Tissue-engineered oral mucosal epithelial grafts on fibrin scaffold for bilateral limbal stem cell deficiency: clinical outcomes with histological follow-up and complementary molecular characterization

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Italiadomani – PIANO NAZIONALE DI RIPRESA E RESILIENZA

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Abstract

Bilateral limbal stem cell deficiency (LSCD) poses a significant therapeutic challenge due to the lack of autologous **limbal stem cells (LSCs)** suitable for transplantation. While approaches such as **Cultivated Limbal Epithelial Transplantation (CLET)** are well established for unilateral disease, bilateral LSCD requires alternative autologous epithelial sources. In this context, we describe a tissue-engineering strategy that employs **autologous oral mucosal epithelial cells** expanded ex vivo on a **fibrin scaffold**.

Two patients with bilateral LSCD were treated using this fibrin-based epithelial graft. The production process adhered to stringent quality, safety, identity, and potency controls, including verification of epithelial lineage markers, assessment of stemness-associated profiles, sterility testing, and evaluation of graft integrity and functionality prior to release for clinical use.

Both patients experienced early functional improvement following transplantation. Each subsequently underwent **penetrating keratoplasty (PK)**, offering the unique opportunity to perform an in-depth histological evaluation of the excised corneal buttons. These specimens were analysed for epithelial differentiation status, stemness markers, neovascularization, myofibroblast-associated fibrosis, and inflammatory cell infiltration, providing a comprehensive view of graft behavior and host–tissue interaction.

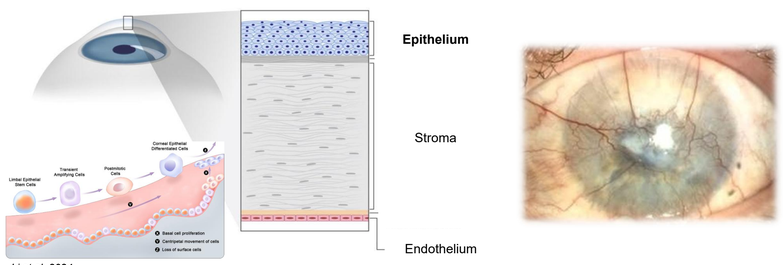
Clinically, the patients were **followed for over one year**. Before the PK, one patient seemed to evolve towards progressive conjunctivalization, whereas the other developing a mixed corneal–conjunctival epithelial profile, whose long-term implications remain to be clarified.

To further elucidate the biological mechanisms underlying these divergent outcomes, we performed **single-cell RNA sequencing** analyses of corneal, conjunctival, and oral mucosal cultured epithelial cells. This molecular profiling aims to identify the pathways governing epithelial identity, plasticity, and regenerative potential, ultimately supporting future innovations in tissue-engineered therapies and direct transdifferentiation strategies.

Overall, this work confirms the clinical feasibility of a fibrin-based, tissue-engineered autologous oral epithelial graft for bilateral LSCD, underscores the value of rigorous process controls and histological follow-up, and lays the groundwork for personalized regenerative strategies informed by high-resolution molecular data.

1 Background

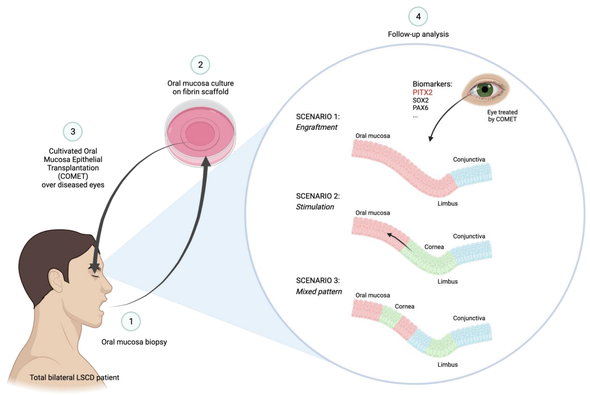
If the **Limbals Stem Cells (LSCs)** are damaged or depleted the condition called **Limbal Stem Cell Deficiency (LSCD)** occurs.



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If both eyes are involved, the condition is called **bilateral LSCD**. A promising therapy relies on **Cultivated Oral mucosal Epithelial Cell Transplantation (COMET)**[1].

