Oral Mucosal Epithelial Cells

Subjects: Materials Science, Biomaterials

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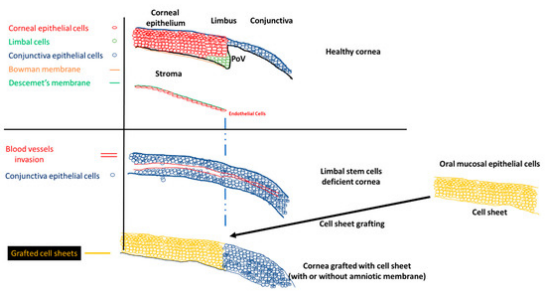
The corneal surface is an essential organ necessary for vision, and its clarity must be maintained. The corneal epithelium is renewed by limbal stem cells, located in the limbus and in palisades of Vogt. Palisades of Vogt maintain the clearness of the corneal epithelium by blocking the growth of conjunctival epithelium and the invasion of blood vessels over the cornea. The limbal region can be damaged by chemical burns, physical damage (e.g., by contact lenses), congenital disease, chronic inflammation, or limbal surgeries. The degree of limbus damage is associated with the degree of limbal stem cells deficiency (partial or total). For a long time, the only treatment to restore vision was grafting part of the healthy cornea from the other eye of the patient or by transplanting a cornea from cadavers. The regenerative medicine and stem cell therapies have been applied to restore normal vision using different methodologies. The source of stem cells varies from embryonic stem cells, mesenchymal stem cells, to induced pluripotent stem cells. This review focuses on the use of oral mucosa epithelial stem cells and their use in engineering cell sheets to treat limbal stem cell deficient patients.

Keywords: cell sheet ; limbal stem cell deficiency ; tissue engineering ; clinical trial ; oral mucosa epithelial cells

1. Introduction

A healthy cornea is essential for proper vision. This part of the eye must be kept clear to be fully functional. The cornea is divided into different parts: (1) the corneal epithelium, (2) the Bowman membrane, (3) the stroma, (4) the Descemet membrane, and (5) the corneal endothelium (Scheme 1). The corneal epithelium is constantly renewed by limbal stem cells, located in the limbus. The corneal epithelial cells completely renew in five to seven days [6]. The asymmetric division of the limbal stem cells generates a limbal stem daughter cell and a transient amplifying cell, which migrate to the central cornea. Limbal stem cells migrate toward the middle of the corneal epithelium, in an X, Y, Z direction [[1]]. During their migration, limbal stem cells differentiate until they become squamous cells and detach from the surface of the cornea [[2]].



Scheme 1. Normal cornea, limbal stem cell cornea, and the grafting of the cell sheets on limbal stem cell deficient corneas to restore the corneal morphology. POV, palisades of Vogt.

2. Clinical Trials of LSCD Treated with Oral Mucosal Epithelial Cells

The stem cell niche is precisely located at the level of palisades of Vogt, in the limbus [[3]]. Injury to the limbal niche prevents corneal epithelial cell renewal and results in the growth of conjunctival epithelium over the cornea. Conjunctival growth over the cornea is accompanied by the neovascularization of the cornea. Many studies reported that the limbal region functions as a barrier between the cornea and conjunctiva. The role of the barrier is to block the conjunctivalization and the neovascularization of the cornea [[4][5][6][7]]. Damage to this barrier leads to the development of limbal stem cell deficiency (LSCD). The process of conjunctivalization, where conjunctival epithelial cells invade and populate the corneal surface, results in neovascularization, opacification, and inflammatory cell infiltration [[8][9][10]]. This limbal stem cell deficiency leads to different levels of visual impairment, as reported by the international Limbal Stem Cell Deficiency

Working Group [8], [11],[12]. LSCD can be caused by exogenous trauma, such as thermal burns, chemical injury (alkali burn), or endogenous eye diseases (e.g., Stevens–Johnson syndrome, ocular pemphigoid, aniridia (a genetic disorder), contact lenses, multiple surgeries, or microbial infection) [11], [13].

