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Comprehensive assessment of corneal microstructural changes following V4c implantable collamer lens surgery using in vivo confocal microscopy

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Abstract

Background Implantable Collamer Lense (ICL) presents a viable alternative to conventional refractive surgeries, but their impact on corneal microstructure remains unclear. By employing in vivo confocal microscopy (IVCM), we examined changes in stromal and endothelial cells following the insertion of V4c ICLs, with the goal of enhancing post-surgical care and outcomes.

Methods In this longitudinal investigation, we conducted detailed preoperative assessments on 103 eyes from 53 participants. Follow-up evaluations were carried out after surgery at set intervals: one day, one week, one month, three months, six months, and twelve months. We used IVCM to analyze changes in stromal and endothelial cells. To assess differences between pre- and post-surgery variables and to investigate correlations with age, axial length (AL), and spherical equivalent refraction (SER), we applied a repeated measures mixed-effects model, with statistical significance set at $P < 0.05$.

Results No vision-threatening complications were reported post-surgery. Significant reductions in stromal cell density (SCD) were observed postoperatively, with anterior and mid- SCD reaching their lowest values at 3 months and posterior SCD at 1 month, remaining below baseline at 12 months. endothelial cell density (ECD) and percentage of hexagonal cells (PHC) decreased initially, recovering by 12 months. Conversely, endothelial cellular area (ECA) and coefficient of variation of cell size (CoV) increased postoperatively, with the most significant change at 1 week. Endothelial deposits were detected in 49 of 101 eyes on postoperative day 1, half of them were absorbed within 3 months post-surgery. Changes in posterior SCD were negatively related to AL, while AL, SER, lens thickness showed associated with endothelium changes.

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Conclusion Our findings elucidate the corneal microstructural changes following V4c ICL implantation, particularly the significant early reductions in stromal and endothelial cell densities. We recommend careful management of viscoelastics during surgery to minimize endothelial deposits that may harm the endothelium. Enhanced early postoperative monitoring and these surgical adjustments can lead to improved surgical and post-surgical care, ultimately supporting better patient recovery.

Keywords Implantable collamer lens, Endothelial cell density, Visian ICL V4c, Refractive surgery, Myopia correction, Confocal microscopy

Background

The utilization of phakic intraocular lenses (pIOLs) has gained increasing popularity as a preferred option for correcting high levels of myopia in individuals with low corneal thickness, rendering them ineligible for corneal refractive surgery [1]. The advantages of pIOLs in addressing high myopia, as previously documented, encompass favorable refractive outcomes, rapid visual rehabilitation, exceptional visual quality, preservation of accommodation, reversibility, consistent safety records, and a favorable cost–benefit ratio compared to excimer laser surgery [2–4]. Among these, the Implantable Collamer Lens (ICL; STAAR Surgical, Monrovia, CA) has emerged as the most widely adopted posterior chamber phakic intraocular lens (PC-pIOL) globally. The transition from the initial V1 model to the V4c model involved significant design modifications aimed at enhancing visual quality and reducing the occurrence of complications, particularly pupillary block, and lens opacities [5].

While substantial efforts have been made to refine lens techniques, such as the introduction of the V4c model featuring a central port design to enhance aqueous humor flow and preserve anterior segment physiology, it is noteworthy that postoperative corneal endothelial loss of 4.03%±2.20% at the 4-year mark remains a concern [6]. Moreover, existing research has extensively investigated short- and long-term changes in corneal endothelial densities, demonstrating that corneal endothelial loss typically falls within acceptable safety margins [6–9]. However, there exists a notable research gap pertaining to the examination of microstructural alterations in the cornea following ICL implantation. In vivo confocal microscopy (IVCM) represents a non-invasive optical modality capable of generating high-resolution imagery of corneal architectures within the living human eye. This technology facilitates the detailed visualization of cellular structures, microorganisms, inflammatory and epithelioid cells, and fibrosis. It has been widely utilized to monitor corneal wound healing processes following procedures such as laser in-situ keratomileusis (LASIK) and photorefractive keratectomy (PRK) [10–12]. For now, most published reports have used specular microscopy to observe the morphological features of corneal endothelium before and after ICL surgery, few studies had employed IVCM to observe the microstructure alterations of cornea after ICL implementation.

To address this research gap, IVCM was utilized to examine both the superficial and deeper layers of the cornea before and after the implantation of ICLs. The study involved systematic, high-resolution scans at several post-surgical time points to observe changes in cellular density, morphology, and the emergence of pathological conditions. This method provided a detailed analysis of the immediate and ongoing effects of ICL on corneal microstructure, thereby enhancing our understanding of the corneal response to ICL implantation.

Methods

Participates

This prospective and consecutive research was approved by the Institutional Review Board of the EYE & ENT Hospital of Fudan University. The study complied with the ethical standards set forth in the Declaration of Helsinki, and all participants provided written informed consent before their inclusion in the study. In this study, 103 eyes from 53 myopic patients who received ICL V4c implants from September 2019 to January 2020 were examined. Eligibility for participation required individuals to be older than 17, have a stable refractive error, and abstain from contact lens wear for a minimum of two weeks prior to the preoperative assessment. Moreover, a minimum endothelial cell density (ECD) of 2000 cells/mm² was necessary for inclusion. Patients were excluded if they were using ocular medications (other than artificial tears), had current or past ocular infections or inflammations, a history of ocular trauma, progressive retinal disorders, any known allergic hypersensitivities, or any corneal diseases.

