Cellular Therapy With Human Autologous Adipose-Derived Adult Stem Cells for Advanced Keratoconus

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Purpose: The aim of this phase 1 study was to preliminarily evaluate the safety and efficacy of autologous adipose-derived adult stem cell (ADASC) implantation within the corneal stroma of patients with advanced keratoconus.

Methods: Five consecutive patients were selected. Autologous ADASCs were obtained by elective liposuction. ADASCs (3×10^6) contained in 1 mL saline were injected into the corneal stroma through a femtosecond-assisted 9.5-mm diameter lamellar pocket under topical anesthesia. Patients were reviewed at 1 day, 1 week, 1, 3, and 6 months postoperatively. Visual function, manifest refraction, slit-lamp biomicroscopy, intraocular pressure, endothelial cell density, corneal topography, corneal optical coherence tomography, and corneal confocal biomicroscopy were recorded.

Results: No intraoperative or postoperative complications were recorded, with full corneal transparency recovery within 24 hours. Four patients completed the full follow-up. All patients improved their visual function (mean: 1 line of unaided and spectacle-corrected distance vision and 2 lines of rigid contact lens distance vision). Manifest refraction and topographic keratometry remained stable. Corneal optical coherence tomography showed a mean improvement of 16.5 μm in the central corneal thickness, and new collagen production was observed as patchy hyperreflective areas at the level of the stromal pocket. Confocal biomicroscopy confirmed the survival of the implanted stem cells at the surgical plane. Intraocular pressure and endothelial cell density remained stable.

Conclusions: Cellular therapy of the human corneal stroma in vivo with autologous ADASCs appears to be safe. Stem cells survive in vivo with intrastromal new collagen production. Future studies with larger samples are required to confirm these preliminary results.

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Stroma constitutes more than 90% of the corneal thickness. Many features of the cornea, including its strength, morphology, and transparency, are attributable to the anatomy and biomechanical properties of the corneal stroma.[1] Many diseases such as corneal dystrophies, scars, or ectatic disorders induce distortion of its anatomy or physiology, leading to loss of transparency and subsequent loss of vision. Despite the great efforts in the last decade to try to replicate the corneal stroma in the laboratory to look for an alternative to classic corneal transplantation, this has not been accomplished yet because of the extreme difficulty to mimic the highly complex ultrastructure of the corneal stroma, obtaining substitutes that do not achieve enough transparency or strength properties.[2]

In this scenario, in the last few years, cellular therapy of the corneal stroma has gained much interest by the use of mesenchymal stem cells (MSCs) from either ocular or extraocular sources, capable to differentiate in to adult keratocytes in vitro and in vivo in animal models.[1] It has already been demonstrated by several authors, including reports from our research group,[3–5] the capability of these stem cells to not only survive and differentiate into adult human keratocytes in xenogeneic scenarios without inducing any inflammatory reaction but also to produce new collagen within the host stroma,[3,6] to modulate preexisting scars by corneal stroma remodeling[7,8] and to improve corneal transparency in animal models for corneal dystrophies by collagen reorganization and in animal models for metabolopathies by the catabolism of the accumulated proteins.[9–12] MSCs have also shown immunomodulatory properties in syngeneic, allogeneic, and even xenogeneic scenarios.[12,13]

Among all MSCs, human adipose-derived adult stem cells (ADASCs) have been demonstrated to be an ideal source of autologous stem cells because they satisfy all the requirements: easy accessibility to the tissue, high cell retrieval efficiency, and the ability of these stem cells (ADASCs) to differentiate in to multiple cell types (keratocytes, osteoblasts, chondroblasts, myoblasts, hepatocytes, neurons, etc).[1]

To the best of our knowledge, no in vivo corneal stroma cellular therapy studies have been published yet in humans. The aim of this phase 1 pilot study was to preliminarily evaluate the safety and tolerance of autologous ADASC

implantation within the corneal stroma of patients with advanced keratoconus, as well as to analyze the potential benefits of this corneal cellular therapy in such cases.

MATERIALS AND METHODS

Study Approval, Design, and Subjects

This is a prospective consecutive series of cases investigation based on the cooperation between the Research, Development, and Innovation Department of Vissum Instituto Oftalmologico de Alicante (Spain), Optica General (Saida, Lebanon), Laser Vision Center (Beirut, Lebanon), and REVIVA Research and Application Center (Middle East Hospital, Beirut, Lebanon). The Institutional Review Board Ethical committee of the Reviva Research and Application Center (Lebanese University, Beirut, Lebanon) prospectively approved this study. The study was conducted in strict adherence to the tenets of the Declaration of Helsinki, and it was registered in ClinicalTrials.gov (Identifier: NCT02932852). All patients provided written informed consent for all procedures described in this study.

All 5 consecutive patients who were included in the prospective study met the following inclusion criteria: advanced keratoconus defined as stage ≥ IV according to the RETICS keratoconus classification[14]; age ≥18 years; negative human immunodeficiency virus, hepatitis B, hepatitis C serology; and no history of malignancy.