Different methodologies have been developed since 1905, when the first corneal transplantation was completed by Dr. Eduard Zirm [14]. Corneal transplantation is used to repair only the damaged central part of the cornea, but cannot restore the presence of limbal stem cells, which are involved in the renewal of the corneal epithelium, or be used for long term treatment of corneal epithelial defects due to limbal stem cell deficiency. Limbal stem cell transplantation is used to restore the renewal process of the corneal epithelium when the limbal stem cells are damaged and can no longer perform their duty. The curing potency of the limbal stem cell graft can be superior to the corneal transplantation. Grafting of limbal stem cells renews the central part of the damaged cornea to treat corneal epithelial defects [15].

No approved treatment currently exists for LSCD patients other than: (1) autologous grafting of limbal stem cells [16][17] and (2) allografting of limbal epithelium from a deceased donor [18][19]. Autologous grafts produce excellent results in treating the LSCD cornea because the risk of graft rejection from the transplant is reduced. However, this treatment has limitations: (1) this approach cannot be performed if the patient has bilateral LSCD, which is a challenging task for ophthalmologists [20]; (2) a risk exists of damaging the healthy cornea [9]. Donor cornea allograft treatment heavily depends on the supply of donor corneas provided by eye banks. The shortage of donor eyes is well known worldwide as a serious problem [21]. Even when a donor cornea is grafted onto a patient's eye, a long-term immunosuppressant treatment to decrease the graft rejection risk is required [22][23].

Among the cells used for corneal recovery, oral mucosa epithelial cells are the most commonly used for in vitro, in vivo, and translational applications. Different laboratories and hospitals over the world engineered oral mucosal epithelial cell sheets to treated patients afflicted with LSCD, in clinical trials studies. Table S1 summarizes the type of cells used to treat LSCD, and the table S2 reports all the clinical trials published, treating LSCD with oral mucosal epithelial cells.

Table S1	Heterologous			Autologous			
	Donor of Limbus	**iPSC**	**Mesenchymal Stem Cells**	**Limbus**	**iPSC**	**Mesenchymal Stem Cells**	**Oral Mucosal Epithelial Cells**
Pro	Regeneration of the Cornea	High Availability	High Availability	Low Risk of Rejection	High Availability	High Availability	Epithelial Differentiation
	Limited Self-Renewal	Self-Renewal	Self-Renewal	Limited Self-Renewal	Self-Renewal	Self-Renewal	Limited Self-Renewal
	Regeneration of the Cornea	Epithelial Differentiation	Low Immunogenicity	Regeneration of the Cornea	Low Immunogenicity	Low Immunogenicity	Regeneration of the Cornea
			Epithelial Differentiation		Epithelial Differentiation	Epithelial Differentiation	
Con	Potential Rejection	Potential Immuno Rejection	Potential Tumoriginecity	Not Available in case of Bilateral LSCD	Unknown Potential Immuno Rejection	Unknown Potential Immuno Rejection	Unknown Potential Immuno Rejection
	Long Term ImmunoSuppressant Treatment	Potential Tumoriginecity		Risk of Damaging the Healthy Cornea	Potential Tumoriginecity	Potential Tumoriginecity	Low Availability
	Low Availability						
Tested on LSCD patients	Yes	Yes	No	Yes	No	No	Yes
Reference for Clinical Trials	32-36	https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000041628	N/A	32-36	N/A	N/A	Studies reported in Supplementary Data 1

Table S1: Summary of the cells used to treat patients LSCD.

