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Air-guided manual deep anterior lamellar keratoplasty: Long-term results and confocal microscopic findings

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PURPOSE. *To evaluate the long-term results of air-guided manual deep anterior lamellar keratoplasty (DALK) and to perform confocal microscopy on postoperative DALK corneas.*
METHODS. *Seven postoperative consecutive DALK corneas were evaluated 1 year after suture removal. All patients underwent a complete ophthalmologic examination evaluating visual acuity, astigmatism, corneal thickness, and endothelial cell count. Confocal microscopy was performed to examine the corneas of the seven eyes and to obtain the measured interface depth.*
RESULTS. *Eighteen months after surgery, the mean postoperative uncorrected visual acuity was 20/38 and the mean best-corrected visual acuity was 20/23. Postoperative mean value of residual recipient stroma thickness was 65.57 μm ± 28.74.*
CONCLUSIONS. *Maximum depth DALK can lead to significant advantages for quality of vision when compared to other types of anterior lamellar keratoplasty. Still, it remains a challenging procedure. These results show that a deep dissection without baring Descemet membrane makes good visual results possible, preventing corneal perforation and conversion to penetrating graft. (Eur J Ophthalmol 2007; 17: 897-903)*

KEY WORDS. *Keratoconus, Deep anterior lamellar keratoplasty, In vivo confocal microscopy*

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INTRODUCTION

In the past few years, surgeons have expressed great interest in deep anterior lamellar keratoplasty (DALK) as it does not lead to endothelial graft rejection.
A variety of surgical techniques (1-12) for DALK have been proposed. At present, the most important challenge in this type of surgery is the development of a repeatable technique capable of facilitating the deep dissection, improving optical outcomes, minimizing the operation time, and reducing punctures of the Descemet membrane.
Air-guided manual deep lamellar keratoplasty (13, 14) – a modified technique of the original experimental one proposed by Melles et al in 1999 (7) – is relatively easy to perform from a technical point of view. It minimizes the risk of inadvertent puncture of the Descemet membrane and increases the ability to obtain a clear interface, with a good postoperative visual acuity.
The most difficult step in DALK is the removal of deep stromal layers. This is due to the fact that the stromal dissection depth relative to the corneal thickness cannot be optically visualized. Air-guided DALK allows a deep dissection of the corneal stroma with a clinically clear interface, but does not guarantee the strip of the Descemet membrane. In vivo microscopic evaluation of the corneal structure has always been a challenge for ophthalmic clinicians and researchers. In the late 1980s, technological advances led to the development of powerful clinical confocal microscopes that access the living human eye anterior structures in situ at the cellular level. Coronal sections of the in situ corneal epithelium, Bowman membrane, stroma, and endothelium can be visualized at a

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resolution of 1 µm with in vivo confocal microscopy (15). This resolution is sufficient for the visualization and detection of corneal cells; however, internal cellular structures cannot be visually accessed.
In this study we present a case series of patients who underwent an air-guided manual deep lamellar keratoplasty, studied by in vivo confocal microscopy.