Exclusion criteria were corrected distance visual acuity (CDVA) <0.1 in the contralateral eye; active concomitant inflammatory eye disease; other ophthalmic comorbidity such as cataract, retinal diseases, or glaucoma; previous ocular surgical interventions other than cataract; previous corneal hydrops or central corneal scars; history of cognitive impairments or dementia, which may affect the patient's ability to participate in the informed consent process and to appropriately complete evaluations; any immunodeficiency or immunosuppressive therapy; serologic evidence of infection with hepatitis B, hepatitis C, or HIV; and pregnancy or breast feeding. Keratoconus progressive status was not considered as an exclusion or inclusion criterion.

Isolation, Characterization, and Culture of Autologous ADASCs

Patients were subjected to standard liposuction after informed consent was obtained. All procedures were performed in good medical practice conditions. Approximately 250 mL of fat mixed with local anesthetic was obtained from each patient. Adipose tissue was processed according to methods described in previous articles.[15,16] Briefly, adipose tissue was washed in phosphate-buffered saline and digested in collagenase I for 40′ at 37°C in agitation. Then, collagenase was inhibited adding autologous human serum (extracted from each patient). Erythrocytes were lysed in erythrocyte lysis buffer (Gibco-Life Technologies), and then the pelleted cells were cultured in DMEM with Glutamax and Na-Pyr (Gibco), 10% autologous human serum, 1% P/S (Penicillin-Streptomycin, Gibco) + 0.2% amphotericin B (Gibco). Cell characterization was performed by $CD34^+CD45^-CD105^+$ labeling and flow cytometry analysis as requested by the International Federation of Adipose Therapeutics (IFATS).[17] Sixty to 80 hours before transplantation, quiescence was induced by lowering the amount of serum to 0.5% (Fig. 1A) to transplant the ADASC in a physiological status more close to the natural nonproliferative stromal keratocytes, as proliferative stem cells within the corneal stroma could potentially induce stromal scarring or haze. Quiescence and the absence of apoptosis and aneuploidy were verified by propidium iodide labeling (Invitrogen), and cell cycle analysis by flow cytometry as we have described in previous articles from our group.[3,5] Just before injection, cells were harvested by trypsinization (Sigma), and 3×10^6 cells were prepared per patient in saline (Fig. 1B). This high cellular concentration was established according to evidence observed in our previous experimental studies and considering the expected high cellular loss after transplantation due to solution leakage outside the cornea.[3–5]

Surgical Intervention: Autologous ADASC Implantation

Topical anesthesia with sedation was used for all surgeries. The 60-kHz IntraLase iFS femtosecond laser (AMO Inc, Irvine, CA) was used in the single-pass mode for recipient corneal lamellar dissection by creating an intrastromal lamellar cut of 9.5 mm in diameter at half depth of the preoperative thinnest pachymetry point measured by Visante anterior segment optical coherence tomography (OCT) (Carl Zeiss, Germany). The femtosecond laser–assisted corneal dissection ended with a 30-degree anterior side cut as a corneal incision. The femtosecond laser parameter settings, similar to the ones used for laser in situ keratomileusis (LASIK), are

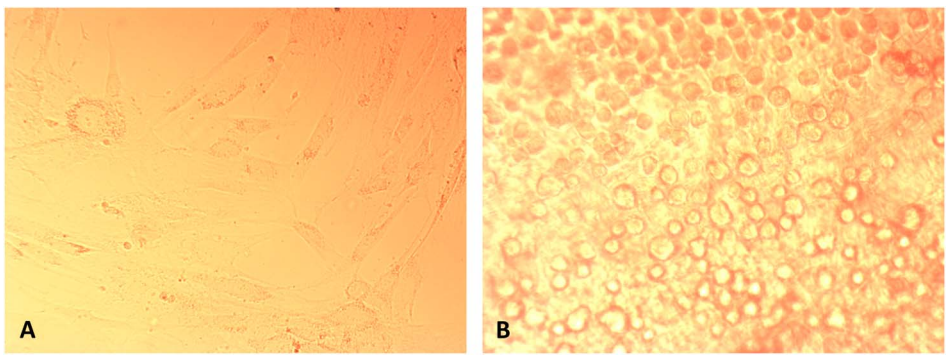

FIGURE 1. Microscopic appearance (phase-contrast photographs) of cells at 80% of confluence before trypsinization attached to the culture flask (A; ×10 magnification). Cells were then trypsinized, counted, and assessed for cell viability, and immunophenotyped before their transplantation (B; ×10 magnification).

TABLE 1. Laser Specifications for the Preparation of the Recipient Cornea

Parameter	Value	Parameter	Value
Lamellar Cut		Anterior Side Cut	
Diameter, mm	9.5	Diameter, mm	9.5
Depth, μm	Thinnest/2	Posterior depth, μm	Thinnest/2 + 10
Energy, μJ	1.50	Energy, μJ	1.7
Tang spot Sep, μm	5	Cut position, °	90
Rad spot Sep, μm	5	Cut angle, °	30
		Spot Sep, μm	3
		Layer Sep, μm	3

Rad, radial; Sep, separation; Tang, tangential.

summarized in Table 1. The corneal intrastromal pocket was then opened by blunt dissection using the Morlet lamellar dissector (Duckworth & Kent, England), and subsequently, 3 million autologous ADASCs contained in 1 mL were injected into the pocket through a 25-G cannula. Before the cellular injection, a 1-mm corneal paracentesis was performed to reduce the intraocular pressure (IOP) and allow a higher volume to be injected into the stromal pocket. Topical antibiotics and steroids (Tobradex, Alcon) were applied at the end of surgery. No patient received corneal sutures.