Before Transplant

Reference	Number of Patients	Diseases	Age Of the Patients (years)	Inclusion Criteria	Exclusion Criteria	Biopsy Size	Number of OMEC Isolated	Number of OMEC seeded per dish	Number of Cell Sheet Engineer	Time to Engineer Cell sheets (Days)	Harvesting Method	Characterization of the Cell Sheet	% of Corneas with Pre-Surgical Treatment	% of Patients with Tear Film Deficiency	% of Corneas with Inflammation before Transplantaiton	Growth Cell Support	Serum Used in Culture Media
Nakamura, 2004	4	3x SJS/CB and 3x CB.	24.5 ± 7.9	Not reported	Not reported	2-3 mm^2	Not Reported	Not Reported	Not Reported	Not Reported	With Amniotic Membrane	H&E, KRT3	100	Not Recorded	Not Reported	Fibroblast Feeder Cells	FBS
Nishida, 2004	4	1x SJS, 3 with OCP. All Eyes have Symblepharon	69.75 ± 8.53	Bilateral LSCD	glaucoma or xerophthalmia of the entire cornea	3 mm x 3 mm	Not Reported	Not Reported	Not Reported	14	Thermo-Responsive Surface	KRT3, Beta1-Integrin, p63	Not Reported	75	100	Fibroblast Feeder Cells	Not Reported
Ang, 2006	10	7xSJS, 1x CB, 1x TB, 1x OCP	57.1 ± 18.9	Not Reported	Not Reported	2-3 mm^2	Not Reported	Not Reported	Not Reported	15-16	With Amniotic Membrane	KRT3, KRT4, KRT13, Desmoplain, Alpha6-Integrin, Laminin 5, Collagen IV, ZO-1	Not Reported	Not Recorded	0	Fibroblast Feeder Cells	FBS and Autologous serum
Inatomi, 2006 (ref 69)	12	5xCB, 7xSJS, 1 Ocular Surface Disorder, 1 TB, 1 pseudo-ocular cicatricial pemphigoid.	47 ± 23.6	Not Reported	Not Reported	2-3 mm^2	Not Reported	0.7 to 1.5x10^5 Cells	Not Reported	15-16	With Amniotic Membrane	Not Reported	58	Not Recorded	Not Reported	Fibroblast Feeder Cells	FBS and Autologous serum
Inatomi, 2006 (Ref 68)	2	1x SJS, 1x CB	55 ± 21.2	Not Reported	Not Reported	3-5 mm^2	Not Reported	1x10^5 Cells	Not Reported	15-16	With Amniotic Membrane	Not Reported	Not Reported	100	Not Reported	Fibroblast Feeder Cells	Not Reported
Nakamura, 2007	5	3x SJS, 3 x CB	58.4 ± 18.12	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Recorded	Not Reported	Not Reported	Not Reported
Satake, 2008	4	2x SJS, 2x pOCP	52 ± 14.1	Not Reported	Not Reported	8 mm diameter	Not Reported	1-2x10^5 Cells	Not Reported	15-16	With Amniotic Membrane	Not Reported	Not Reported	Not Recorded	Not Reported	Fibroblast Feeder Cells	FBS
Ma, 2009	5	2x Acute AB, 1x chronic AB, 2x chronic TB	36 ± 17.67	Not Reported	Not Reported	6 mm x 6 mm	Not Reported	Not Reported	Not Reported	Not Reported	With Amniotic Membrane	KRT3, KRT13, p75, p63, ABCG2, H&E.	100	Not Recorded	20	Fibroblast Feeder Cells	FBS
Nakamura, 2010	17	11x SJS, 4x OCP, 2x Squamous Cell Carcinoma, 1 TB, 1 graft versus host disease.	54 ± 21	Not Reported	Patients having been grafted with the cell sheet and had penetrating keratoplasty and conjunctival fornix reconstruction.	2-5 mm^2	Not Reported	1-2x10^5 Cells	Not Reported	15-16	With Amniotic Membrane	Not Reported	Not Reported	Not Recorded	Not Reported	Fibroblast Feeder Cells	Unspecific Serum
Priya, 2011	10	9x CB, 1x SJS	31 (8 to 65)	Not Reported	Not Reported	4 mm x 2 mm	Not Reported	1-2x10^5 Cells	Not Reported	Not Reported	With Amniotic Membrane	Not Reported	100	Not Recorded	Not Reported	Fibroblast Feeder Cells	Autologous Serum
Satake, 2011	36	12x JSJ, 11x CB, 9x OCP, 7x pOCP, 1x gelatinous drop like dystrophy.	58.5 (14 to 81)	Not Reported	Not Reported	8 mm diameter	Not Reported	1-2x10^5 Cells	Not Reported	Not Reported	With Amniotic Membrane	Not Reported	2.7	Not Recorded	Not Reported	Fibroblast Feeder Cells	Autologous Serum
