American Journal of Ophthalmology Case Reports 37 (2025) 102270

Contents lists available at ScienceDirect

American Journal of Ophthalmology Case Reports

journal homepage: www.ajocasereports.com/

Intrastromal autologous implantation of adipose derived adult stem cells for the management of established corneal scars

Jorge L. Alio del Barrio [a,b], Alberto Parafita-Fernandez [c,d], Daniel Gomez Plaza [a,e], Maria Eugenia Fernandez [f], Jorge L. Alio [a,b,*]

[a] Cornea, Cataract and Refractive Surgery Unit, Vissum (Grupo Miranza), Alicante, Spain
[b] Division of Ophthalmology, School of Medicine, Universidad Miguel Hernández, Alicante, Spain
[c] Ophthalmology Department, Complexo Hospitalario Universitario de Pontevedra, Pontevedra, Spain
[d] Clínica Oftalmológica Dr Parafita, Ribeira, A Coruña, Spain
[e] Instituto de Microcirugía Ocular (IMO), Barcelona, Spain
[f] Unidad de Terapia Celular. Hospital Gregorio Marañon, Madrid, Spain

ARTICLE INFO

Keywords:
Stem cells
Corneal transplant
Cornea
Cellular therapy
Keratoconus
Mesenchymal stem cells

ABSTRACT

Purpose: To describe a case of well-documented corneal stroma transparency improvement following the intrastromal implantation of autologous adipose-derived mesenchymal stem cells (ADASC) in a keratoconic patient with established stromal scars.

Observations: ADASC were isolated by elective liposuction, and a solution composed of 3x106 ADASC contained in 1mL saline was used to soak a corneal intrastromal pocket created with femtosecond laser at mid-depth. No signs of inflammation or rejection were observed. One year after surgery, we observed a complete restoration of the pre-existing corneal stroma scars, observed both clinically and by anterior segment OCT. The rest of the visual and topographic parameters did not show relevant changes except for the patient's refractive sphere. OCT showed a thin new layer of neocollagen deposited at the surgical plane. Total stroma optical density (OD) improved from 51.5 to 41.2 GSU, anterior stroma OD improved from 55.9 to 42.8 GSU, and posterior stroma OD improved from 46.9 to 39.6 GSU.

Conclusions and importance: This clinical case provides new clinical evidence supporting the use of intrastromal mesenchymal stem cell implantation to solve or alleviate established corneal scars.

1. Introduction

Preclinical studies have demonstrated the ability of stem cells (SC) to improve corneal transparency and thickness in animal models for corneal dystrophies.[1] The mechanism of action is not fully understood but is assumed to be related to the promotion and acceleration of the corneal stromal remodeling. The natural turnover of the collagen lamellae appears to be promoted either through the direct differentiation of the implanted stem cells into adult keratocytes, a host keratocyte activation by some paracrine effect from secreted extracellular vesicles, or a combination of both.[2,3]

In 2017, our group published the first clinical trial assessing the safety and efficacy of autologous adipose-derived adult stem cell (ADASC) implantation within the corneal stroma of patients with advanced keratoconus.[4] In that study, we described for the first time a case of a patient with documented corneal scarring improvement after autologous ADASC implantation within the corneal stroma. In a later study, we demonstrated that this approach induces a progressive improvement in the optical density of the anterior corneal stroma, observed for up to 36 months, a parameter that is directly linked to corneal transparency.[5]

In this report, we present the second clinical case of well-documented corneal stroma transparency improvement following the corneal intrastromal implantation of autologous ADASC.

2. Case report

We present a case of a 39-year-old female patient with bilateral keratoconus. Her right eye unaided distance visual acuity (UDVA) was 0.15 (decimal), and the corrected distance visual acuity (CDVA) was

* Corresponding author. Avda de Denia s/n, Vissum (Miranza Group), 03016, Alicante, Spain.
E-mail address: jlalio@vissum.com (J.L. Alio).

https://doi.org/10.1016/j.ajoc.2025.102270
Received 13 August 2024; Received in revised form 18 December 2024; Accepted 1 February 2025
Available online 3 February 2025

0.36. Best corrected vision with rigid contact lens was 0.6. Refraction of -4D of sphere and −6 at 55° of cylinder. Topography (MS-39, CSO) of her right eye showed the following parameters: SimK1 44.13D, SimK2 48.71D, corneal cylinder −4.58D, thinnest 474 μm, and Kmax 58.62D. Central and anterior corneal stroma haze was observed (Fig. 1 preop).

