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Comparative evaluation of autologous tissue-engineered ocular and oral mucosal tissue grafts- a prospective randomized controlled trial

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Abstract

Background Bilateral ocular surface disease resulting from Stevens Johnson Syndrome (SJS) and chemical injuries are visually debilitating and difficult to treat. Ocular surface reconstruction by various means has been reported with variable results. This study addresses an unmet need for a prospective clinical trial comparing the outcomes of transplanting autologous oral and conjunctival epithelial cell constructs on human amniotic membrane by ex vivo tissue engineering.

Methods A prospective, randomized controlled clinical trial was prospectively applied for registration, with the clinical trial registry of India (CTRI), with the approval of the Institute Ethics Committee number IEC/NP-99/11.04.2014 and CTRI No. REF/2018/10/021791, the study also registered with the WHO-recognized trial registry, International Standard Randomised Controlled Trial Number (ISRCTN) registration reference number 45780. The study was conducted to compare clinical outcomes of two different tissue-engineered cell grafts, Cultivated Oral Mucosal Epithelial Transplantation (COMET) and Conjunctival Cultivated Epithelial Transplantation (CCET) for ocular surface reconstruction in patients with bilateral ocular surface disease due to Stevens-Johnson Syndrome or chemical injuries. Fifty patients were enrolled and randomized to either the COMET or CCET group. A uniform pre-op and post-op protocol using standard medications was followed for all patients Parameters assessed at baseline, day 1, 1 week, 2 weeks, 1 month, 2 months, 3 months and 6 months postoperatively included patient comfort, best corrected visual acuity (BCVA), ocular surface status and corneal clarity. The efficacy was measured in terms of improvement of vision, reduction in vascularization, symblepharon and corneal clarity.

Results In the study, 50 patients (50 eyes; mean ages of 29 ± 15.86 years and 26.36 ± 10.85 years, respectively; range, 12–65 years) were enrolled, with 25 patients each in the COMET and CCET groups. Out of them, 36% were female and 64% were male; the causes were Steven Johnson syndrome (48), and chemical injury (2). Mean pre-operative BCVA was log MAR 1.73 ± 0.57 for COMET and 1.99 ± 0.33 for the CCET group. Pre-operatively all 50 enrolled patients

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had opaque corneas pre-operatively, symblepharon that extended to the cornea categorised as grade 3 and corneal vascularization that went beyond the pupil's boundary into the central zone encluaching on the visual axis. The minimal follow-up time was six months. Following surgery postoperatively, the BCVA considerably improved in the COMET group by 1.51 ± 0.58 compared to the CCET group by 1.91 ± 0.33 at 3 months. BCVA at 6 months was 1.73 ± 0.56 in the COMET group and 1.99 ± 0.31 in the CCET group, which is not statistically significant and comparable to the BCVA before surgery. The corneal clarity was significantly improved in COMET group 25 eye (100%) at 2 month, 3month and 19 eye (76%), 6eye (24%) at 6 months when compared to CCET group 15 eye improved (60%), 9 eyes (36%) not improved and one eye with opaque cornea (4%) at 2 months. 22 eye (88%) had not improved, 2 eye (8%) opaque cornea and 1 eye (4%) improved at 3 months. At 6 months 21 eye (84%) were not improved, 4 eye (16%) eye became opaqued at 6 months. Compared to preoperative conditions, both groups had improved corneal clarity significantly ($p > 0.005$). Of the 50 patients with grade 3 symblepharon extended to the cornea, were completely resolved 19 (76%) in COMET group when compared to CCET group 22 eye (88%) not improved. Similarly, 19 eye (76%) had a improvement in corneal vascularization when compared to the CCET group not improved 25 eye (100%) at 6months. No adverse event was observed in any of either group during the follow up periods.

Conclusion Both cell types are effective to restore the ocular surface integrity in bilateral ocular surface disease. Whereas COMET is safe and efficacious in terms of improvement of clinical parameters including, BCVA, corneal clarity, reduction in vascularization and preventing the recurrence of symblepharon postoperatively 3months and 6 months. In addition, the CCET group maintained the stability of the ocular surface and had improvement in corneal clarity and a decrease in vascularization at 3 months compared to their pre-operative characteristics.

Keywords Tissue engineering, *ex vivo* expansion, Oral mucosal epithelial cells, Conjunctival epithelial cells, Chemical injury, Ocular surface disease (OSD), Cultivated oral mucosal epithelial transplantation (COMET), Conjunctival cultivated epithelial transplantation (CCET), Stevens-johnson syndrome (SJS)

Introduction

The transparent cornea's physiological function and the preservation of the integrity of the ocular surface are both dependent on the healthy corneal and conjunctival epithelium. Limbal epithelium cells (LEC) in the limbus basal epithelium regulate the turnover of the corneal epithelium, which protects the integrity of the ocular surface [1]. Ocular cicatricial pemphigoid (OCP), Steven Johnson syndrome (SJS), extensive limbal surgery, orbital radiotherapy and cytotoxic agents can all result in limbal stem cell deficiency (LSCD), a condition that compromises the integrity of the corneal epithelium and leads to vascularization, conjunctivalization, corneal fibrous ingrowth, and eventually chronic ocular surface inflammation and vision loss. Stevens Johnson syndrome and ocular cicatricial pemphigoid (OCP) are two of the most frequent cause of bilateral LSCD [2, 3]. The symptoms of limbal stem cell deficiency (LSCD)- related ocular surface disease included ocular pain, discomfort and vision loss. Numerous eye disorders have showed considerable promise for the treatment using stem cell therapy. The last few decades have seen the emergence of a number of procedures as potential options for limbal and mucosal epithelial stem cell transplantation in the case of bilateral LSCD including allogenic Keratolimbal Allograft (KLAL), autologous Cultivated Oral Mucosal Epithelial Transplantation (COMET), autologous Conjunctival Cultivated Epithelial Transplantation (CCET). The treatment of corneal disorders encompasses a variety of modalities, each with its own set of advantages and disadvantages. Some of the main treatment options are for corneal disorders including medication therapy, amniotic membrane transplantation, stem cell therapy, surgical therapy, and medical devices, etc. While traditional treatments for corneal disorders have their significant advantages, they also sometimes come with disadvantages such as graft rejection, long recovery periods, and the need for immunosuppression. COMET and CCET offer promising alternatives by using the patient's own cells to regenerate the corneal surface, and thus minimizing rejection risks, shortening recovery times, and avoiding long-term immunosuppression [4, 5]. However, no randomized controlled trials have been carried out so far to compare the different surgical methods of limbal stem cell transplantation (LSCT) in bilateral LSCD. The objective of our study was to examine the morphological and functional clinical results of autologous COMET and CCET in eyes with bilateral ocular surface disease and limbal stem cell deficiency (LSCD) and the purpose of the study is to compare the efficacy and post-operative changes in primary and secondary outcomes of COMET and CCET.