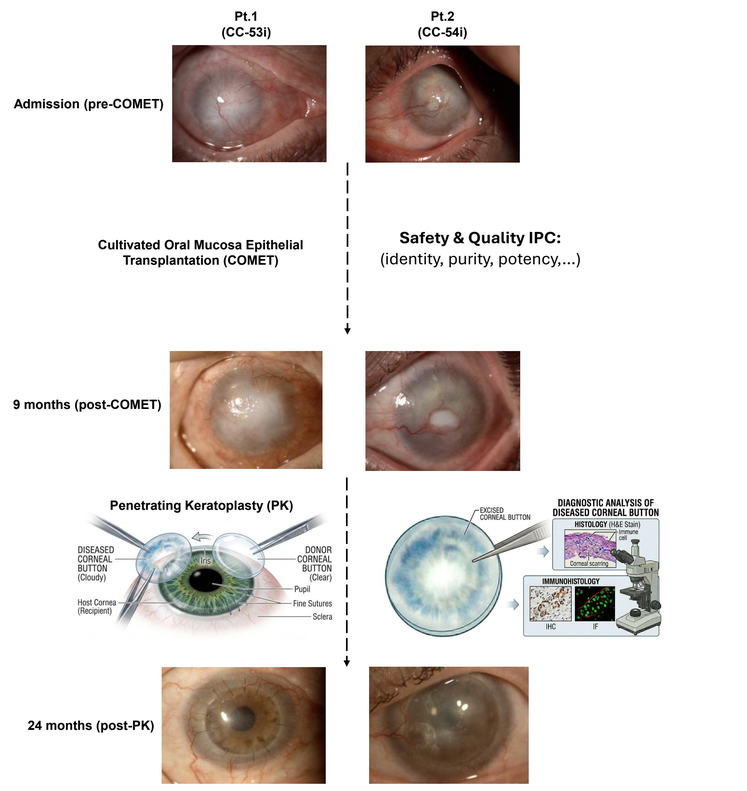
The related **regenerative mechanisms** are still unknown and need to be clarified.



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2 Patients' admission & follow-up

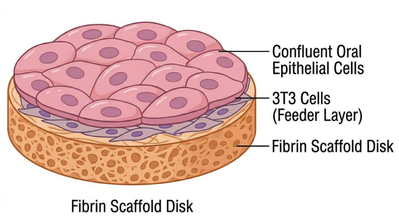
Two patients suffering by bilateral LSCD due to chemical burns were treated by COMET and by **Penetrating Keratoplasty (PK)** and followed for up **2 years**.



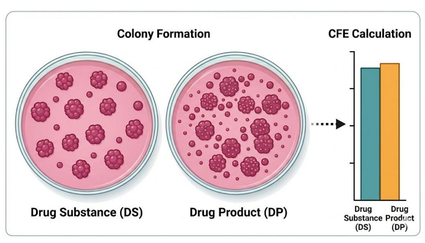
3 Graft manufacturing & In-Process Controls

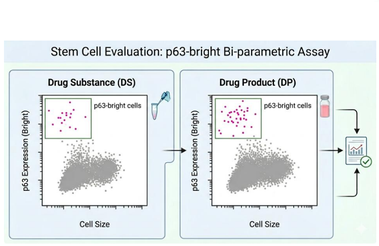
Oral mucosal epithelial cells were isolated and cultured onto a **feeder layer** of lethally irradiated murine 3T3-J2 fibroblasts and the resulting primary culture **cryopreserved**.

After thawing, the oral mucosal cells *(drug substance)* were seeded onto a **fibrin matrix** covered with lethally irradiated feeder cells, to generate the final product *(drug product)*.



The **percentage of clonogenic cells** of the two oral mucosa tissues was evaluated by **Colony Forming Efficiency (CFE) assay**.



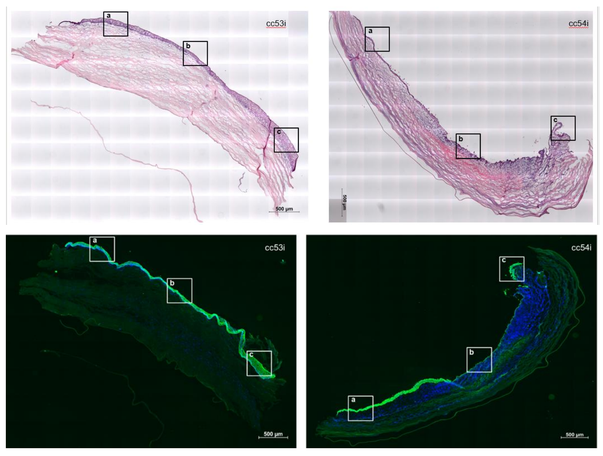


The **percentage of stem cells** of the two oral mucosa grafts was evaluated by analyzing the presence of **p63-bright cells** through a bi-parametric assay.

