Bioengineering sclerocorneal limbal substitutes via cell-laden hydrogels functionalized with growth factors

Miguel Pérez-Garrastachu[1], Ainhoa Agirrebengoa-Arrieta[2], Ander Martin[2], Maddalen Rodriguez-Astigarraga[1], Cristina Romo-Valera[1], Noelia Andollo[1].
[1]Department of Cell Biology and Histology / BEGIKER Research Group. University of the Basque Country (UPV/EHU) / Biobizkaia Health Research Institute, Leioa / Barakaldo (Bizkaia) – Spain. [2]Department of Cell Biology and Histology. University of the Basque Country (UPV/EHU), Leioa (Bizkaia) - Spain.

INTRODUCTION

The sclero-corneal limbus is a transitional region housing the niche of limbal epithelial stem cells (LESCs), supported by a specialized architecture known as Vogt's palisades. Damage to this region, as in limbal stem cell deficiency, impairs corneal regeneration and leads to conjunctival invasion, pannus formation, and progressive vision loss.

Tissue engineering offers promising strategies to restore limbal function through biomimetic substitutes. Our work focuses on hydrogels as core components of such constructs. In this study, we analyze LESC behavior on methacrylated gelatin and collagen hydrogels containing primary corneal stromal cells and functionalized with serum from plasma rich in growth factors (sPRGF). We also assess cellular responses under inflammatory conditions relevant to corneal disease.

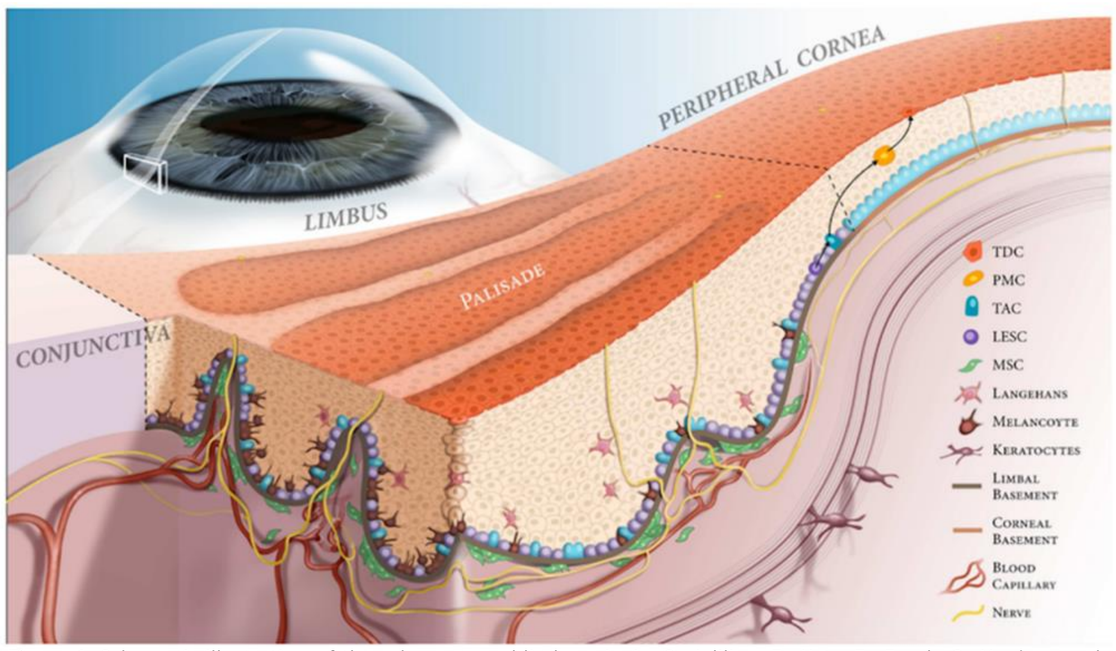


Figure 1. Schematic illustration of the sclero-corneal limbus**.** Amin, S., Jalilian, E., Katz, E., Frank, C., Yazdanpanah, G., Guaiquil, V. H., Rosenblatt, M. I., & Djalilian, A. R. (2021). The Limbal Niche and Regenerative Strategies. *Vision*, *5*(4), 43. https://doi.org/10.3390/vision5040043

METHODOLOGY

1. Isolation, culture and expansion of Limbal Epithelial Stem Cells (**LESCs**) and Corneal Stromal Keratocites (**CSKs**) from donated human corneas.
2. Acquisition of serum rich in growth factors (sPRGF) through calcium-mediated coagulation of blood plasma from healthy donors (n=6).
3. Fabrication of cellular (10^6 CSK/mL) or acellular hydrogels supplemented or not with 10% sPRGF:
   1. 5% Methacrylated gelatin (50% DS) + 0,25% LAP (CELLINK). Photocured under 405 nm LED lamp at 20 mW/cm^2 during 45 seconds.
   2. High density (5 mg/mL) or low density (2 mg/mL) rat-tail acid-extracted type I collagen. Col5 and Col2 respectively.
4. Hydrogels cultured in a CO_2 incubator in basal culture medium under normal or pro-inflammatory conditions (TNF-α 50 ng/mL).
5. Analysis.