Postoperative Care and Follow-up Schedule

Topical antibiotics and steroids (Tobradex, Alcon) were applied every 6 hours for 1 week, followed by a descending dose of topical dexamethasone 0.1% (Maxidex, Alcon) for 3 more weeks.

Patients were reviewed at 1 day, 1 week, 1, 3, and 6 months postoperatively. All the following data were recorded at the preoperative assessment and first, third, and sixth postoperative months: unaided visual acuity (UVA), CDVA, rigid contact lens visual acuity (CLVA), manifest refraction, slit-lamp biomicroscopy, fundoscopy, IOP, endothelial cell count by specular microscopy (Nidek, Japan), corneal topography (Pentacam, Oculus Inc, Germany), anterior segment OCT-Visante (Carl Zeiss, Germany), and corneal confocal biomicroscopy with the Heidelberg Retinal Tomograph (HRT3) Rostock Cornea Module (HRT3; Heidelberg Engineering Inc, Germany).

RESULTS

The 5 consecutive patients had a mean age of 34.2 years (range: 30–42 years). The study sample comprised 3 men and 2 women as well as 3 right eyes and 2 left eyes. None of these eyes had received corneal collagen cross-linking or other ophthalmic interventions in the past. All surgeries were performed as previously described without any intraoperative complication. Four patients completed the full follow-up (6 months). One patient was lost to follow-up after the first postoperative month because of inability to attend further follow-up visits (motivations not related to the study), so this patient was excluded from the subsequent analysis. No complication had been recorded before this exclusion, and information obtained from the patient directly indicated no subjective negative findings and no complications as described by other specialized ophthalmic professionals. Results are summarized in Table 2.

Visual Acuity

All patients improved their visual function regarding the UVA, CDVA, and CLVA (Figs. 2A–C). UVA and CDVA showed their peak at the first postoperative month (with 2 lines of mean improvement), followed by mild regression up to the sixth month, with an overall improvement of more than 1 line compared with the preoperative month. Best visual acuity with rigid contact lenses (CLVA) showed an important and progressive improvement up to the sixth month, with a total mean improvement of more than 2 lines.

Manifest Refraction

The refractive sphere improved in 2 patients (1 and 2) and remained stable in the rest (patients 3 and 4), showing an overall mean improvement of 0.5 D at the end of the

TABLE 2. Visual, Refractive, Keratometric, and Pachymetric Outcomes (Mean Parameters and Range)

	Preoperative	1 mo	3 mo	6 mo
UVA (decimal)	0.1 (0.05 to 0.2)	0.3 (0.1 to 0.4)	0.295 (0.15 to 0.365)	0.233 (0.1 to 0.333)
CDVA (decimal)	0.325 (0.2 to 0.4)	0.525 (0.2 to 0.8)	0.487 (0.2 to 0.75)	0.481 (0.4 to 0.625)
CLVA (decimal)	0.512 (0.4 to 0.6)	0.65 (0.5 to 0.8)	0.737 (0.5 to 0.95)	0.762 (0.5 to 0.875)
Rx Sphr, D	−4.06 (−0.5 to −7)	−3.69 (−0.75 to −7)	−3.812 (−0.75 to −8)	−3.562 (−0.75 to −6.5)
Rx Cyl, D	−2.937 (−2.25 to −3.5)	−3.562 (−2.25 to −4.5)	−3.19 (−2.25 to −4)	−3.25 (−2.5 to −4)
Anterior Km, D	55.95 (47.9 to 64.9)	56.95 (50.2 to 65.6)	56.1 (49.6 to 63.4)	56.82 (50.8 to 65.4)
Posterior Km, D	−8.3 (−6.6 to −9.7)	−8.55 (−7.10 to −10.2)	−8.37 (−6.8 to −9.8)	−8.52 (−7 to −10.2)
Kmax, D	66.3 (56.7 to 79.4)	68.8 (63.2 to 81.3)	68.325 (62.1 to 82.1)	67.95 (61.8 to 81.2)
Topo Cyl, D	−2.95 (−0.4 to −5.8)	−3.47 (−1.5 to −5.8)	−3.37 (−1.2 to −5.3)	−3.1 (−0.7 to −5.7)
CCT, μm	463 (438 to 503)	456 (435 to 484)	465 (439 to 509)	460 (434 to 512)
Thinnest point, μm	405.75 (394 to 432)	407.75 (406 to 438)	411 (378 to 446)	404.5 (364 to 449)
Cornea Vol, mm^3	54.7 (52.9 to 58.1)	54.75 (42.8 to 58.9)	55.02 (53 to 57.8)	55.25 (52.2 to 59)
Visante CCT, μm	429.5 (407 to 464)	428.75 (406 to 468)	439.75 (421 to 478)	446 (419 to 481)

Cornea Vol, cornea volume; Km, mean keratometry; Kmax, maximum keratometry; Rx Cyl, refractive cylinder; Rx Sphr, refractive sphere; Topo Cyl, topographic cylinder.