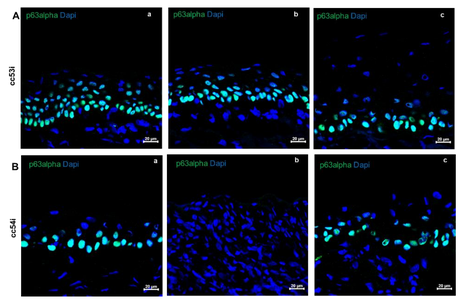
4 Morphology of the graft

PK corneal buttons were widely analyzed.

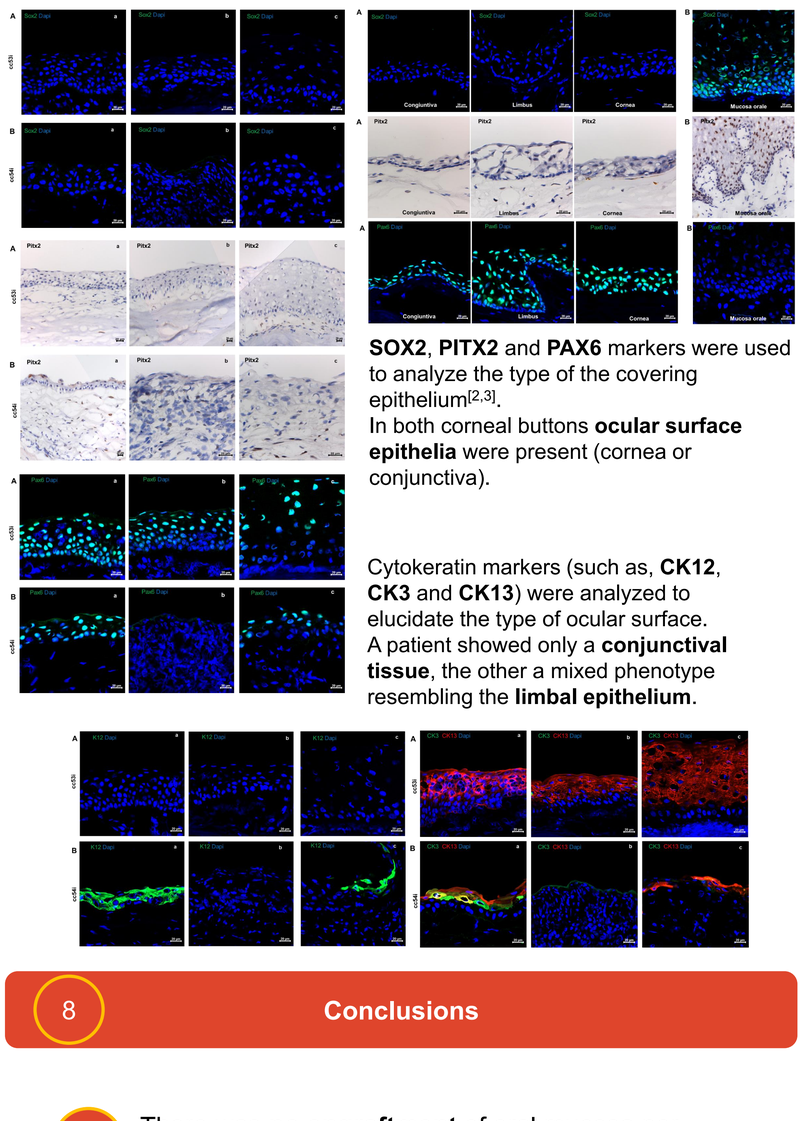
A patient had a **continue epithelial layer**, the other showed a **central discontinuity with fibrotic tissue** (H&E and IF for pan-keratin staining).



The **proliferative potential** of the two regenerated tissues was evaluated by analyzing the expression of **p63α**.



5 Epithelial types characterization



SOX2, **PITX2** and **PAX6** markers were used to analyze the type of the covering epithelium[2,3].

In both corneal buttons **ocular surface epithelia** were present (cornea or conjunctiva).

Cytokeratin markers (such as, **CK12**, **CK3** and **CK13**) were analyzed to elucidate the type of ocular surface.

A patient showed only a **conjunctival tissue**, the other a mixed phenotype resembling the **limbal epithelium**.