Materials and methods

Study design, ethical compliance and patients

The study was a prospective, interventional, randomized controlled clinical trial carried out at our Institute from January 2018 to December 2022. The study was done in accordance with the tenets of the Declaration of Helsinki

and ethical clearance was obtained from Institute Ethics Committee prior to the commencement of the study. The intended use of the potential risk of the procedures was informed. The written Informed consent form was obtained from patients and similarly Legally authorized representative (LAR) consent was obtained from the legal guardians for the minor age group < 18 years patients, who underwent COMET and CCET for ocular surface reconstruction from the year 2018 to 2022 and were included in the study. Patients with a history of ocular inflammation, autoimmune disease, systemic or autoimmune disorder, or lid deformities were excluded from the study. The study included total of 50 patients of bilateral LSCD with limbal affected area at least 6–9 clock hours with Schirmer test value less than 5 mm and with no systematic disorder contraindication surgical intervention. Autologous regenerative transplantation procedure, randomized into two treatment groups, i.e. COMET and CCET. There were 25 patients in the COMET group and 25 patients in the CCET group. The sample size was calculated in the study by using a clinical superiority design. The outcome measures for both groups considered for sample size analysis is graft survival rate, improvement in visual acuity and corneal clarity. The effect size of study is assumed difference is 0.05% Statistical package for Social Sciences (SPSS) version 15.0 is used to generate the random number sequences and participants were randomized to two study groups. All patients underwent comprehensive ophthalmic examination at baseline and every follow-up visit. All patients underwent surgery in one eye only. The study is an open-label design. However, the outcome assessor-qualified ophthalmologist (clinician) will be kept blinded regarding the study group allocation of the patients. Clinical picture documentation, symblepharon status, corneal vascularization, and corneal epithelialization were also assessed during the study by using slit lamp examination, clinical picture documentation, and a grading system based on standard protocol. The primary clinical parameters used for analysis were corrected Best Corrected Visual Acuity (BCVA), which is scored by using the logarithm of the minimum angle of resolution (logMAR) method. The primary outcomes and main result will be a success indicator that includes the following: conjunctivalization (complete/partial-absence/mild conjunctivalization), vascularization (full success-avascular cornea, partial success-mild vascularization), and epithelization (complete/partial). Improving Best Corrected Visual Acuity (BCVA) and corneal transparency will be measured as the study secondary outcome.

Outcome measurement

The primary outcome measures included ocular surface stability in terms of epithelization, extent reduction of vascularization and absence or presence of mild conjunctivalization. Secondary outcome measures were improvement in BCVA and transparency of the cornea.

Surgical technique cultivated oral mucosal epithelial transplantation (COMET)

After sterilization of the oral cavity by using a povidone-iodine 5% oral solution, a thin tissue strip of approximately 4 mm x 4 mm was first harvested from the buccal mucosa of the patient under local anaesthesia, and mucosa tissue was collected in a transport medium containing DMEM and antibiotics (penicillin-streptomycin 50IU/mL and 5 µg/ml amphotericin b). Harvested tissue washed with sterile phosphate-buffered saline to remove blood. Then the tissue was cultured over the denude amniotic membrane and submerged with DMEM/F12 (Gibco) with 10% autologous serum and 1% antibiotics (5 mg/mL gentamicin, 100 mg/mL streptomycin, 100 units/mL penicillin and 0.25 mg/mL amphotericin B (Gibco). Tissue cultures were incubated at 37 °C in a 5% CO_2-95% air incubator was maintained under cGMP (current good manufacturing practice) in a compliant stem cell facility, the media of culture was changed daily and cell growth and their expansion were observed at 24 h, day3, day5, day7 and day11 [6]. After two weeks of tissue culture epithelial cells grew to form a confluent sheet of cells on the amniotic membrane, and were then transplanted into the recipient's eye. After dissection of the fibrovascular pannus, the cultured cells' membrane was gently spread over the cornea and limbus without damaging or dislodging the cells. The membrane was then secured to the ocular surface with fibrin glue (TISSEEL, Baxter Healthcare SA, Switzerland) followed by the placement of a bandage contact lens [7].

Conjunctival cultivated epithelial transplantation (CCET)

In this technique, a tissue strip of approximately 4 mm x 2 mm was first harvested from the conjunctival fornix of either eye and cultured over the denude amniotic membrane in cGMP compliant stem cell facility, the method for tissue culture was follow as described and published previously [6]. After two weeks when the stem cells grew to form a confluent sheet of cells, these cells were then transplanted into the recipient's eye by following the same surgical steps as described above for the COMET procedure.

Post-operative management and follow-up

All patients underwent comprehensive ophthalmic examinations for both eyes at every follow-up visit. The patients were reviewed on the post-op first day, one week, two weeks, one month, two months, three months, and six months. Patients were prescribed standard medications for donor and recipient eyes. In the CCET group for patient donor eye moxifloxacin 0.5% eye drop thrice

a day and gatifloxacin 0.3% eye ointment at bedtime for one week; and lubricant eye drops (carboxymethylcellulose 0.5%) four times a day for 3 months was prescribed post tissue harvesting, whereas in patients harvested with oral mucosa advised maintain the sterilize condition of the oral cavity with povidone-iodine 5% rinse and gargle for 4days along with mild painkiller twice in a day for 3days. The recipient eye in the COMET and CCET groups were prescribed moxifloxacin 0.5% eye drop thrice a day for four weeks, carboxymethylcellulose 0.5% eye drop six times a day for two months and then continued at four times a day, and prednisolone phosphate 1% eye drop four times a day for two weeks and tapered gradually to once a day over three months, followed by fluorometholone 0.1% eye drop four times a day for two weeks and tapered gradually to once a day over three months.

Histopathological and scanning electron microscopy of cultured graft

For histologic investigation samples were formalin-fixed and embedded in paraffin. Subsequently, they were cut into slices of 4 µm, deparaffinized and rehydrated. Sections of COMET and CCET were stained with haematoxylin and eosin.