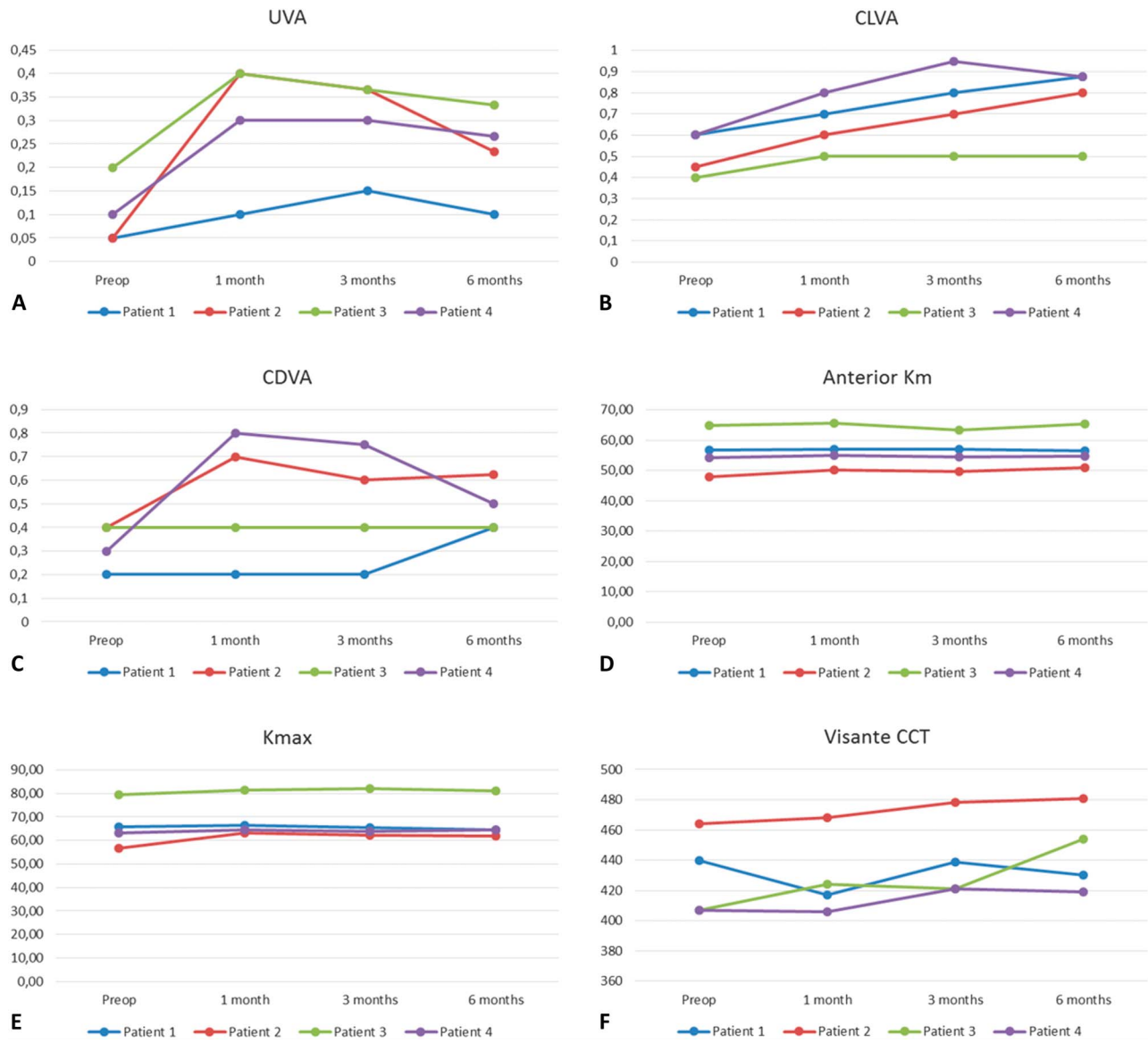

FIGURE 2. Visual (A-C), keratometric (D-E) and pachymetric (F) results along the full follow-up. Unaided visual acuity (UVA), corrected distance visual acuity (CDVA), rigid contact lens visual acuity (CLVA), mean keratometry (Km), maximum keratometry (Kmax), central corneal thickness (CCT).

follow-up (Table 2). The refractive cylinder showed mild deterioration in 3 of the 4 patients (1, 3, and 4), with an overall mean worsening of 0.3 D at the end of follow-up.

Slit-Lamp Biomicroscopy

All corneas were, preoperatively, free of posterior stromal or predescemetic scars and presented a clear visual axis. Only patient 2 presented a couple of paracentral dense anterior stromal scars (Fig. 3A). None of the patients developed any sign of inflammation or rejection during the follow-up, and corneal transparency was fully recovered within 24 hours after the surgical procedure and kept during the whole follow-up period (Fig. 3B). After the third postoperative month, we could observe progressive improvement of the preoperative corneal scars seen in patient 2, confirming this finding at the end of follow-up (Figs. 3B, C).