MATERIALS AND METHODS

This study comprised seven consecutive patients (four male, three female), referred to the Department of Ophthalmology and Neurosurgery of the University of Siena. The mean age of the patients was 35.4 (range 27–52). All patients had keratoconus of moderate degree (stages I and II in the Krumeich and Daniel clinical staging) (4). All patients were intolerant to contact lenses and had no important corneal scarring. All patients had a corneal thickness greater than 400 µm. All patients underwent a deep lamellar keratoplasty without complications with manual dissection from a limbal side port after an air bubble injection in the anterior chamber according to the Caporossi technique (13). A central trephination was performed with 8.0 mm Hessburg-Barron disposable vacuum trephine. The Hessburg-Barron trephine was set to leave 5% to 10% of residual stromal thickness at the thinnest part of the cornea. A button of tissue was removed with a Golf knife on a cleavage plane corresponding to approximately 60% to 70% of the depth of the incision, with the aim of facilitating the subsequent surgical steps. Following the Melles technique, a side port was created by a 15° knife through which air was introduced into the anterior chamber with a 30 g needle. With a 450-µm calibrated diamond knife a nonpenetrating incision was carried out in the corneal limbus through which a bevelled spatula was gradually inserted into the deep stromal layers until the distance between the edge of the spatula and the air reflex in the anterior chamber was reduced and a new deeper cleavage plane was obtained (Fig. 1). The thin smooth spatula was inserted in the deep stromal pocket, which was moved tangentially toward the center of the cornea. At this point it was necessary to be sure that the new cleavage plane passed below the trephine incision, otherwise the deep dissection needed to be repeated.
The following investigations were performed after 18 postoperative months (12 months after the suture removal): in vivo confocal microscopy, assessment of the cornea, visual acuity, corneal thickness by ultrasound pachymetry, endothelial cell count, and topographic astigmatism (EyeTop, CSO, Italy).
In vivo confocal microscopy of the cornea was evaluated using an HRT II Cornea, a confocal laser microscope which combines HRT II laser scanning technology and the Rostock Cornea Module (Heidelberg Engineering, Germany). The following parameters were evaluated by confocal microscopy: cell density, morphology and activation, presence of reflecting inclusions, presence of inflammatory cells, presence of tissue folds, nerve regeneration, and mean thickness of the residual recipient stroma.

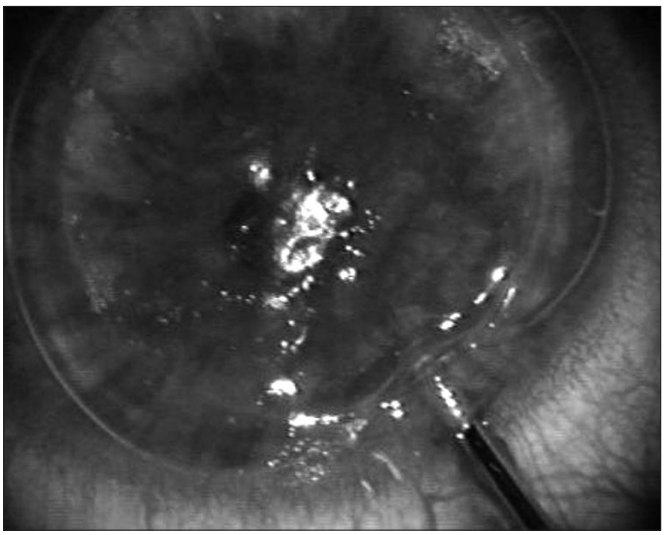

Fig. 1 - *Deep stromal layers dissection with a smooth spatula.*

RESULTS

Eighteen months after surgery, the mean postoperative uncorrected visual acuity was 20/38 and the mean best-corrected visual acuity was 20/23.
The mean preoperative endothelial cell count was 2446/mm^2 (SD 69).
Specular microscopy 1 year after the suture removal revealed an average endothelial cell count of 2003/mm^2 (SD 117). The 18 postoperative months cell loss was 18.12% (p=0.007, unpaired *t* test).
The mean sim-K average topographic corneal curvature value was 45.49 diopters (SD 2.28).
The mean topographic astigmatism was 3.11 diopters (SD 2.32).
Confocal microscopy examination of the lamellar donor

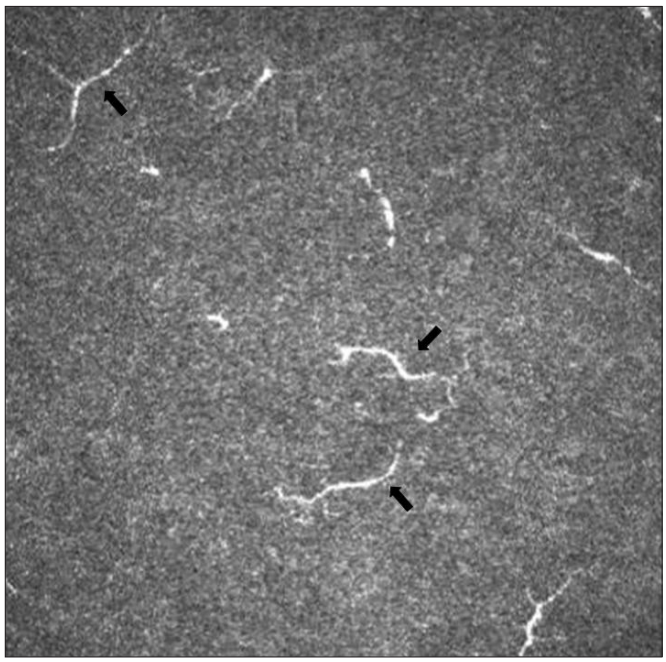

Fig. 2 - *In vivo confocal microscopy of the donor subepithelium. Note the nerve fibers (arrows) of the subepithelial plexus.*