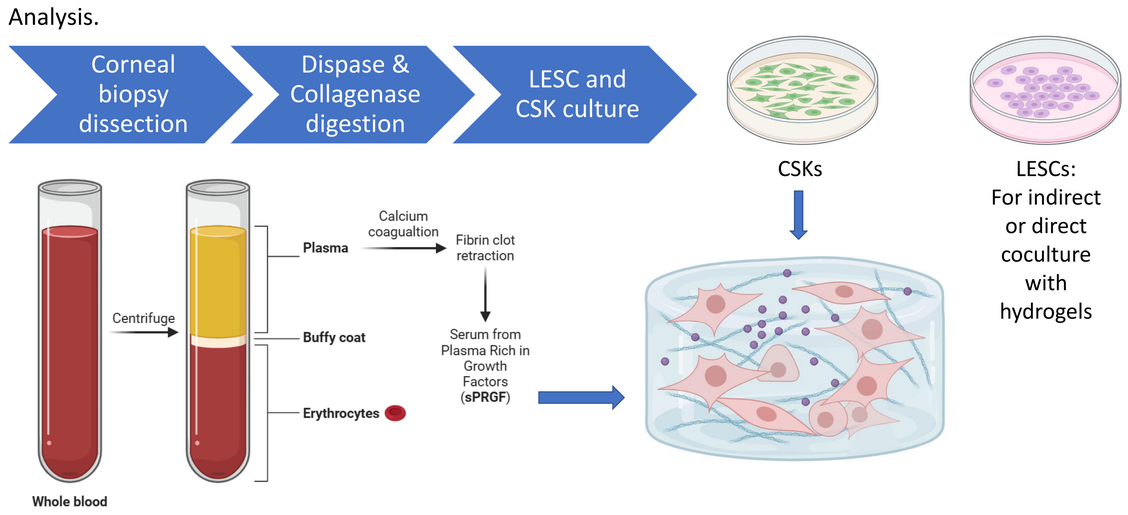
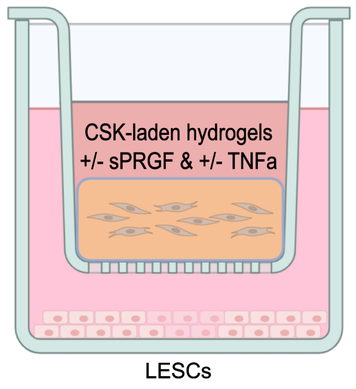


Figure 2. Procedural outline.

RESULTS

I) Hydrogels are effective carriers of growth factors that promote wound healing.



ANOVA - Normalized wounded area

	Sum of Squares	df	Mean Square	F	p
Overall model	169569.1	8	21196.1	10.0201	< .001
sPRGF	69386.9	1	69386.9	32.8015	< .001
Cells	27487.3	1	27487.3	12.9942	< .001
Time (h)	71011.1	3	23670.4	11.1898	< .001
Hidrogel	1610.8	2	805.4	0.3808	0.684
Treatment	73.0	1	73.0	0.0345	0.853
Residuals	590183.9	279	2115.4		