Corneal Topography

All keratometric parameters presented relative stability without differences over 1 D (Table 2 and Fig. 4). Anterior and posterior mean keratometry remained stable but in patient 2 who presented a deterioration of anterior mean keratometry of 2.9 D (Fig. 2D). A deterioration over 1 D in maximum keratometry was observed in 2 of the 4 patients (2 and 3), with a mean overall deterioration of 1.65 D at the sixth month (Fig. 2E). Regarding the topographic anterior cylinder, patients 1 and 4 remained stable, patient 2 showed significant improvement (from −2 D preoperatively to −0.7 D at the

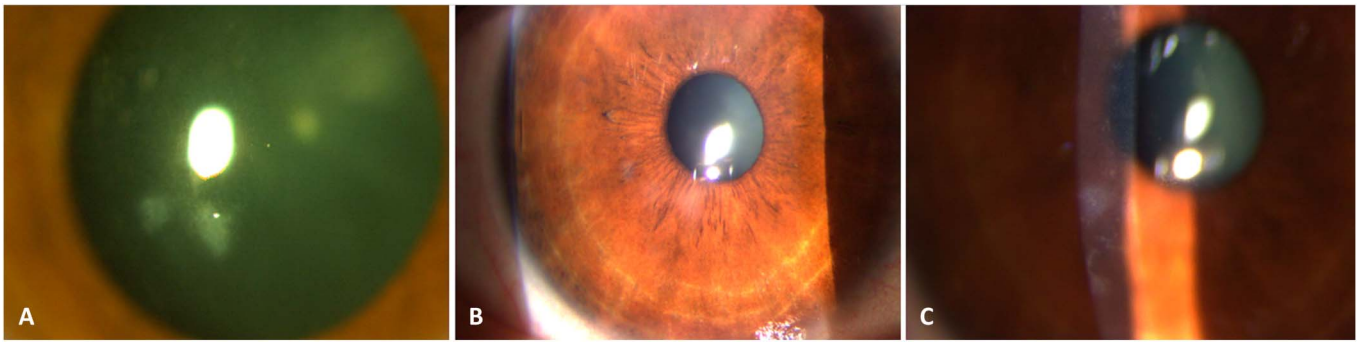

FIGURE 3. Slit-lamp images from patient 2, preoperatively (A) and at the sixth postoperative month (B and C). Observe the postoperative improvement in the density and severity of the inferior paracentral anterior stromal scars.

sixth month), and patient 3, significant worsening (from −0.4 D preoperatively to −2.3 D at the sixth month).

The pachymetric values measured by the Pentacam remained stable, without detecting any obvious enhancement in the corneal thickness parameters. The central corneal thickness (CCT) remained stable in all but patient 4 (from 449 μm preoperatively to 434 μm at the sixth month) and patient 2 (from 503 μm preoperatively to 512 μm at the sixth month). The thinnest point improved or remained stable in all but patient 3 (from 394 μm preoperatively to 364 μm at the sixth month). The corneal volume improved in all but patient 4 (from 52.9 to 52.2 mm^3).

Anterior Segment OCT

Mild improvement in the CCT measured by anterior segment OCT (Visante) was observed in 3 of the 4 patients (but patient 1), with a mean increase of 16.5 μm at the sixth