Autologous ADASC injection was recommended to improve corneal transparency. This case was performed in strict adherence to the tenets of the Declaration of Helsinki and in the context of a registered clinical trial (clinicalTrials.gov identifier: *NCT05279157(*www.clinicaltrials.gov. Patients gave informed written consent for the procedures.

ADASC were isolated by elective liposuction from her hips, following our previously published protocol.[4]A solution composed of 3×10^6 ADASC contained in 1mL saline was used to soak a corneal intrastromal pocket created with femtosecond laser (Visumax 500, Zeiss) at 200 μm and 9.2mm diameter under topical anesthesia.

Preoperative corneal transparency was restored after 24 hours, and we did not observe any intraoperative or postoperative complications during the observation period of 12 months. No signs of inflammation or rejection were observed. One year after surgery, we observed a complete restoration of the pre-existing corneal stroma scars (Fig. 1 postop), observed both clinically and by anterior segment OCT. However, the rest of visual and topographic parameters did not experience relevant changes, except for the refractive sphere: UDVA 0.15 (decimal), CDVA 0.34, best corrected vision with rigid contact lens of 0.6, refractive sphere of -1D, refractive cylinder of −6.5D at 55°, SimK1 44.31D, SimK2 49.2D, corneal cylinder −4.9D, and Kmax 59.5D. By corneal anterior segment OCT, a thin new layer of neocollagen was deposited within the surgical plane, as observed in our previous studies (Fig. 1 postop).[4] Corneal volume changed from 47.1mm^3 preoperatively to 47.7 at 1 month and 47.4 mm^3 at 3 months, remaining stable thereafter (differences up to one year postop of ±0.2 mm^3). A similar pattern was observed with the corneal thinnest point and the corneal stroma thinnest point, changing from 474/435μm (respectively at preop) to 475/433 at 1 month, and 480/440μm at 3 months, remaining stable thereafter (differences up to one year postop of ±3μm).

To objectively assess changes in corneal stroma transparency, optical density (OD) through the follow-up visits were studied, as in previous studies by our group.[6,7] We used ImageJ.JS (online applet, National Institutes of Health) for the analysis of the AS-OCT images obtained with MS-39 (CSO, Italy). The Region of Interest Manager (ROI Manager) tool was used to delineate the corneal stroma at the central 7mm area, excluding Bowman's and Descemet's layers (Fig. 2). The corneal stroma was divided into two halves: one anterior, which included the surgical plane where the pocket was created (as the opacities were mainly anterior and the stem cells were implanted), and the another posterior. where mainly healthy stroma was present. Next, the OD of the three areas (total and halves) were determined in gray scale units (GSU), automatically generated by the software, ranging from 0 (white) to 255 (black). Thus, OD serves as a surrogate for corneal backscatter. Total stroma OD improved from 51.5 to 41.2 GSU, anterior stroma OD improved from 55.9 to 42.8 GSU, and posterior stroma OD improved from 46.9 to 39.6 GSU.

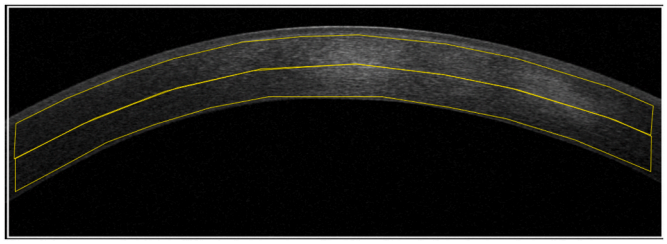

Fig. 2. Delineation and division of the corneal stroma in the central 7mm area, excluding Bowman's and Descemet's layers, to study the optical density of the corneal stroma. The anterior half includes the surgical plane.