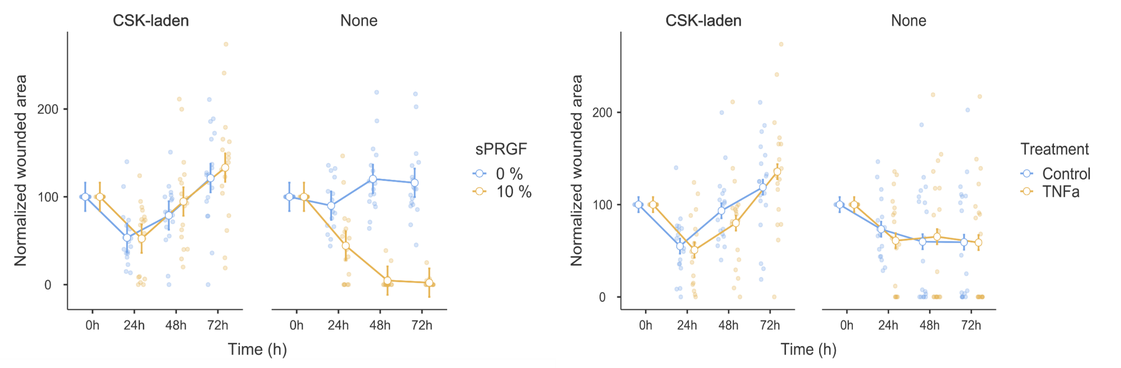


Figure 3. Wound-healing assays of LESCs indirectly exposed to different hydrogels (Col2, Col5, and GelMA). Experimental variables included: (i) presence or absence of CSKs within the hydrogels, (ii) supplementation with sPRGF, and (iii) in vitro inflammatory conditions.

In acellular hydrogels (None), sPRGF-derived growth factors can diffuse toward adjacent LESCs, promoting wound closure. In contrast, when hydrogels are loaded with CSKs, these cells consume sPRGF, reducing the availability of growth factors to LESCs at the bottom of the well and impairing wound healing. Under these conditions, neither residual growth factors nor CSK-derived paracrine signals reach sufficient levels to support wound closure. This effect is consistent across all hydrogel types tested and is independent of inflammatory treatment.

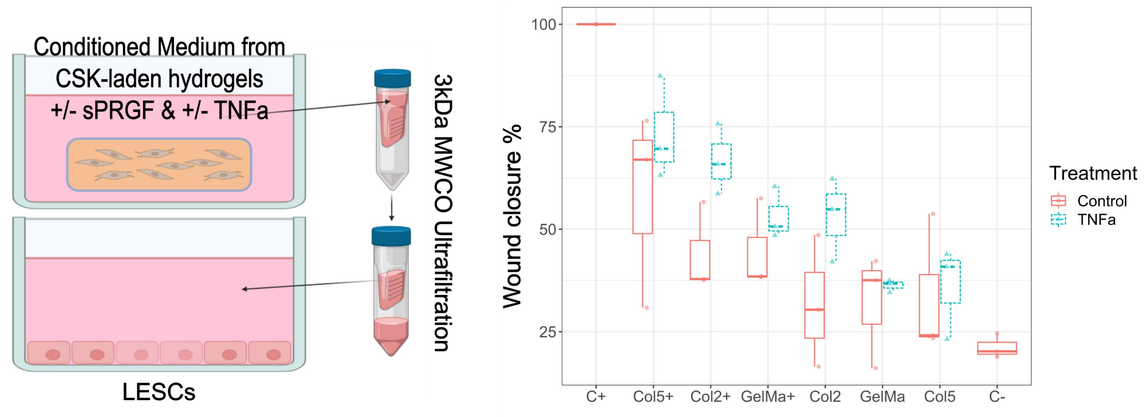


Figure 4. Wound-healing assays of LESCs exposed to concentrated conditioned medium from CSKs cultured in different hydrogels (Col2, Col5, and GelMA), with or without sPRGF supplementation, under normal or pro-inflammatory conditions (TNF-α).

In all cases, conditioned medium derived from CSKs cultured in sPRGF-supplemented hydrogels enhanced LESC wound closure. Notably, this effect is further amplified under TNF-α-induced inflammatory conditions, suggesting that sPRGF promotes an even greater wound-healing response in the presence of inflammation.

C+: Positive control (LESC growth medium); C−: Negative control (DMEM-F12). The "+" suffix indicates hydrogels supplemented with 10% sPRGF.

II) LESC adhesion to GelMA requires the presence of CSKs within the hydrogel. Activation of CSKs by sPRGF promotes LESC expansion.