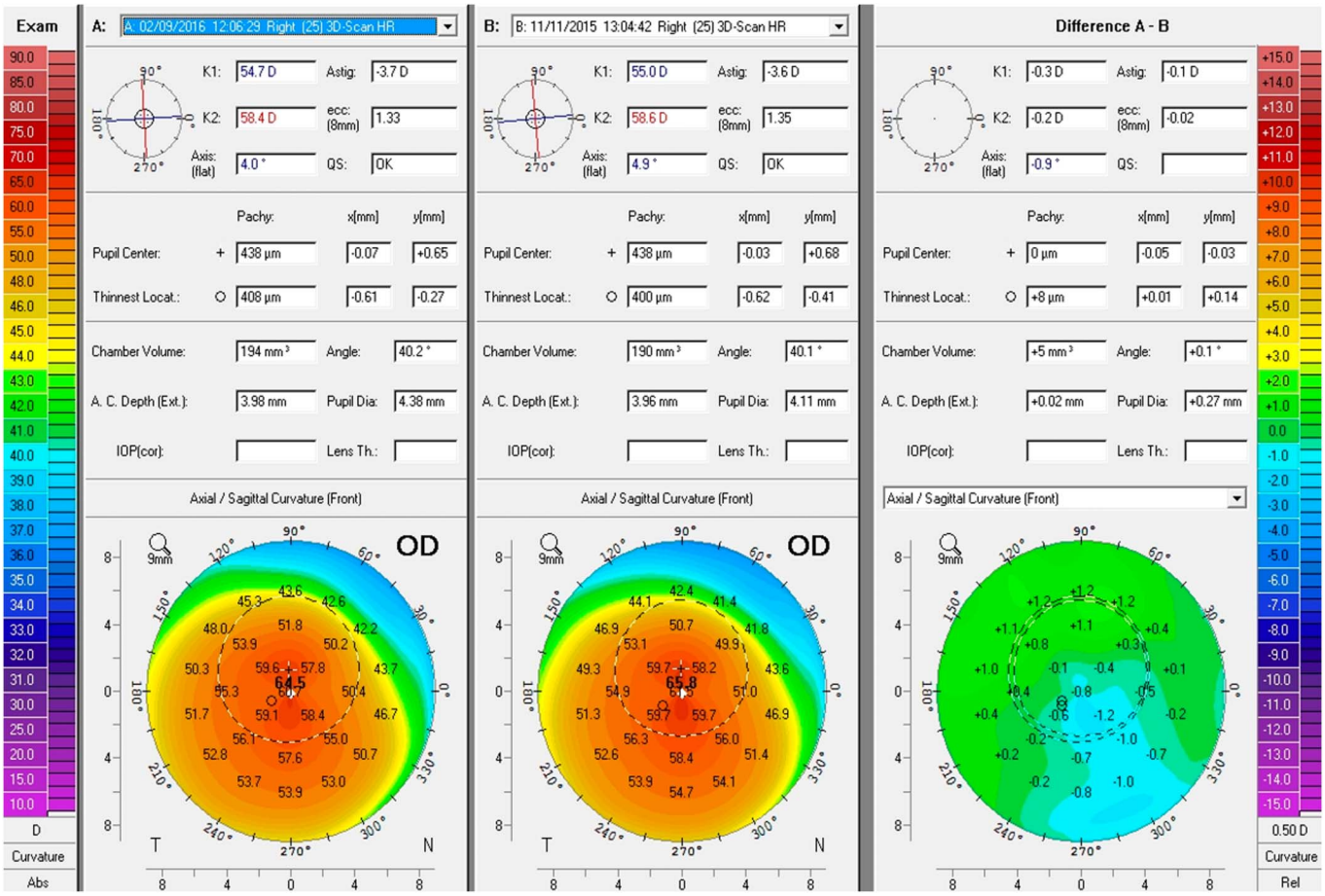

FIGURE 4. Corneal topography (Pentacam) comparison between preoperative and 6 months after surgery in patient 1. Observe the stability of the keratometric parameters.

month. This improvement in the thickness occurred after the first month (Fig. 2F).

After the third postoperative month, we also observed in 3 of the 4 patients' (1, 2, and 4) patchy hyperreflective areas at the level of the stromal pocket compatible with areas of new collagen production. These areas were not homogeneously distributed along the stroma, appearing as isolated islands (Fig. 5).

Confocal Biomicroscopy

Up to the third postoperative month, round cells were observed in the surgical plane in all cases (Fig. 6B). These cells showed a different morphology than the usual dendritic or fusiform shape presented by the anterior and posterior stromal keratocytes (Fig. 6A). At the sixth month, these cells at the surgical level had already a fusiform shape and were not different from those observed in other stromal planes. This rounded cellular morphology was considered a landmark for cellular survival during the early postoperative period.

Other Parameters

No changes in IOP were detected. Endothelial cell density measured by specular microscopy remained stable, without evidence of endothelial cell damage (mean values of 2612 preoperatively and 2821 cells/mm^2 at the sixth month postoperatively).

DISCUSSION

Nowadays, corneal collagen cross-linking has radically changed the long-term prognosis of keratoconus, although many cases are still diagnosed in an advanced state in which classic corneal transplantation (either by full-thickness or lamellar techniques) is the only treatment option. The aim of our group was to find an alternative therapy to classic corneal transplantation to regenerate the corneal stroma, thus avoiding the important intraoperative and postoperative risks associated with those techniques.[18] Cellular therapy of the corneal stroma by the use of stem cells has gained relevant scientific interest in the last few years. All MSCs seem to have similar behavior in vivo, being able to achieve keratocyte differentiation and modulate the corneal stroma with also immunomodulatory properties.[1] Corneal stromal stem cells (CSSCs) may have enhanced functions, as they are already corneal cells with more directed differentiation potential. However, the number of CSSCs that can be obtained from human corneas is quite limited and technically very demanding, with an inefficient cell subculture and impossible to obtain without damaging the donor cornea. All these major drawbacks have significantly limited their use for clinical practice and preclude their autologous application, so an extraocular source of cells that could replace CSSCs is necessary to solve all these limitations. Human adult adipose tissue has been demonstrated to be an ideal source of autologous stem cells, as it satisfies all the requirements: easy accessibility to the tissue, high cell retrieval efficiency, and ability of its stem cells (ADASCs) to differentiate into multiple cell types.[3] Bone marrow MSCs have the same profile as ADASCs, but their extraction by bone marrow puncture is a more complicated and painful procedure that requires general anesthesia. Umbilical MSCs present an attractive alternative, but their autologous use would be expensive and currently impossible. Embryonic stem cells also present important ethical issues. A new and exciting possibility is offered by the induced pluripotent stem cells, obtained from adult cells, as recent studies[19,20] have demonstrated their ability to differentiate into corneal keratocytes with similar immunomodulatory properties as MSCs.