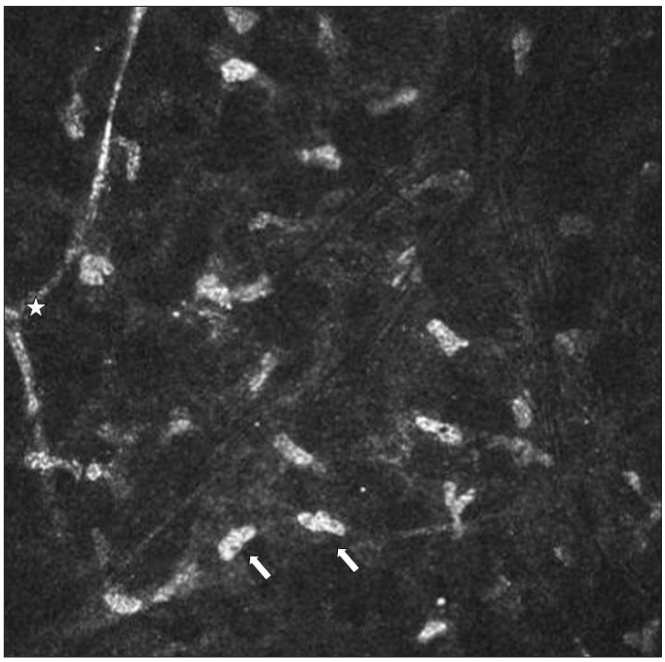

Fig. 3 - *In vivo confocal microscopy of the donor stroma. Note the keratocytes (arrows) and the nerve fibers (asterisk).*

buttons showed normal superficial and intermediate epithelium layers.

In the Bowman layer region important alterations were not evident. In addition, the subepithelial nerve plexus was detectable (Fig. 2). Only in one case (Case 3) the regeneration of subepithelial nerve plexus had remained at almost the initial stage.

Anterior stroma showed the presence of keratocytes (Fig. 3) and corneal nerves had regenerated (Fig. 4). Keratocytes density was slightly reduced and presented an irregular distribution compared to normal cornea.

Deep stromal interface is easily visualized using confocal microscopy as highly reflective particles (microdots) (Fig. 5, A–D) appear to be deposited at the interface of the button. In this region the examination detects linear hyporeflective microfolds (Fig. 5C).

The deep recipient stroma presents a normal distribution and morphology of the keratocytes (Fig. 6, A and B). In this region the linear hyporeflective microfolds are also evident.

Endothelium analysis shows a good cell density but a slight pleomorphism if compared to normal corneas (Fig. 6, C and D).

Postoperative mean value of the thickness of the residual recipient bed was 65.57 µm ± 28.74.

Table I shows postoperative uncorrected and best-corrected visual acuity, topographic sim-k readings, ultrasound and optic pachymetry, endothelial cell count, and confocal interface depth.