6 Neovascularization and inflammation

The subepithelial or stromal **neovascularization process** was identified using vascular endothelial markers, such as **CD31** and **α-SMA**.

In a patient α-SMA marked also new myofibroblast cells showing **fibrovascular tissue**.

Presence of **lymphocytes T (CD3)**, **B (CD20)** and **monocytes/macrophages (CD68)** was observed in the fibrovascular tissue of a patient showing a deep **inflammatory environment**.

7 Outcomes & future perspectives

At 24 months post-PK, Best Corrected Visual Acuity **(BCVA)** improved **from Hand Motion (HM) to 2/10 in Pt.1** and **from Count Fingers (CF) to 1/10 in Pt.2**, highlighting a meaningful functional visual gain.

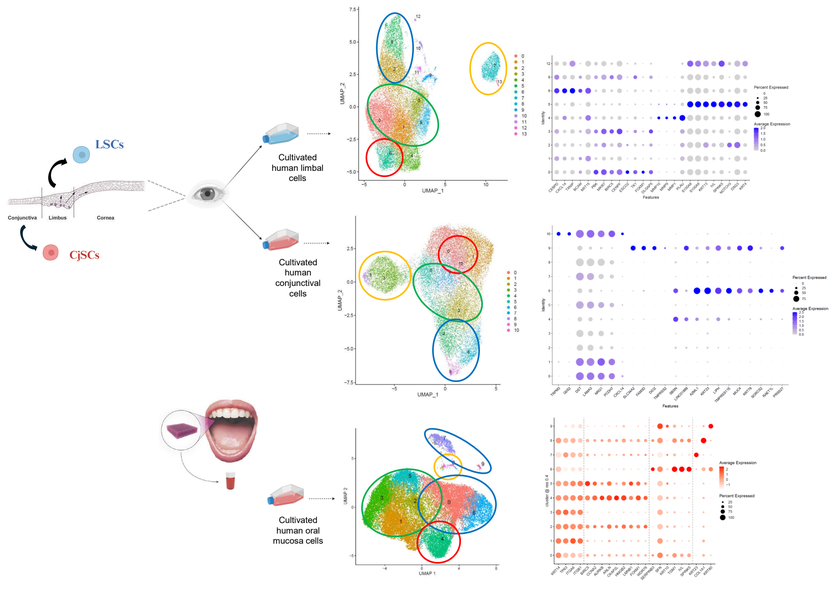
Despite persistent epithelial instability requiring permanent therapeutic contact lens use, superficial neovascularization did not involve the central cornea.

The treatment was considered a **partial success** given the significantly **improvement in quality of life** for both patients.

However, further studies are needed to refine and optimize the therapy.

Ongoing experiments include **scRNA-seq analysis** of the three cultivated epithelia involved in this regenerative scenario: limbo-corneal, conjunctival and oral mucosa tissues.

Stem cells, transit-amplifying cells, differentiated cells and feeder layer cells clusters and **related specific factors** were individuated.



8 Conclusions

a. There was **no engraftment** of oral mucosa nor complete corneal regeneration.

b. Despite **minimal visual recovery**, both patients are independent, very satisfied, and **able to manage on their own**.

c. The underlying biology of the regenerative mechanism needs **further in-depth study** to understand it and improve the therapy.

d. Exploring direct or indirect **interactions between the involved cell types** could provide new insights.

References

[1] Attico E, Galaverni G, Pellegrini G. Clinical Studies of COMET for Total LSCD: a Review of the Methods and Molecular Markers for Follow-Up Characterizations. *Current Ophthalmology Reports.* 2021; 9, 25–37. https://doi.org/10.1007/s40135-020-00263-9

[2] Attico E, Galaverni G, Torello A, Bianchi E, Bonacorsi S, Losi L, Manfredini R, Lambiase A, Rama P, Pellegrini G. Comparison Between Cultivated Oral Mucosa and Ocular Surface Epithelia for COMET Patients Follow-up. *International Journal of Molecular Sciences.* 2023; https://doi.org/10.3390/ijms241411522.

[3] Attico E, Galaverni G, Bianchi E, Losi L, Manfredini R, Lambiase A, Rama P, Pellegrini G. SOX2 Is a Univocal Marker for Human Oral Mucosa Epithelium Useful in Post-COMET Patient Characterization. *International Journal of Molecular Sciences.* 2022; 23(10):5785. https://doi.org/10.3390/ijms23105785

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