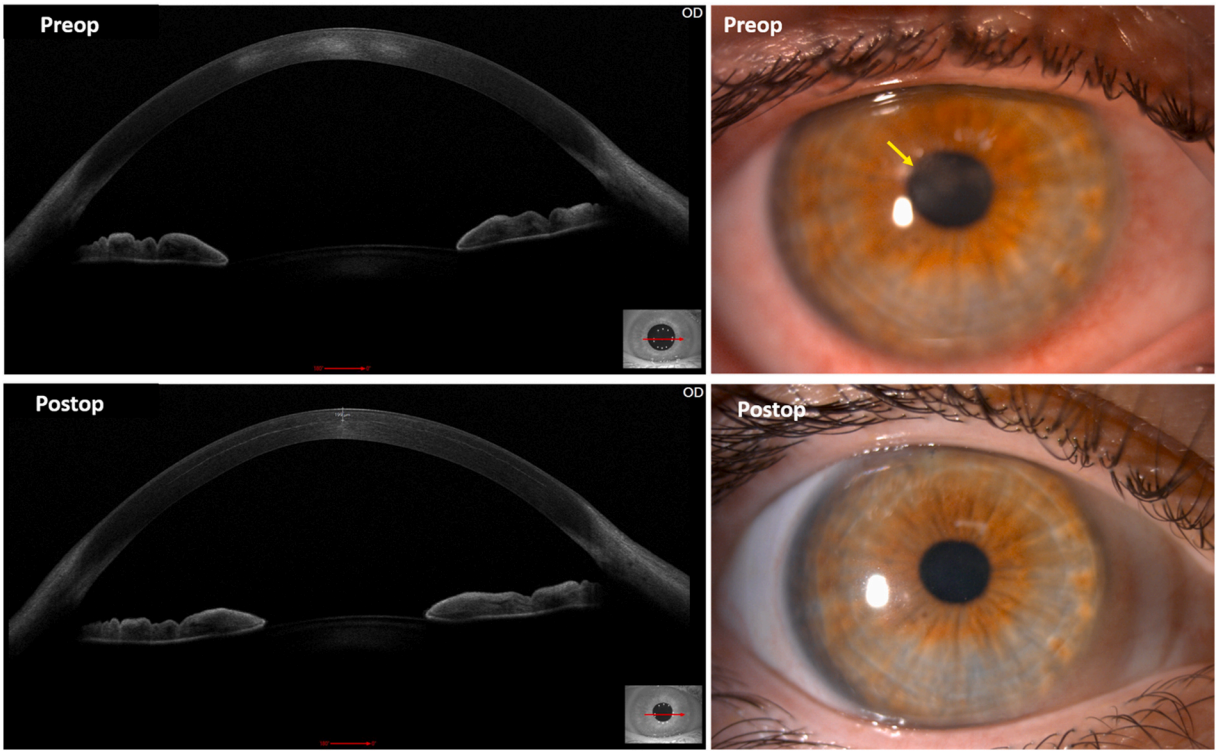

Fig. 1. Anterior segment OCT image (left) and slit lamp photograph (right) before and 6 month after autologous ADASC implantation within a corneal stroma femtopocket dissected at 200 μm. Please observe the new layer of neo-collagen deposited at the surgical plane seen by OCT imaging postoperatively. The arrow points the location of the preoperative corneal haze at the slit lamp photo.

3. Discussion

Cellular therapy of the corneal stroma has gained interest in recent years, since recent clinical evidence supports findings observed previously in animal models regarding the ability of mesenchymal stem cells to prevent or alleviate pre-existing corneal scars.[1,4] We previously demonstrated that the intrastromal implantation of these cells within the cornea of real patients induces a progressive improvement of the optical density of the corneal stroma.[5] We also demonstrated that this improvement coincides with a progressive increase in keratocyte density within the corneal stroma, as measured by confocal microscopy after the SCimplantation.[8] We hypothesize that mesenchymal SC implantation within the corneal stroma induces a keratocyte cellular density increase by the direct differentiation of the autologous SC into an adult keratocyte, and possibly also due to a SC paracrine activation of the host corneal stroma stem cells with keratocyte migration. This increase in keratocyte density seems to promote the corneal stroma remodeling, accelerating the natural turnover of the collagen fibres. This leads to an enhanced optical density and improved corneal stroma transparency.

Despite the large evidence from animal models, clinical evidence is still limited. Ramin et al. described a case of herpes keratitis scarring with CDVA of hand movements. Using the same approach as our previous studies (autologous ADASC injected into a corneal stroma femtopocket) they report a CDVA visual improvement to 20/60 at 6 months.[9] However, they did not provide corneal OCT or densitometry evidence to support their findings.