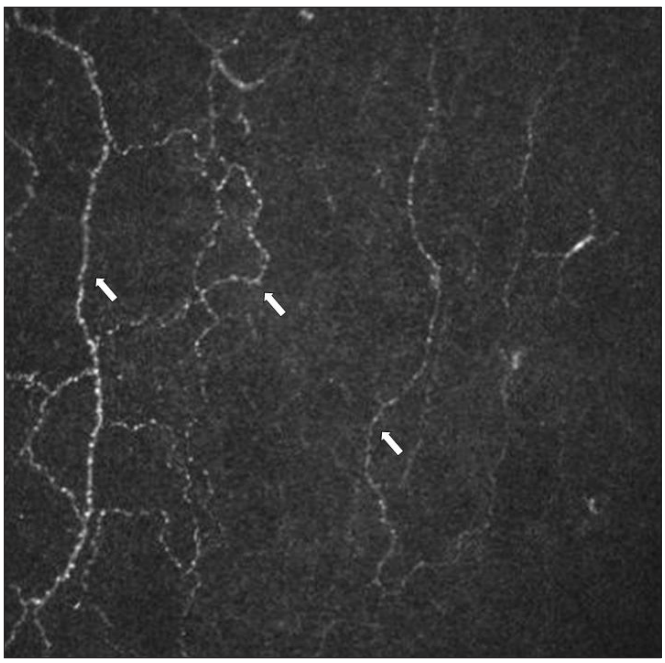

Fig. 4 - *Regenerated corneal nerves (arrows) in the donor stroma by confocal microscopy.*

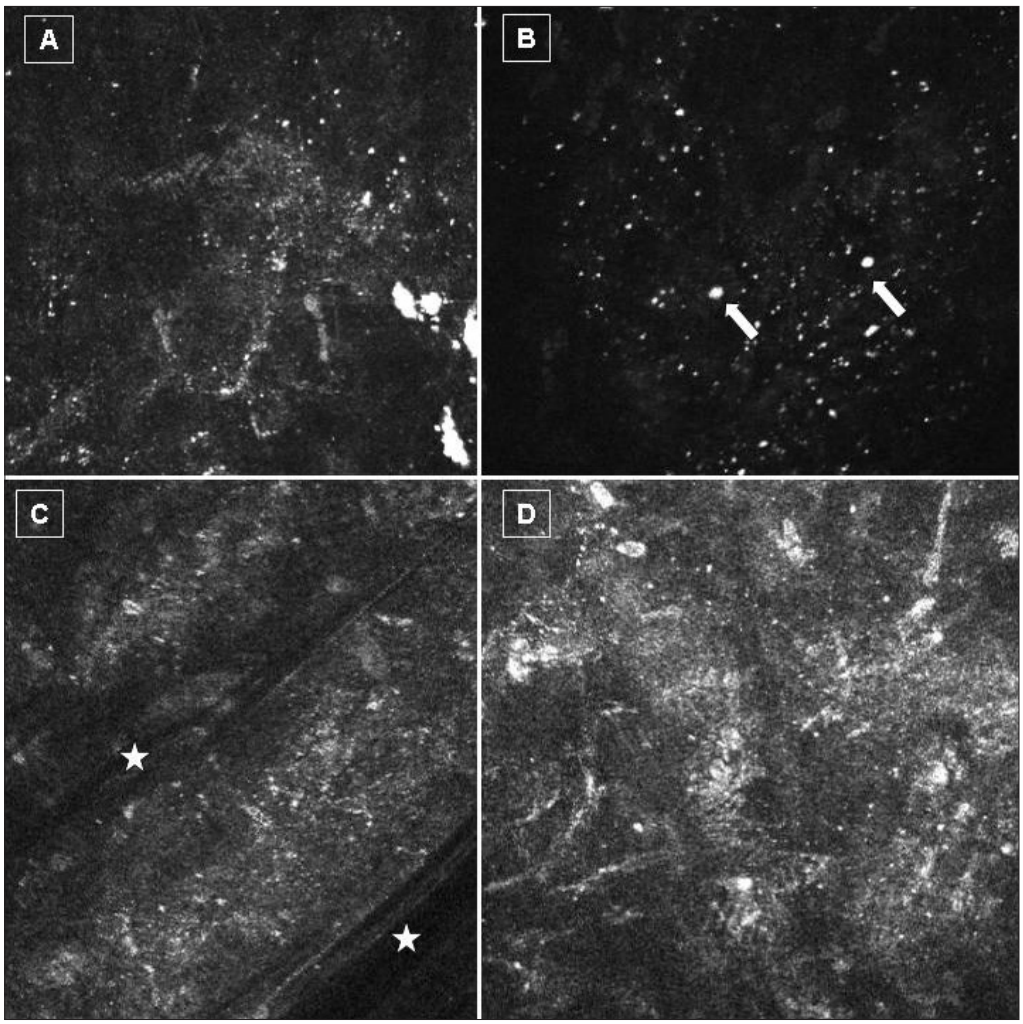

Fig. 5 - *In vivo confocal microscopy of the interface region in 4 different cases (***A-D***). Note the highly reflective particles (arrows) and stromal folds (asterisks).*