The oral mucosal epithelial cells and conjunctival stem cells grew on the HAM scaffold and gently washed in 0.1 M phosphate buffer (pH 7.4) twice and then fixed with Karnovsky's fixative in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4 °C. After washing, these cells were fixed in 1% osmium tetroxide for 2 h at 4 °C, dehydrated in ascending grades of acetone and embedded in araldite CY 212. Thin Sect. (70 nm) was cut with a glass knife and mounted onto nickel grids. Thin sections mounted on grids contrasted and viewed under a scanning electron microscope.

Outcome measures and statistical analysis

The two techniques were compared in terms of anatomical and functional success. The outcome measures for anatomical success were improvement in corneal clarity and clinically stable corneal surface and absence of conjunctivalization/vascularization. The procedure was considered a failure if there was progressive conjunctivalization/vascularization after six weeks of interventional surgical procedure. The functional outcome was assessed in terms of BCVA by using the logMAR. The statistical analysis was done using a t-test and Wilcoxon rank sum test by using GraphPad prism software (5.01).

Table 1 Demographic details of patients in each group

Parameter/Group	COMET	CCET	*p* value
Number of patients n (%)	25 (50%)	25 (50%)	1.0
Mean age ± SD years (range)	29 ± 15.86 (6–68)	26.36 ± 10.85 (11–51)	0.49
Sex **Number of Females(%):Number of Males(%)**	9 (36%): 16 (64%)	9 (36%): 16 (64%)	1.0
Etiology of LSCD **SJS** **Chemical injury**	24 1	24 1	1.0

Results

The study included fifty patients with bilateral ocular surface disease of which 25 underwent the COMET and 25 the CCET procedure. Steven Johnson syndrome (SJS) was the cause of bilateral LSCD in 48 individuals, while chemical injury was the cause in 2 cases. The study was prospectively registered with the Clinical Trial Registry of India (CTRI) on October 2018 and the patient recruitment started in January 2018, anticipated end of the recruitment in December 2022. In the COMET and CCET groups, the median age of the patients was 29 ± 15.86 years and 26.36 ± 10.85 years, respectively ($p=0.49$) (Table 1). Clinical measures including corneal clarity, anatomical scores, recurrence of symblepharon, BCVA, corneal vascularization and corneal epithelization were coded and masked during the follow up to evaluate the outcomes of the post-operative groups. A qualified ophthalmologist (clinician) had performed a clinical examination of coded and masked parameter.

Figure 1; Table 2 display the findings of each groups best corrected visual acuity (BCVA). Although the COMET group performed marginally better at 2 and 3months follow-ups, the BCVA did indicate modest improvement in both groups at those points (statistically insignificant); nevertheless, at the 6month follow up, both groups BCVA had reverted to baseline ($p>0.005$). Regarding visual recovery, there was no statistically significant difference between the two groups ($p>0.005$) was observed at 6months.

At each follow up, every patient underwent a slit lamp biomicroscopy examination, clinical pictures documentation, and grading system by using the standardized protocol to check for corneal clarity, symblepharon status, corneal vascularization and corneal epithelialization. The result showed significant improvement ($p<0.05$) in all these anatomical parameters in both groups at 6 months follow up (Tables 3, 4, 5 and 6). At the 2 month, 3month, and 6month follow ups the COMET group corneal clarity was substantially better than the CCET group ($p>0.005$) (Table 3). At 2 months, 3 months and 6 months follow-ups, the COMET group symblepharon status was substantially better than CCET group ($p<0.05$) (Table 4). At 6 months follow up, the COMET groups corneal vascularization status was substantial than the CCET group ($p<0.05$) (Table 5). The corneal epithelialization was significantly better in the COMET group compared

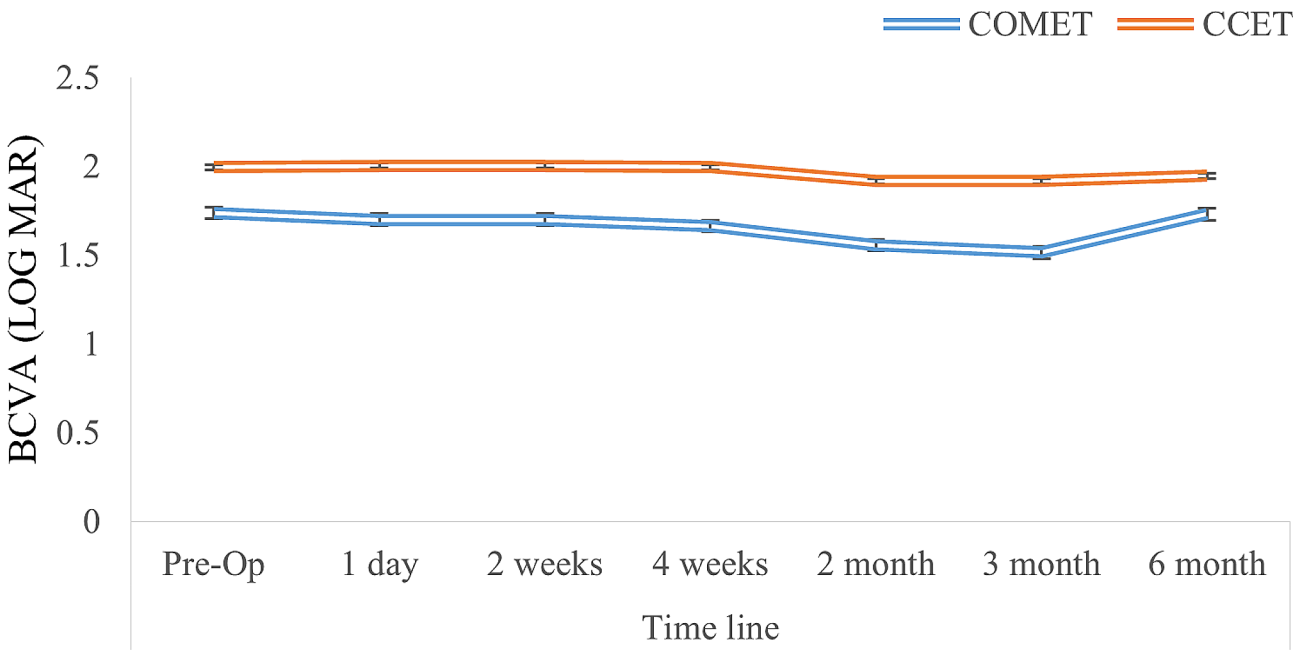


Fig. 1 Line diagram showing change in Best Corrected Visual Acuity (BCVA) of patients in each group over 6 months follow up (log MAR)