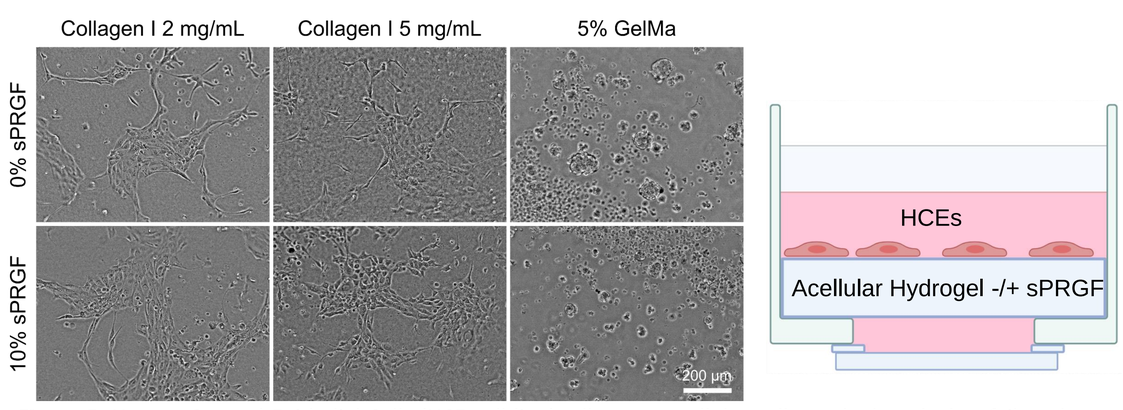


Figure 5. Human Corneal Epithelial Cells (HCE cell line) adhere to acellular collagen hydrogels, but fail to do so over GelMA, regardless of sPRGF supplementation.

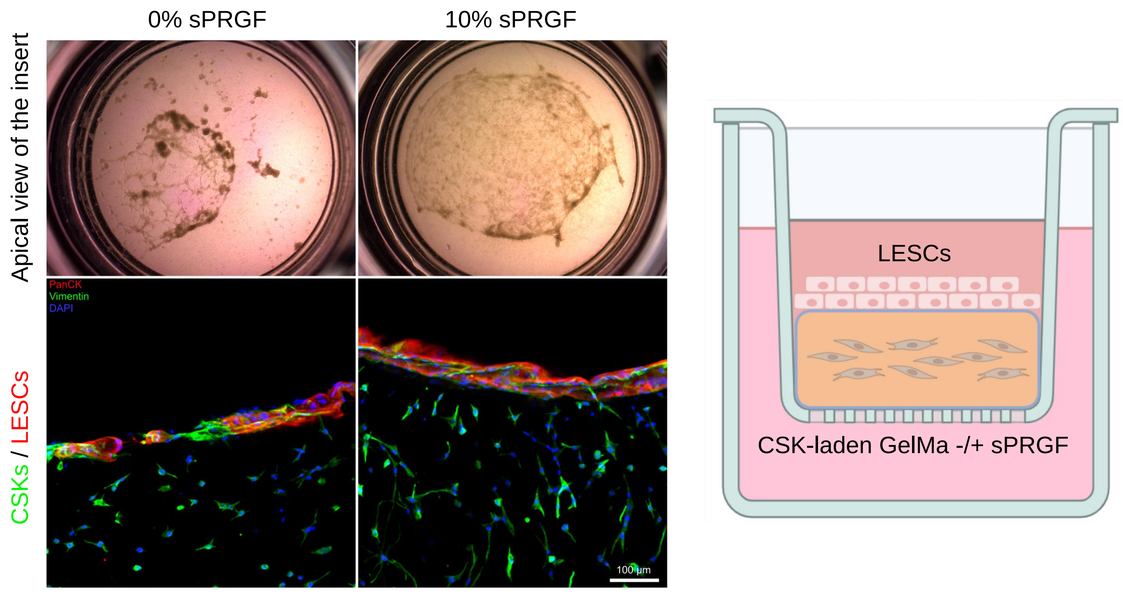


Figure 6. To adhere to GelMA, LESCs require the presence of CSKs within the hydrogel and close interaction at the apical region. Activation of the CSKs by adding sPRGF to the formulation promotes the expansion of the LESCs. After one week in culture, the differences are noticeable. Note that in the presence of sPRGF, the CSKs are polarized and the LESCs form a continuous epithelium.

CONCLUSIONS

1. Collagen- and methacrylated gelatin-based hydrogels sustain the availability of growth factors to surrounding cells.
2. sPRGF promotes LESC re-epithelialization and expansion, as well as CSK activation.
3. LESCs successfully adhere to methacrylated gelatin only when it is populated with CSKs, suggesting that close cell–cell interaction is essential.
4. sPRGF-mediated activation of CSKs further enhances LESC expansion.

Aknowledgements: This research study was supported by grants from the Department of Health of the Basque Government (2023111027, IT524-22) and the Spanish Ministry (PID2023-152436OB-100). Pérez-Garrastachu, M. was supported by a post-doctoral fellowship Margarita Salas.
Contact: Miguel Pérez-Garrastachu: miguel.perez@ehu.eus / Noelia Andollo: noelia.andollo@ehu.eus

TERMIS EU-Chapter 2026 PALMA DE MALLORCA 21-24 APRIL / SPAIN
BIOBIZKAIA EUSKAL OSASUN IKERKUNTZA INVESTIGACIÓN VASCA EN SALUD BASQUE HEALTH RESEARCH
eman ta zabal zazu Universidad del País Vasco Euskal Herriko Unibertsitatea
termis EUROPE