Takeda, 2011	3	2x TB, 1x CB	41 ± 21	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	15-16	With Amniotic Membrane	Not Reported	Not Reported	Not Reported	Not Reported	Fibroblast Feeder Cells	Not Reported
Burillon, 2012	25	9x CB, 2x Neuroparalytic Keratitis, 3x Rosacea Keartitis, 4x Lyell Syndrome, 1x Severe Trachoma, 3x Contact Lens Hypoxia, 3x Congenital aniridia, 1x Cystinosis, 1x Hepatitis C	51.9 ± 13.8	Not Reported	Acute Systemeic Infection, History of Acute phase of Ocular Inflammation, History of Neoplastic Disease, Glaucoma, Symblepharon, Hypersensibility or allergy to antibiotics or serum, pregnancy, infection disease.	3 mm x 3 mm	Not Reported	Not Reported	Not Reported	Not Reported	Thermo-Responsive Surface	KRT3, KRT76, p63, beta1-integrin, Laminin 5, CFE	Not Reported	Not Reported	Not Reported	Fibroblast Feeder Cells	Not Reported
Hirayama, 2012	32	12x CB, 4x SJS, 12x OCP, 6x pOCP	59.3 ± 16.3	Not Reported	Previous Patients having been grafted with cell sheet (diagnosied with absence of Palisades of Vogts and conjunctivalization); Patients that had cell sheet grafted with amniotique membrane.	8 mm diameter	Not Reported	Not Reported	Not Reported	Not Reported	With Amniotic Membrane	Not Reported	Not Reported	Not Reported	Not Reported	Fibroblast Feeder Cells	Autologous Serum
Sotozomo, 2013	40	21x SJS, 10x OCP, 7x CB, 3x Idiopathic Stem Cells Deficiency, 1x Radiation Keratopathy, 1x graft versus host disease, 1x congenital aniridia, 1x LSCD due to drug toxicity, 1x Slazmann's corenal degenration	57 (from 9 to 86)	Not Reported	Not Reported	6 mm diameter	Not Reported	Not Reported	Not Reported	7-9	With Amniotic Membrane	Not Reported	48.9	Not Reported	Not Reported	Fibroblast Feeder Cells	FBS and Autologous Serum
Gaddipati, 2014	1	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported
Kolli, 2014	2	Chemical Burn	44 and 76	Not Reported	Not Reported	3 mm diameter	Not Reported	Not Reported	Not Reported	14 (6 days shorter with USP grade product, in GMP facility)	With Amniotic Membrane	Pax6, KRT12, KRT13, ABCG2, C/EBPg, DeltaNp63	Not Reported	No for the 76 years old patient	Not Reported	No	Autologous Serum
Sotozono, 2014	9	3x SJS, 5x CB, 2x OCP	45.1 ± 25.23	LSCD, history of acute episode, of epithelial defect, persistent epithelial defect, persistent ocular surface inflammation, resistance to conventional therapies, fibrovascular tissue surrounding the persistent epithelial defect.	Not Reported	6 mm diamter	Not Reported	Not Reported	Not Reported	8-9	With Amniotic Membrane	Not Reported	Not Reported	Not Reported	100	Fibroblast Feeder Cells	FBS and Autologous serum
Kim, 2015	6	3x SJS, 2x CB, 1x Ocular Malignant melanom	Not Precise	Not Reported	Not Reported	Not Reported	Not Reported	1-2x10^5 Cells	Not Reported	8-10	Dispase	CFE, EGF levels	Not Reported	Not Reported	Not Reported	No	Not Reported
Baradaran-Rafii, 17	14	14x CB	38.9 ±12.59	Successful treatment of chemical burn with Cell Sheet, absence of significant symblepharon or eye lide abnormalities, presence of reflex tear, no corneal inflammation, visual acuity at least equal to 20/120.	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	14-21	With Amniotic Membrane	p63, KRT3, KRT12	85.7	Not Reported	0	Fibroblast Feeder Cells	FBS
Kim, 18	8	6x SJS, 1 CB, 1x OCP	42 ± 15.04	Complete LSCD with conjunctivalization, Failure of limbal or amniotic membrane, vision acuity less than counting fingers, over 16 years old, problems in using immunosuppressive agents.	Not Reported	0.8 - 1.5–1.0 - 2.0 cm^2	Not Reported	1-2x10^5 Cells	Not Reported	7-12	Dispase	Not Reported	75	Not Reported	Not Reported	Fibroblast Feeder Cells	FBS