Table 2 Best corrected visual acuity (BCVA) of patients in each group (log MAR)

Group	Pre-op (Va 0)	Day 1 post-op (Va 1)	1 week post-op (Va 2)	2 weeks post-op (Va 3)	1 month post-op (Va 4)	2 months post-op (Va 5)	3 months post-op (Va 6)	6 months post-op (Va 7)
COMET	1.73 ± 0.57	1.7 ± 0.60	1.7 ± 0.60	1.68 ± 0.60	1.66 ± 0.58	1.55 ± 0.57	1.51 ± 0.58	1.73 ± 0.56
CCET	1.99 ± 0.33	2.0 ± 0.33	2.0 ± 0.33	2.0 ± 0.33	1.99 ± 0.33	1.91 ± 0.32	1.91 ± 0.33	1.99 ± 0.31
p value	0.60	0.67	0.67	0.45	0.47	0.47	0.05	0.12

Table 3 Corneal clarity (CC) of patients in each group

Group	Grade*	Pre-op (CC 0) (*n*)	Day 1 post-op (CC 1) (*n*)	1 week post-op (CC 2) (*n*)	2 weeks post-op (CC 3) (*n*)	1 month post-op (CC 4) (*n*)	2 months post-op (CC 5) (*n*)	3 months post-op (CC 6) (*n*)	6 months post-op (CC 7) (*n*)
COMET	1	0	25	25	25	25	25	25	19
	2	0	0	0	0	0	0	0	6
	3	25	0	0	0	0	0	0	0
CCET	1	0	23	25	25	25	15	1	0
	2	0	0	0	0	0	9	22	21
	3	25	2	0	0	0	1	2	4
p value		1.0	0.14	1.0	1.0	1.0	**0.002**	**0.000**	**0.000**

* Corneal clarity grading:

Grade 1: Improved

Grade 2: Not improved

Grade 3: Opaque cornea

with the CCET group at 3 months and 6 months follow-up ($p<0.05$) (Table 6). Representative pre-operative and 6-month post-operative clinical images of patients treated under both treatment groups are shown in Fig. 2. Bright field microscopy, histology and scanning electron microscopy of graft revealed that epithelial cells began to proliferate under culture conditions, showing outgrowth from the harvested *oral mucosal* and *conjunctival* tissue explants on the human amniotic membrane (COMET and CCET groups) on day 1, epithelial cells were clearly distinguishable by day 4–5, and the cells reached full confluence by 11days. Three out of 25 harvested oral mucosal and 7 out of 25 conjunctival tissues, failed to proliferate to produce new cells even until 14 days had passed. For all these patients, tissues were harvested again from a

Table 4 Symblepharon status in patients in each group

Group	Grade†	Pre-op (Sa 0) (*n*)	Day 1 post-op (Sa 1) (*n*)	1 week post-op (Sa 2) (*n*)	2 weeks post-op (Sa 3) (*n*)	1 month post-op (Sa 4) (*n*)	2 months post-op (Sa 5) (*n*)	3 months post-op (Sa 6) (*n*)	6 months post-op (Sa 7) (*n*)
COMET	1	0	25	25	25	25	25	25	17
	2	0	0	0	0	0	0	0	8
	3	25	0	0	0	0	0	0	0
CCET	1	0	25	25	25	25	21	12	3
	2	0	0	0	0	0	4	13	22
	3	25	0	0	0	0	0	0	0
p value		1.0	1.0	1.0	1.0	1.0	**0.03**	**0.000**	**0.000**

† Symblepharon grading:

Grade 1: Improved

Grade 2: Not improved

Grade 3: Extending to cornea

Table 5 Corneal vascularization status in patients in each group

Group	Grade‡	Pre-op (V 0) (*n*)	Day 1 post-op (V 1) (*n*)	1 week post-op (V 2) (*n*)	2 weeks post-op (V 3) (*n*)	1 month post-op (V 4) (*n*)	2 months post-op (V 5) (*n*)	3 months post-op (V 6) (*n*)	6 months post-op (V 7) (*n*)
COMET	1	0	25	25	25	254	25	25	19
	2	0	0	0	0	0	0	0	6
	3	25	0	0	0	0	0	0	0
CCET	1	0	25	25	25	25	25	25	0
	2	0	0	0	0	0	0	0	25
	3	25	0	0	0	0	0	0	0
p value		1.0	1.0	1.0	1.0	1.0	1.0	1.0	**0.000**

‡ Corneal vascularization grading:

Grade 1: Improved

Grade 2: Not improved

Grade 3: Extending beyond the margin of pupil into central cornea

Table 6 Corneal epithelialization status in patients in each group

Group	Grade§	Pre-op (E 0) (*n*)	Day 1 post-op (E 1) (*n*)	1 week post-op (E 2) (*n*)	2 weeks post-op (E 3) (*n*)	1 month post-op (E 4) (*n*)	2 months post-op (E 5) (*n*)	3 months post-op (E 6) (*n*)	6 months post-op (E 7) (*n*)
COMET	1	0	25	25	25	25	25	23	22
	2	0	0	0	0	0	0	2	3
	3	25	0	0	0	0	0	0	0
CCET	1	0	25	25	25	25	25	12	10
	2	0	0	0	0	0	0	13	15
	3	25	0	0	0	0	0	0	0
p value		1.0	1.0	1.0	1.0	1.0	1.0	**0.001**	**0.000**

§ Corneal epithelialization grading:

Grade 1: Improved

Grade 2: Not improved

Grade 3: Clinically unstable ocular and corneal surface

different location and effective cell proliferation was seen thereafter in tissue culture.

There was Regardless of tissue type, adequate cell proliferation was achieved and the transplantation procedure was carried out in all 50 patients after 14 days. COMET samples were noted to have complete cell confluency by the end of the 11th day, while in the CCET group there was some lack of confluence in extreme periphery in five out of 25 samples even upto day 14 (Figs. 3, 4 and 5).