TABLE I - UNCORRECTED AND BEST-CORRECTED VISUAL ACUITY, TOPOGRAPHIC ASTIGMATISM, ULTRASOUND AND OPTIC PACHYMETRY, CONFOCAL RECIPIENT STROMA THICKNESS, AND ENDOTHELIAL CELL COUNT

Case	Visual acuity		Corneal topography, sim-K	Pachymetry		Confocal donor lamella thickness/total cornea thickness (residual recipient stroma thickness), µm	Endothelium cell count, cells/mm^2
	Uncorrected	Best-corrected		U/S µm	Optic µm		
1	20/40	20/23 –1.50 cyl (130°)	42.64 ax 21°; 43.95 ax 111°	611	640	573/633 (60)	1980
2	20/30	20/20 –3 cyl (30°)	44.67 ax31; 48.17 ax121	580	630	542/630 (88)	2617
3	20/32	20/23 –2.50 cyl (175°)	44.76 ax5; 47.89 ax95	594	602	515/555 (40)	1996
4	20/200	20/32 –3.50 sph = –6.50 (80)	42.12 ax90; 49.37 ax180	612	651	474/520 (55)	1801
5	20/20	20/20	43.64 ax162; 45.68 ax72	660	683	570/640 (70)	1626
6	20/30	20/23 –1 sph = –2.50 (20°)	44.13 ax 21°; 48.63 ax 111°	599	613	589/620 (31)	1903
7	20/200	20/20 –1.50 sph = –2.50 (180)	43.81 ax 165°; 47.43 ax 75°	776	791	590/705 (115)	2100