To date, many studies in animal models for different corneal abnormalities have demonstrated the possible benefits of cellular therapy to improve corneal transparency by their ability to reorganize the stromal collagen lamellas and new collagen production.[1–13] However, to the best of our knowledge, no in vivo data have been published yet in humans studying the safety and efficacy of such therapies. With this purpose, we selected patients with advanced keratoconus as candidates for classic corneal transplantation because of unsatisfactory visual function and contact lens intolerance because, in case of failure, standard treatment for such patients could still be performed. In such cases, disease progression is hard to evaluate because of the lack of reliability from manifest refraction and corneal topography, also it loses relevance, as corneal collagen cross-linking is not an alternative and corneal transplantation is usually the only treatment option. Thus, keratoconus progression was not analyzed in our study.

Stem cell delivery to the corneal stroma was performed through a femtosecond laser–assisted lamellar pocket at half depth. Although we were dealing with advanced keratoconic corneas with severe thinning, we did

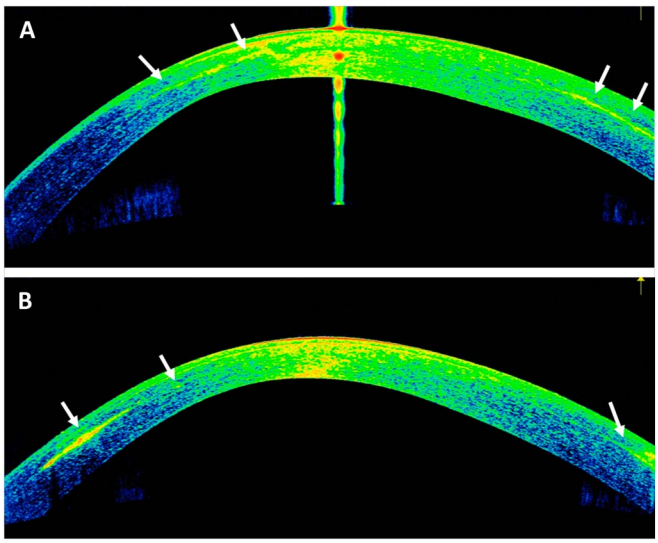

FIGURE 5. Corneal OCT images from patients 2 (A) and 4 (B) at the sixth postoperative month. Observe the patchy hyperreflective areas (white arrows) at the level of the stromal pocket compatible with areas of new collagen production. These areas were not homogeneously distributed along the stromal surface.

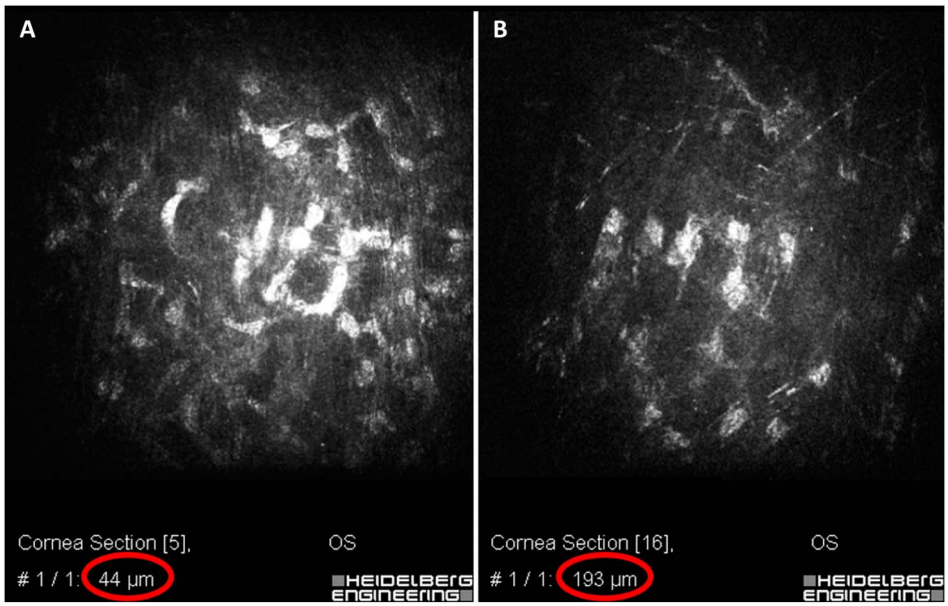

FIGURE 6. Corneal confocal biomicroscopy images from patient 4 at the third postoperative month. Cellular presence is confirmed at the level of the surgical plane with cells showing a more round shape (B) compared with the usual fusiform shape presented by the corneal keratocytes in the stroma anterior (A) and posterior to the surgical plane.