Discussion

The management of limbal stem cell deficiency (LSCD) is dependent on the degree of involvement (partial or total), laterality (unilateral or bilateral), the severity of ocular

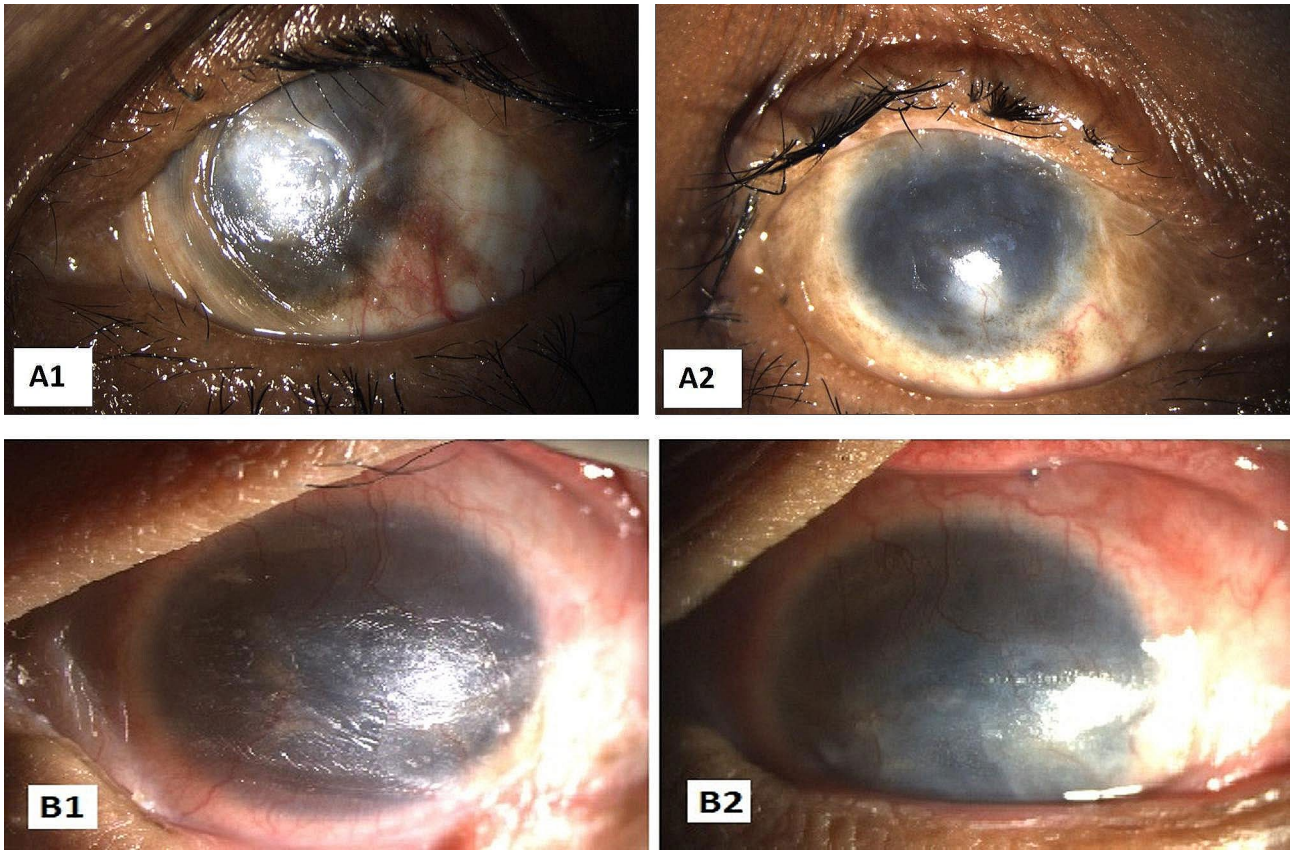
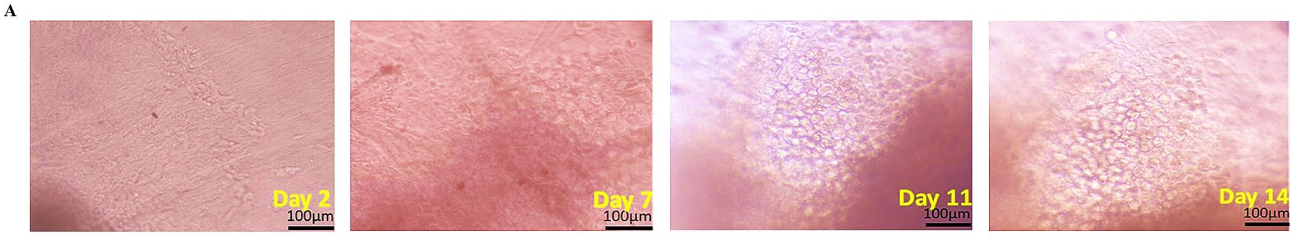
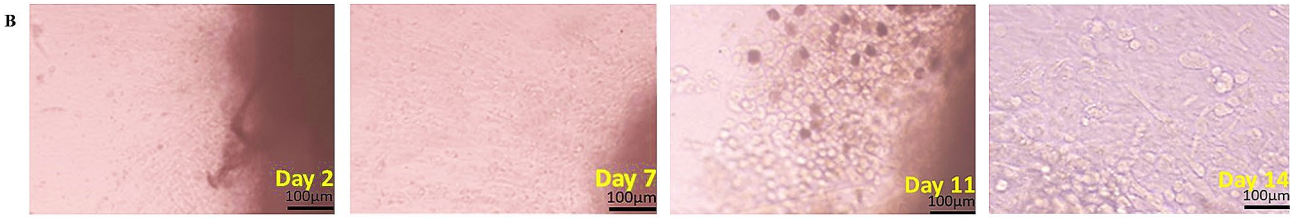


Fig. 2 Representative pre-operative and 6-month post-operative clinical images of patients treated under each treatment group; (A1,A2) COMET, and (B1,B2) CCET



A. Bright field microscopy images of oral mucosal tissue culture at different days.



B. Bright field microscopy images of conjunctival cultivated limbal epithelial tissue culture at different days.

Fig. 3 (**A**)Bright field microscopy representative COMET tissue culture image at different days. (**B**)Bright field microscopy representative CCET tissue culture image at different days