Table S2: Summarizes of data related with the patients recruited to treat LSCD in clinical trials, with oral mucosal epithelial cells.

In general, the results are very positive and encouraging, but the technology of oral mucosa epithelial cell sheet can be improved. The average time for the follow-up of the patients was around two years, with a maximum of 7.5 years, for all the clinical trials involving oral mucosal epithelial cell sheets to treat LSCD. The visual acuity improved for the majority of the patients treated with oral mucosal epithelial cell sheets. For 246 transplanted corneas, 52.8% of the corneas demonstrated an improvement, 8.2% were steady, and 2.9% deteriorated. Based on a review, the transplant of limbal stem cells was over 70% successful, and it is thought that this success rate can be increased. We think that oral mucosal epithelial cell sheet technology to treat LSCD can also be improved, because only 52.8% of the transplanted cornea resulted in improved visual acuity. Neovascularization of the grafted cornea occurred [[24][25][26]], but never grew over the cornea, indicating that cultured autologous oral mucosal epithelial cell sheet (CAOMECS) can block the neovascularization of the cornea of the healthy cornea [[27]]. The mechanism of action of the cell sheet is not well understood, but it could involve a combination of the physical barrier and the production of anti-angiogenic factors. The physical barrier function is indicated by the decrease in the corneal conjunctivalization level after the epithelium cell sheet graft, but this is not always the case [[28][29]]. Corneal opacity is another aspect of patient outcome. Corneal opacity decreased over time after a cell sheet graft in some studies [[30],[31]]. If the opacity did not improve and was persistent, penetrating or deep lamella keratoplasty (PKP and DALK, respectively) was performed [[32][33][34][35][36][37]]; in some cases, two years after the initial grafting [[28], [38]]. PKP and DALK should only be performed after cell sheet graft and once the cornea surface is stable.

A total of 55 adverse events (AEs) was reported, with only nine severe adverse events (SAEs) among the 249 treated patients. Among the 55 AEs, persistent epithelial defects (PEDs) were the most frequent (30 cases), and they were recurrent after the initial cell sheet transplantation (54.54% of the total AEs). A review explained how persistent epithelial defects are treated [[39]]. In some of the clinical studies, the PEDs resolved by themselves with the help of grafted cell sheets or conjunctival cells [[33], [40][41]]; antibiotics were used [[25]], a second reoperation was performed (oral mucosa epithelial cell sheet, amniotic membrane, allogenic corneal, or limbal stem cells transplants) [[33], [37], [40]], with a contact lens bandage [[26], [42]]; or they did not resolve [[29], [32], [43][44][45]]. Intraocular pressure was reported for 14 of the patients (5.62% of all patients), which resolved with specific treatment, such as antiglaucoma medication or carbonic anhydrase inhibitor [[28], [29]]. Infection of the cornea was detected in four patients (1.6% of the total number of patients), and the

infections resolved with antibiotic treatment [[25], [29], [32], [37]]. Some other AEs were recorded, such as cataract, pain, corneal recurrence, Meibomian cyst, keratitis, symblepharon formation, drug induced allergy, and liver dysfunction, which resolved after stopping a systemic drug treatment [[32],[35], [43]]. Nine SAEs were reported: two corneal perforations, where one cornea healed with a small patch graft [36], and the patient in the other study was withdrawn [[43]]; seven cell sheets were rejected [[33], [35], [37], [43]]. Follow up and reports of adverse events are reported in the table S3.

