM can be used for surgery after 2 weeks when achieving more than 80% confluence [56]. Whether the cultivated cells obtained from the deceased donors for allograft CLET attain the same morphologic criteria during the 2-week interval and the same outcome after transplantation, needs further investigations.

In conclusion, the results of the current meta-analysis revealed no significant difference between allogeneic and autologous CLET in terms of success rate and two-line visual improvements in patients with LSCD. Future research in the field of CLET will aim to optimize and modify LESC cultivation from ex vivo to in vivo conditions and implement GMP parameters for LESC cultivation and scaffold preparations. The use

of "no touch graft surgery" in future clinical trials may improve the overall success rates after both allogeneic and autologous CLET techniques.

Compliance with ethical standards

Conflicts of interest The authors have no commercial or proprietary interest in any concept or product described in this article.

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