not observe intraoperative complications such as corneal tears. Nevertheless, to be able to safely perform this technique, a minimal thinnest pachymetric point of 250 µm is probably required (that thickness would leave an anterior cap close to a LASIK flap). Some concerns may arise about the possible weakening effect of this corneal dissection on an already severely weakened and pathologic cornea. However, according to John Marshall's important findings, vertical side cuts through corneal lamellae rather than horizontal delamination incisions contribute to loss of structural integrity during LASIK flap creation.[21] In our study, only a 30-degree anterior side cut was performed; so considering these findings, the weakening effect of this dissection should be marginal. However, it would be interesting to demonstrate John Marshall's data again in the keratoconic cornea, but as previously discussed, progression analysis in our advanced ectatic corneas was not performed because a follow-up period of 6 months is not enough to establish conclusions in this regard, and also, this analysis would not be reliable in such corneas because of the lack of repeatability of topography and refraction.[22]

Three million autologous ADASCs (contained in 1 mL) were prepared for injection into the stromal pocket. This high cellular concentration was decided as high cellular loss was expected during delivery because of solution leakage outside the cornea. Before the injection, we performed a corneal paracentesis to reduce the IOP and allow more volume within the pocket, although no more than 10% to 30% of the injected volume was expected to remain within the stroma. Further studies are necessary to assess precisely the real amount of cells that remain immediately after transplantation.

Visual function improved in all patients. This benefit was modest but observed in the 4 study patients, with a pattern in which this improvement was mostly observed within the first month followed by mild subsequent regression. This early postoperative finding should be theoretically attributed to the surgical procedure itself and not to the presence of stem cells, because they have not differentiated yet into adult keratocytes (considering their lack of the usual dendritic shape at confocal biomicroscopy and the absence of new collagen production on corneal OCT at the first month visit). However, we could not observe early changes in manifest refraction or topographic keratometry that may explain this early visual improvement.

Keratometric and pachymetric data obtained by corneal topography (Pentacam) showed overall stability (Fig. 4), with a possible progression of ectasia in patient 3. This patient presented the most advanced cone, so cellular therapy may not be capable to halt the progression of ectasia in such cases. Nevertheless, we should take into account the low reliability of the data measured by conventional scanning-slit corneal topographers in advanced keratoconus due to eye fixation errors and light scattering from corneal haze and opacity.[22] Some studies suggest superiority of anterior segment OCT over conventional topographers in advanced keratoconic eyes, as its scanning beam penetrates opaque tissues more deeply, providing clearer images of cloudy corneas allowing for better and more reliable preoperative assessment.[23] Pachymetry measurement by anterior segment OCT in our study demonstrated a mild but real improvement of the CCT (mean: 16.5 µm at the sixth month), correlated with the presence of patchy areas of new collagen production in the majority of the patients. This new collagen production appears in low amount and not homogeneously distributed along the surgical plane, thus this procedure is not able to restore large amounts of tissue if that is the purpose as in corneal thinning diseases. As previously observed in animal models, the addition of hyaluronic acid–derived scaffolds or acellular corneal stroma may assist to achieve this goal.[5,6]

We did not observe any intraoperative or postoperative complication, the surgery being well tolerated in all cases with complete restoration of corneal transparency within 24

hours. We could also observe significant clinical improvement of corneal opacity in patient 2 (Fig. 3), as has been suggested in previous studies in animal models.[7] However, larger case series with corneal opacity are required to demonstrate this finding.

Within experimental studies, it is easy to demonstrate, by postmortem analysis, the survival of the previously transplanted marked cells (through immunofluorescence analysis) and their differentiation into adult keratocytes (by detecting keratocan expression).[3,5] However, in vivo human studies have the important drawback of being limited to confocal biomicroscopy findings (vulnerable to subjective interpretations) to observe the behavior of the transplanted cells in vivo. A perfect patient collaboration is not always possible in such a diagnostic technique, which reduces the accuracy of the depth measurement at the evaluated plane. Taking into account all these limitations, we could observe the presence of round cells at the surgical plane in all patients compatible with the transplanted ADASCs, later showing a progressive dendritic shape change until the point of being not possible to be differentiated from normal keratocytes at the end of the follow-up.

Nevertheless, the conclusions of this pilot study should be considered carefully, as they are vulnerable to bias because the study included a small sample, was not masked, and was not a controlled study. So future studies with a larger sample and longer follow-up should confirm the preliminary results observed here. Other studies should follow as well to demonstrate the role of this therapy in other nonectatic corneal dystrophies with progressive opacification of the corneal stroma due to deposit diseases, confirming the preliminary data observed in animal models.[9,11] The use of heterologous instead of autologous ADASCs would be interesting since a recent study observed gene expression differences between the induced pluripotent stem cell–derived keratocytes generated from fibroblasts of both keratoconic and normal human corneal stroma, influencing cellular growth and proliferation.[24] In this scenario, immune rejection is feasible, although the demonstrated immunomodulatory properties of these stem cells even in xenogeneic scenarios may avoid chronic steroid use and may enhance the clinical and anatomical results. Finally, it would still be interesting to evaluate the possible benefits of this therapy in mild or moderate progressive keratoconus, studying its impact on the natural course of the disease.

As a conclusion, with this phase 1 study, we show preliminarily the apparent safety of corneal stromal transplantation of autologous ADASCs in humans, their survival in vivo, and their ability to produce a low amount of new collagen. This study includes a small sample of patients, so future studies with larger samples are required to finally confirm the safety and efficacy of the procedure and to clarify the relevance of the observed findings.

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