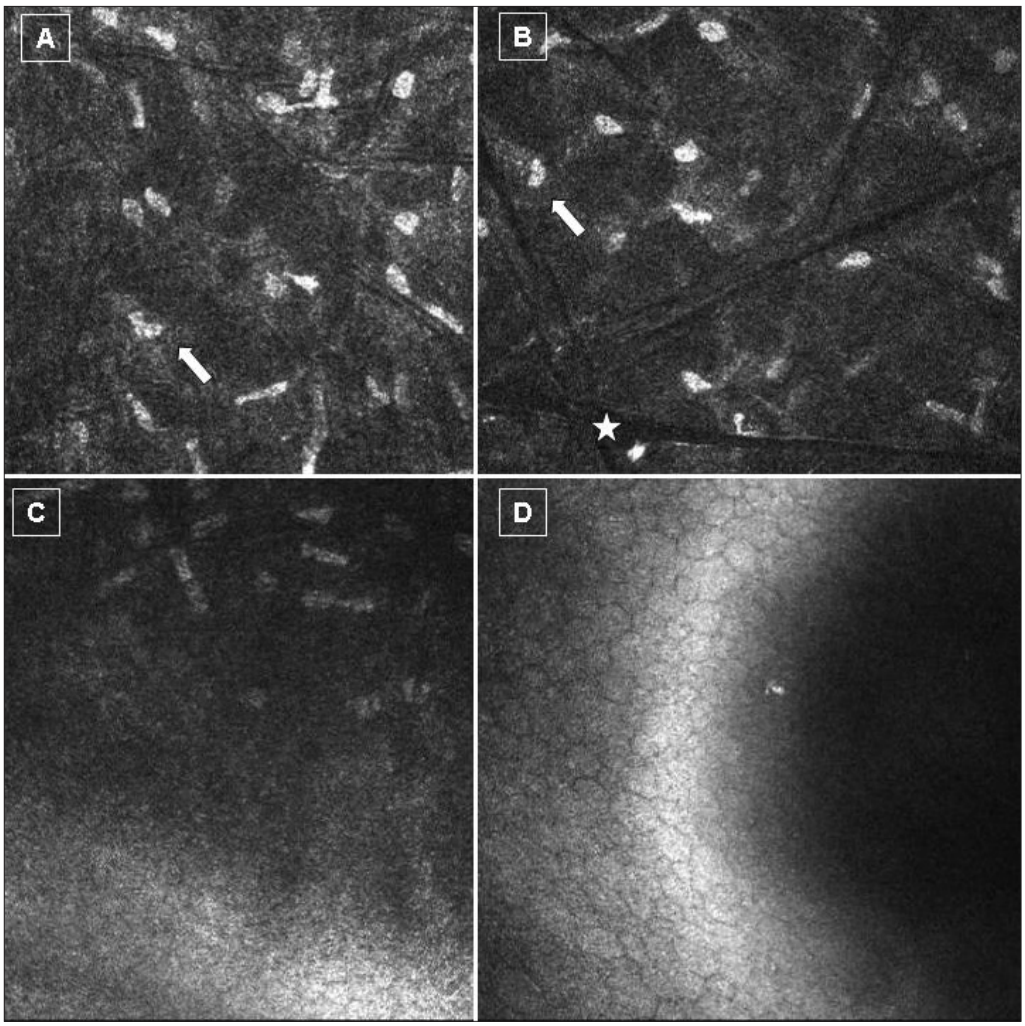

Fig. 6 - *In vivo confocal microscopy of the recipient stroma (***A, B***) and of the endothelial region (***C, D***). Note the highly reflective keratocytes (arrows) and stromal folds (asterisk).*

DISCUSSION

Several deep lamellar keratoplasty surgical techniques have been developed, modified, or improved. These include manual dissection, intrastromal air injection, intrastromal balanced salt solution (BSS) injection, trypan blue injection, or viscodissection after air injection into the anterior chamber (1-12).

Air-guided manual deep lamellar keratoplasty (13) was proposed in 2002 as a combination of an air injection in the anterior chamber and a manual dissection with a thin bevelled spatula.

In vivo confocal microscopy allows ophthalmic clinicians and researchers to visualize living tissues at greatly increased resolutions.

The use of confocal microscopy has been reported for various corneal diseases. This tool allows real time in vivo examination of all layers of the cornea, and thus visualization of microscopic alterations.

Recently a confocal microscopic study after a manual DALK technique similar to the air-guided has been published with a 12-month follow-up (16).

In this study confocal microscopy examination was carried out on seven patients 18 months after an air-guided manual DALK.

Eighteen months after DALK, epithelium appeared without significant morphologic changes and subepithelial nerve plexus was regenerated. However, as reported after laser in situ keratomileusis (LASIK), the morphology and density of regenerated subepithelial nerve fibers in the donor button never completely returned to the normal stroma levels (15).

Donor stroma analysis showed a good keratocytes presence. Interestingly, in certain zones of the button, kerato-

cyte densities appeared chronically reduced relative to normal corneas.

The interface region was characterized by the presence of highly reflective particles and stromal folds (17). These folds were probably due to the mechanical effect of the donor button apposition.

Confocal microscopy enables the calculation of the interface depth (18). This is a very significant parameter since several studies (1-12) point out the need of baring the Descemet membrane when performing DALK. Descemet membrane baring seems to be important to obtain visual results similar to penetrating keratoplasty.

With air-guided DALK the mean residual recipient stroma was 65 m. Despite this value, in our case series mean best-corrected visual acuity was 20/23; this finding is similar to those of other studies investigating visual outcome after DALK obtained with lamellar techniques that bare the Descemet (19-22).

With regard to thickness of the residual recipient stroma our results are similar to the data published by Marchini and coauthors (16) with the exception of a different higher standard deviation. This difference is probably due to the smaller population that we investigated, especially considering that our technique has been carried out only in patients with keratoconus.

Maximum depth anterior DALK (19) may lead to significant advantages in quality of vision (20) when compared to other types of anterior lamellar keratoplasty. However, it remains a challenging procedure (21-23).

Our results show that a deep dissection without baring Descemet membrane can lead to good visual results preventing corneal perforation and conversion to a penetrating graft.

None of the authors has a financial or proprietary interest in any material or method mentioned.

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