Reference	Time for the Follow Up (months)	AE	SAE
Nakamura 2004	13.8 ± 2.9	In case 3 (both eyes), a small epithelial defect with minimal cell infiltration suggested a low toxic bacterial infection which was controlled by the frequent use of ofloxacin and cefmenoxime eye drops.	None Reported
Nishida 2004	13-15	None Reported	None Reported
Ang 2006	12.6 ± 3.9	None Reported	None Reported
Inatomi, 2006 (ref 65)	20 ± 11.05	They observed 1 type of complication: epithelial defect for 2 SJS treated with cultivated corneal epithelial transplantation, 1 SJS pretreated with (amniotic membrane transplantation+keratoepithelioplasty) and treated with phacoemulsification+intraocular lens, 1 SJS treated with phacoemulsification+intraocular lens, 1 thermal burn (acute) treated with lid plastic surgery.	None Reported
Inatomi, 2006 (Ref 64)	19 and 26	None Reported	None Reported
Nakamura 2007	Not Reported	For 1 patient, with bilateral LSCD, the transplantation failed. Three allogenic cultivated corneal epithelia were transplanted on the right eye, and two were transplanted on the left eye, and all failed. This patient had recurrent infections Staphylococcus aureus. The corneal epithelia had many recurrent small epithelial defects. Even by treatment well the patient, opaque epithelium always covered its eyes. Same AE were reported after the transplant of the CAOMECS. Then, 16 months and 8 months after the failure of the CAOMECS transplant, the patient was grafted with allogeneic limbal transplant. For the 4 other patients, after CAOMECS transplant, the ocular surface was stable, without inflammation BUT corneal stromal opacity affected the patients and required penetrating keratoplasty (these harvested samples were studied by light and electron microscopy, but also immunohistochemical staining).	None Reported
Satake 2008	up to 16 months	For 1 patient, the intraocular pressure increased, and it was resolved with an antiglaucoma medication.	None Reported
Ma, 2009	29.6 ± 3.6	None Reported	For 1 patient, microperforation over the cornea was observed (descementocele).
Nakamura, 2010	50 (up to 90 months)	Ocular hypertension was recorded for 16% of the transplanted corneas (3 corneas). One infection occurred.	None Reported
Priya, 2011	18.6 (1 to 38 months)	None Reported	None Reported
Satake, 2011	25.5 (2 to 54.9)	None Reported	None Reported
Takeda, 2011	30 ± 19.5	None Reported	None Reported
Burillon, 2012	12	Different AE were reported during the study: pain and corneal recurrence, meibomian cyst, eye inflammation, increased intraocular pressure, keratitis, amniotic membrane graft, conjunctival operation, and symblepharon formation.	One Corneal perforation and Corneal graft rejection were reported, and the patients were withdrawn from the study.
Hirayama, 2012	147 weeks maximun	Three ocular hypertensions were reported	None Reported
Sotozomo, 2013	28.7 (6.2 to 85.6)	Sixteen persistent epithelial defect (10 for SJS, 3 for OCP, 2 for thermal-chemical burn), 2 corneal stromal melting (1 for OCP), 2 ocular infection (keratitis, endophthalmitis), 3 infiltrations (2 for SJS, 1 for OCP), 4 elevation of intraocular pressure (1 OCP, 2 thermal-chemical burn), One hepatic dysfunction (for 1 SJS), 1 drug-induced allergy.	None Reported
Gaddipati, 2014	13	None Reported	None Reported
Kolli, 2014	9 and 41	None Reported	None Reported
Sotozono, 2014	23.3 (5.6 to 39.7)	Intraocular pressure occurred for 2 patients due to the steroid's treatment, but it was resolved. Methicillin-resistant Staphylococcus aureus corneal infection occurred for 1 patient, but the infection was healed, with no perforation.	None Reported
Kim, 2015	Not Reported	None Reported	None Reported
Baradaran-Rafii, 17	28.2 ±8.03	Forty months after grafting, the percentage of cell sheet rejection is around 30%. Different AE were reported: Cataract (3 corneas), Rejection of endothelial graft (from cadaver donors)(4 corneas), failure (1 cornea), corneal epithelial defect (3 corneas), elevated intra-ocular pressure (2 corneas)	None Reported
Kim, 18	10.1 ± 4.8	Only epithelial defects were observed on 50% of the corneas. One transplantation failed (1 OCP because of symblepharon recurrence): 12.5%.	None Reported

Table S3: Summarize of the patients follow up and reports of the severe and adverse events.

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