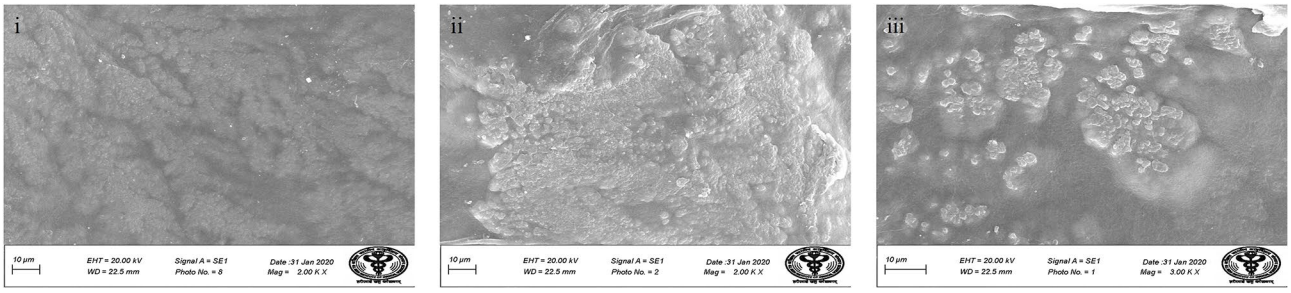


Fig. 4 (**i**)Characterization of substrate (HAM) (**ii**) Cultivated oral mucosal epithelial cells on HAM (iii) Conjunctival cultivated limbal epithelial cells on HAM

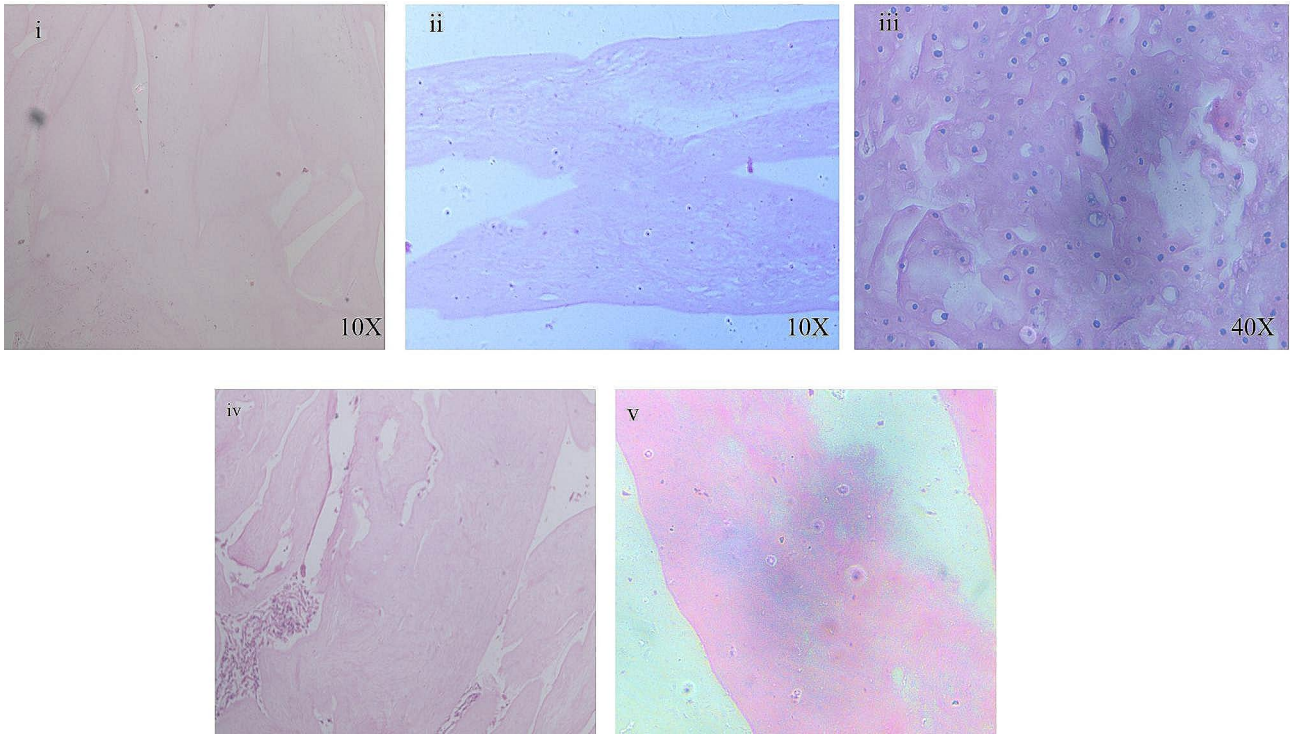


Fig. 5 (**i**)Haematoxylin and eosin stained images at different magnifications of the substrate (HAM) (**ii**)10x(**iii**)40x image of cultivated oral mucosal epithelial cells on HAM (**iv**)10x and (**v**) 40x image of conjunctival cultivated limbal epithelial cells on HAM

surface inflammation, presence of symblepharon, tear status, ocular surface keratinization, and systemic factors such as age and general health of the patient [8–10]. Limbal stem cell transplantation (LSCT) is the sole effective treatment for LSCD. The major goal is to keep providing a fresh corneal epithelium for a long – if not indefinite-period of time so that patients can be freed from irksome photophobia and regain useful visual acuity. In case of LSCD that are bilateral, both Cultivated Oral Mucosal Epithelial Transplantation (COMET) and Conjunctival Cultivated Epithelial Transplantation (CCET) are considerable options [8]. However, the efficacy of COMET versus CCET in such patients has not been assessed in a randomized controlled clinical trial.

The transplantation of cultivated oral mucosal epithelial sheets offers a viable and safe alternative in the reconstruction of a stable ocular surface [8, 9]. Re-establishment of a stable and transparent corneal epithelium, regression of corneal conjunctivalization/vascularization, and resolution of the persistent epithelial defect (PED) has been generally considered as a criterion for clinical success after LSCT procedures [9] In one study on the excised corneal buttons following COMET, cytokeratins, MUC5AC (mucin expressed by conjunctival goblet cells but not oral mucosal epithelial cells), ABCG2, and p63 expression were examined [11]. All specimens were positive for K3, K4, and K13 but negative for K8 and MUC 5AC, which suggests that the keratinocytes were derived from the oral mucosa. In addition, the basal cells were small, compact keratinocytes that preferentially expressed pan-p63, ABCG2, and p75. These findings suggest re-integration and long-term survival

of transplanted progenitor cells [11, 12]. In COMET, the cells are autologous; therefore, there is no risk of immunologic rejection and thus no need for immunosuppression. The oral mucosa is less differentiated than epidermal keratinocytes [13, 14]. They proliferate rapidly and can be kept in culture for prolonged periods without keratinization. Cytokeratin K3 is expressed by both corneal epithelium and oral mucosa but not by the epidermis, suggesting closer gene expression between oral and corneal epithelium [13, 14]. In a study by *Nakamura T et al.* [15] the long-term outcomes of autologous cultivated oral mucosal epithelial transplantation (COMET) for the treatment of the scar phase of severe ocular surface disorders were investigated in 19 eyes of 17 patients. Clinical efficacy was evaluated by BCVA at the postoperative 36th month. The clinical results (clinical conjunctivalization, corneal opacification, corneal neovascularization and symblepharon formation) were evaluated and graded on a scale from 0 to 3 according to their severity. Infection and persistent epithelial defects were evaluated for clinical safety. During the long-term follow-up period, postoperative conjunctivalization and symblepharon were significantly inhibited. All eyes manifested various degrees of postoperative corneal neovascularization, but it gradually abated and its activity was stable at 6 months after surgery.