In the current article, we describe a second case with evidence of corneal scarring improvement after intrastromal autologous mesenchymal SC implantation demonstrated clinically (Fig. 1), by anterior segment OCT (Fig. 1) and by objective corneal stroma optical density measurement. However, we could not demonstrate a relevant objective visual improvement secondary to the corneal scarring resolution, likely because of underlying ectasia and subsequent corneal irregularity. Up to one year of follow-up, corneal ectasia demonstrated morphological stability. Finally, the potential impact of the SC-deposited layer of new collagen (consistently observed in all cases of intrastromal ADASC implantation in previous series)[4,10] in the natural progression of the ectatic disease remains unclear.

CRediT authorship contribution statement

Jorge L. Alio del Barrio: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Alberto Parafita-Fernandez:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis. **Daniel Gomez Plaza:** Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Maria Eugenia Fernandez:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Conceptualization. **Jorge L. Alio:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

4. Consent to publish

Patient Consent: Written consent to publish this case has not been obtained. This report does not contain any personal identifying information.

Funding

The study has been financed by the Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III (FIS 00127), Ministerio de Sanidad (Spain) FIS 17/00523 (Nº EudraCT 2018-000523-14) and has been registered as a clinical trial with the international number NCT05279157 (www.clinicaltrials.gov).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None.

References

1. Alió Del Barrio JL, Arnalich-Montiel F, De Miguel MP, El Zarif M, Alió JL. Corneal stroma regeneration: preclinical studies. *Exp Eye Res*. 2021;202, 108314. https://doi.org/10.1016/j.exer.2020.108314.
2. Arnalich-Montiel F, Pastor S, Blazquez-Martinez A, et al. Adipose-derived stem cells are a source for cell therapy of the corneal stroma. *Stem Cell*. 2008;26(2):570–579. https://doi.org/10.1634/stemcells.2007-0653.
3. Shojaati G, Khandaker I, Funderburgh ML, et al. Mesenchymal stem cells reduce corneal fibrosis and inflammation via extracellular vesicle-mediated delivery of miRNA. *Stem Cells Transl Med*. 2019;8(11):1192–1201. https://doi.org/10.1002/sctm.18-0297.
4. Alió Del Barrio JL, El Zarif M, de Miguel MP, et al. Cellular therapy with human autologous adipose-derived adult stem cells for advanced keratoconus. *Cornea*. 2017;36(8):952–960. https://doi.org/10.1097/ICO.0000000000001228.
5. El Zarif M, Alió Del Barrio JL, Mingo D, A Jawad K, Alió JL. Corneal stromal densitometry evolution in a clinical model of cellular therapy for advanced keratoconus. *Cornea*. 2023;42(3):332–343. https://doi.org/10.1097/ICO.0000000000003152.
6. Alio Del Barrio JL, Parafita-Fernandez A, Canto-Cerdan M, Alio JL, Teus M. Evolution of corneal thickness and optical density after laser in situ keratomileusis versus small incision lenticule extraction for myopia correction. *Br J Ophthalmol*. 2021;105(12):1656–1660. https://doi.org/10.1136/bjophthalmol-2020-316601.
7. Parafita-Fernandez A, Garcia-Gonzalez M, Gros-Otero J, Alvarez-Rementería Capelo L, Blázquez Sánchez V, Teus M. Evolution of visual acuity, flap thickness, and optical density after laser in situ keratomileusis performed with a femtosecond laser. *J Cataract Refract Surg*. 2020;46(2):260–266. https://doi.org/10.1097/j.jcrs.0000000000000008.
8. El Zarif M, A Jawad K, Alió Del Barrio JL, et al. Corneal stroma cell density evolution in keratoconus corneas following the implantation of adipose mesenchymal stem cells and corneal laminas: an in vivo confocal microscopy study. *Investig Ophthalmol Vis Sci*. 2020;61(4):22. https://doi.org/10.1167/iovs.61.4.22.
9. Ramin S, Abbasi A, Ahadi M, Rad LM, Tafti ZF. Restoration of corneal transparency in a patient with corneal scarring using mesenchyme stem cells: a case report. *Korean J Ophthalmol*. 2023;37(5):429–431. https://doi.org/10.3341/kjo.2023.0037.
10. El Zarif M, Alió JL, Alió Del Barrio JL, et al. Corneal stromal regeneration therapy for advanced keratoconus: long-term outcomes at 3 years. *Cornea*. 2021;40(6):741–754. https://doi.org/10.1097/ICO.0000000000002646.