The ocular surface was improved in 18 eyes (95%) during the follow-up periods, and visual acuity at the postoperative 36th month was improved in 10 eyes (53%). The result of the study strongly supports the use of tissue-engineered cultivated oral mucosal epithelial sheets for reconstructing the scarred ocular surface. The ocular surface became stable postoperatively 6 months in COMET operated patients with a reduction in neovascularization. The vision was stable in patients in comparison to preoperative conditions, and no ocular surface inflammation and scarring was observed during the completion of follow-up. In another study by *Satake Y et al.* [16]. Long-term outcomes of COMET in the treatment of total limbal stem cell deficiency in forty eyes in 36 patients were evaluated for a mean follow-up period of 25.5 months. Kaplan-Meier analysis of corneal surface stability revealed an early decline in transplanted oral mucosal epithelial stability over the first 6 months, remaining comparatively stable thereafter (1 year, 64.8%; 2 years, 59.0%; and 3 years, 53.1%). In 9 eyes, persistent epithelial failure developed within 3 months. In eight eyes, fibrovascular tissue invaded the corneal surface. Fornix survival decreased until approximately six months after implantation. A corneal opacity seemed to affect postoperative vision. The complications reported included stromal melting or perforation in 8 eyes, infectious keratitis in 2 eyes, glaucoma in 8 eyes, and herpetic keratitis recurrence in 1 eye. Moreover, in another study, Prabhasawat P et al. [17] treated 20 eyes (18 patients) with bilateral severe ocular surface disease with COMET. It was found that 15 of 20 eyes examined had a successful clinical outcome, defined as being free of epithelial defects, with a clear cornea, free of fibrovascular tissue invasion, and free of ocular surface inflammation, 75%). The clinical success rate at 1 year was 79.3%, and that at 4 years (end of follow-up) was 70.5%. Fourteen of 20 (70%) eyes exhibited improvement in visual acuity after COMET. Preoperative symblepharon was eliminated in most eyes (8 of 13, 61.5%) after COMET combined with eyelid reconstruction when needed. The only complication was corneal perforation (1 eye) induced by a severe eyelid abnormality; treatment with a tectonic corneal graft was successful. This study also showed that COMET can successfully restore ocular surface damage in most eyes with corneal LSCD. In patients enrolled in the study more than half of eye, preoperatively visual acuity was limited to finger counting or hand movements. It is notable that patients operated with COMET post 24 weeks were able to do their work along with a reduction in care assistance.

The rationale for using cultivated conjunctival forniceal cells for the treatment of LSCD was established by the study by *Wei ZG et al.* [18], where they evaluated the cell kinetic properties of epithelial cells from various zones of the conjunctiva in neonatal and adult mice. To examine the proliferative rate of the conjunctival epithelium, a single administration of tritiated thymidine (3 H-TdR) was used to detect cells in "S" phase. This study found that slow-cycling cells, detected as label-retaining cells (LRCs), were present in bulbar, forniceal, and palpebral epithelia, as well as in limbal epithelium. The greatest number of LRCs was found in the forniceal epithelium. These findings proved that forniceal epithelium is a zone enriched in conjunctival epithelial stem cells. In 2010, *Ang LP et al.* [19], evaluated the feasibility of cultivated conjunctiva as a viable epithelial sheet for transplantation and corneal resurfacing in eyes with limbal stem cell deficiency (LSCD). Human corneal epithelial (HCE) and human conjunctival epithelial (HCjE) cells were cultivated on the human amniotic membrane (AM) and then denude AM and cultivated HCE and cultivated HCjE cells were then transplanted into 18 eyes of rabbits with induced LSCD. The cultivated and engrafted epithelia were examined by transmission electron microscopy (TEM) and immunohistochemistry. Two weeks after transplantation, the eyes were examined by slit lamp biomicroscopy and scored on epithelial integrity, corneal haze, and corneal neovascularization. Both cultivated and engrafted HCjE sheets demonstrated confluent epithelial sheets with five to six layers of the well-stratified epithelium. TEM examination of engrafted HCjE revealed numerous microvilli, desmosomes, and

hemidesmosomes, identical to in vivo corneal epithelium. Immunohistochemical analysis of both HCjE and HCE cells showed the presence of CK3, CK4, and CK12, with the absence of Muc5AC. Clinical outcomes for eyes receiving HCjE transplants and HCE transplants were comparable, with most having transparent, smooth corneas, free of epithelial defects. In another study by *Tan DT et al.* [20], the use of a serum-free derived cultivated conjunctival epithelial sheet for ocular surface transplantation and reconstruction was investigated. Seven subjects with various ocular surface disorders were selected for the procedure: one patient had an extensive conjunctival nevus, three patients had pterygium, two patients had persistent leaking trabeculectomy blebs, and one patient had bilateral superior limbic keratoconjunctivitis. Conjunctival epithelial cells were harvested from the forniceal conjunctiva of patients 2 weeks before the definitive surgery. Cultivation of conjunctival epithelial cells on the human amniotic membrane (HAM) was carried out under serum-free conditions. At the time of transplantation, the area of diseased conjunctiva was excised and the cultured conjunctival epithelium-HAM composite was transplanted onto the surgical defect. Patients were followed up with serial slit-lamp examinations, fluorescein staining, and photographic documentation. The mean follow-up period of the study was 11.6 months. A successful outcome, defined as resolution of the disease, maintenance of conjunctival epithelialization, maintenance of graft integrity, and absence of significant complications, was obtained in all seven patients. A good functional and cosmetic result was achieved in all eyes.

This study aimed to compare the clinical outcomes of cultivated oral mucosal epithelial transplantation (COMET) with cultivated conjunctival epithelial transplantation (CCET) in patients with bilateral limbal stem cell deficiency (LSCD) predominantly caused by Stevens-Johnson syndrome (SJS) and, in a minority, by chemical injury. Our results and findings demonstrate that both COMET and CCET procedures result in significant anatomical improvements over six months, though COMET shows some advantages in specific parameters. Based on the demographic distribution of enrolled patients in the COMET and CCET groups was similar, with no significant difference in median age, ensuring that the baseline characteristics did not bias the outcomes ($p=0.49$). This similarity provides a reliable foundation for comparing the efficacy of the two interventions. Based on the result the BCVA outcomes indicated a modest, albeit statistically insignificant, improvement in both groups at 2 and 3 months postoperatively. However, by the 6-month follow-up, the BCVA had reverted to baseline levels in both groups. The result suggests that both COMET and CCET may offer short-term improvements in visual acuity, these effects do not sustain long-term. The lack of significant differences between the groups ($p>0.005$) underscores that neither procedure offers a superior benefit in terms of visual recovery over the six-month period. Moreover; the anatomical parameters showed more promising and sustained improvements. Significant enhancements in corneal clarity, symblepharon status, corneal vascularization, and corneal epithelialization were observed in both groups at the six-month follow-up ($p<0.05$). The findings reveals that COMET group patients had substantially better corneal clarity at all follow-up time points ($p>0.005$), suggesting that COMET may be more effective in maintaining corneal transparency. A significant improvement in symblepharon status was also observed in the COMET group at each follow-up ($p<0.05$), indicating a potential advantage in preventing conjunctival adhesions. At the six-month follow up, COMET patients showed significantly less corneal vascularization compared to the CCET group ($p<0.05$), highlighting a potential benefit in reducing neovascularization. During the analysis it also observed that the COMET group demonstrated significantly better corneal epithelialization at both 3 and 6 months postoperatively ($p<0.05$), suggesting a more robust epithelial healing process. However, based on secondary outcome the anatomical success rate in terms of corneal clarity was found to be statistically significant in both groups post-operatively. Based on primary outcome the COMET group was found to be significantly better than the CCET group at 3months in terms of anatomical success and epithelization. Patients enrolled in the study under COMET and CCET groups received medication including steroids, lubricants, antibiotics and oral tablet of vitamin C. During follow-up of the patients, none of the patients were observed with lid keratinization under the COMET group. In another study reported by chie et al. 2013 showed that patient with severe ocular surface disease with corneal stromal opacity can achieved better vision when treated with COMET in combination with penetrating or deep lamellar keratoplasty. Moreover; COMET alone helps in the reconstruction of the ocular surface integrity with the help of an amniotic membrane as a substrate which may help in preventing the cornea from melting [21, 22]. Based on our study findings we observed that the confluence rate of oral mucosal epithelial cells increased as a result of the formation of our tissue-engineered graft. For ocular surface restoration employing cell expansion-tissue engineering technology, it is advantageous for ophthalmologists to harvest the tissue from oral mucosa instead of the conjunctiva. Our study strength is that it is a randomized clinical trial and that it demonstrates the potential of tissue-engineered cultured grafts COMET and CCET. According to our research and randomized controlled clinical trial, tissue-engineered COMET and CCET have the benefit of being autologous in origin.

Within 7 to 11 days after tissue harvesting, for the cells to develop under cGMP conditions, COMET demonstrated a supply of epithelial cells that were abundant compared to CCET. The results of this study have several important implications for clinical practice. While both COMET and CCET are effective in improving anatomical outcomes in LSCD patients, COMET appears to offer superior benefits in certain parameters such as corneal clarity, symblepharon status, and epithelialization. These findings suggest that COMET may be a preferable option for patients with bilateral LSCD, particularly in cases where maintaining corneal transparency and minimizing neovascularization are critical. Additionally, it was noted that patients who underwent COMET did not have pain or complication at the donor site and that only a small amount of tissue needed to be removed. In conclusion, both tissue-engineered autologous graft surgical procedures are effective in restoring the integrity of the ocular surface in cases of bilateral ocular surface disease when combined with supportive medications. No adverse events or complications were observed during surgery or postoperative. Comparing the tissue-engineered autologous COMET and CCET groups, the COMET group had a higher rate of graft survival without any rejection., Additionally, to draw any further conclusions research with larger cohorts and extended follow-up periods is required and necessary to confirm these findings and to assess the long-term efficacy and safety of both procedures. Future studies should explore the underlying mechanisms that contribute to the observed differences between COMET and CCET, potentially leading to further optimization of these techniques.

Conclusion

In conclusion, both COMET and CCET are viable options for treating bilateral LSCD, with significant improvements in anatomical parameters observed in both groups. However, COMET demonstrates certain advantages, particularly in terms of corneal clarity, symblepharon status, and epithelialization. These findings contribute to the growing body of evidence supporting the use of epithelial transplantation in LSCD and offer valuable insights for clinicians in selecting the most appropriate treatment modality for their patients.

Abbreviations

CCET	Conjunctival Cultivated Epithelial Transplantation
COMET	Cultivated Oral Mucosal Epithelial Transplantation
KLAL	Keratolimbal Allograft
LSCD	Limbal Stem Cell Deficiency
OCP	Ocular Cicatricial Pemphigoid

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Author contributions

RT: Conceptualization, arranging financial support as principal investigator (PI), overall supervision, clinical and scientific input, plan for data analysis and interpretation, administrative support, manuscript writing and revision. PKP: tracking of study material, coordination of patient visits, facilitating data collection, data entry, data analysis, and manuscript writing. TAK: clinical data capture and documentation, coordinating surgical visits of patients, data collection and assembly, data analysis and interpretation, manuscript writing. AKD: clinical data input by masked analysis of images, data analysis and interpretation, manuscript writing. MK: data analysis and interpretation, manuscript writing. MM: cell culture, laboratory data collection and analysis, manuscript writing. SK and SS: Provision of study material or patients, data analysis and interpretation, manuscript writing. NL, NG and MV: Provision of patients, clinical support, data analysis and interpretation, manuscript writing. SM: assisted in conceptualization as co-, supervision of cell culture work study, scientific and administrative support, manuscript writing and modification. All the authors provided substantial scientific contributions, read and approved the final manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are not publicly available because we are not able to permit any possibility of identifying persons from treatment history regardless of data anonymity, but data are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Institute Ethics Committee of All India Institute of Medical Sciences, New Delhi, India. (Reference No. IEC/NP-99/11.04.2014). The informed consent form obtained from all the patients. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki by following the ICH-GCP guideline and national ICMR guidelines for Stem Cell Research and Therapy.Our study adheres to CONSORT guidelines and includes a completed CONSORT checklist.

Consent for publication

Not Applicable.

Clinical trial registry number

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Competing interests

The authors declare no competing interests.

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