Preoperative assessments

All participants were subjected to a comprehensive set of preoperative evaluations. These assessments included measurements of best-corrected visual acuity (BCVA), spherical equivalent refraction (SER), and examinations using a slit-lamp and fundus photography after pupillary dilation. Non-contact intraocular pressure (IOP) was determined using a Canon Full Auto Tonometer TX-F (Canon, Inc., Tokyo, Japan). Additional parameters such as axial length (AL), corneal thickness, white-to-white

corneal diameter, lens thickness (LT), and anterior chamber characteristics were measured using the Pentacam HR (Oculus Optikgeräte, Wetzlar, Germany). Furthermore, the corneal microstructure was analyzed using IVCM.

Confocal microscopy

IVCM was employed to examine all enrolled eyes, complemented by Heidelberg retinal tomography (HRT II) with the Rostock cornea module (Heidelberg Engineering GmbH, Dosenheim, Germany), in accordance with established protocols detailed in the existing literature [10, 11]. To ensure the comfort and cooperation of patients, a topical anesthetic solution (0.4% oxybuprocaine, Santen Pharmaceutical Co. Ltd.) was gently instilled into the lower conjunctival fornix. Subsequently, a high-viscosity contact gel (Comfort Gel; Bausch & Lomb, GmbH, Berlin, Germany) was applied to the front surface of the microscope lens, with attention to preventing the formation of any air bubbles. A single-use polymethylmethacrylate cap (TomoCap; Heidelberg Engineering GmBH) was firmly affixed to the holder to encompass the microscope lens. Subsequently, patients were positioned using chin and forehead rests, and instructed to maintain a consistent gaze directly at a fixed object at the center of the objective lens, ensuring uniformity in focal area across all examinations. The digital micrometer was calibrated to zero upon the initial visualization of superficial cells, after which a series of central corneal images were meticulously recorded. This imaging procedure extended from the corneal superficial epithelial layer to the posterior stroma, covering a central diameter of ≤9 mm.

To facilitate the comparative analysis of corneal stromal cell density(SCD) and morphology before and after surgery, we categorized the stromal measurements into three distinct zones: 1.the anterior stroma, located below Bowman's membrane; 2. the mid-stroma, positioned within the middle layer spanning the entire corneal thickness (from epithelium to endothelium); 3. the posterior stroma, situated anterior to the endothelium [13]. The cell densities within these three stromal layers were determined based on the cells present in one image (400×400 μm). In contrast, ECD was calculated within a region of interest (ROI) comprising more than 50 cells. These results are expressed in cells per square millimeter. Additionally, we assessed the endothelial cellular area (ECA), the coefficient of variation of cell size (CoV), and the percentage of hexagonal cells (PHC). CoV(%), calculated as the standard deviation of cell size to mean cell size, was employed to quantify the variability in endothelial cell size. All image analyses were conducted independently by three experienced physicians (W.T.C, X.J.Z, and Q.L.W) with the final parameters derived as an average of their results, utilizing ImageJ software (Version 1.47, National Health Institute, Bethesda, MA).

Surgical procedures

The surgeries were executed by Dr. Y.Z.Q, who followed strict protocols regarding the operative techniques. Preoperative preparation included administering ophthalmic antibiotics four times daily for three days prior to surgery. On the day of the procedure, agents were used to prepare the eyes for surgery. During the procedure, the anterior chamber was filled with 1% sodium hyaluronate to facilitate surgical maneuvers, which was carefully evacuated at the operation's conclusion. The implantation of an ICL V4c lens was achieved via a small incision in the cornea using a specialized injector system. Precise placement of the lens haptics in the desired location was accomplished using a specialized tool, and any remaining viscoelastic material was cleansed from the eye using a saline solution. After surgery, a regimen of antibiotics and anti-inflammatory drops was initiated, tapering off over the following weeks.

Postoperative Assessment Timeline

Follow-up evaluations were conducted at specified intervals post-surgery, including at one day, one week, and at one, three, six, and twelve months after the procedure. These comprehensive follow-up assessments encompassed the examination of several crucial parameters: (1) BCVA, SER, IOP; (2) an in-depth analysis of the corneal microstructure was performed based on the captured IVCM images, focusing on cell density and morphology of both the stromal and endothelial layers.

Statistical analysis Approach

Data analysis was conducted with a well-known statistical software (SPSS version 23.0). Descriptive statistics were presented as means with their respective standard deviations. A sophisticated statistical model was employed to assess variations over time within subjects, allowing for a robust analysis of the pre- and post-surgical measurements [14]. A significance level was set at $p<0.05$ for all tests. The model also explored potential relationships among various demographic and ocular measurements, including changes in corneal metrics following the operation.

Results

This study encompassed 53 patients scheduled for ICL surgery, involving assessments of 103 eyes (52 right, 51 left). The participant group featured a predominant female majority ($n=42$) compared to males ($n=11$). The average age of the participants was 26.7±6.1 years, ranging from 17 to 44 years. The mean spherical equivalent refraction was recorded at -9.84±2.9 diopters, with

values extending from −18.25 to -4.5 diopters. The average axial length of the eyes measured 27.31±1.4 mm, spanning from 24.23 mm to 30.75 mm. The corneal endothelial cell density was approximately 2992.26±250.89 cells/mm², with a range from 2448 to 3652 cells/mm². Scheduled follow-up evaluations were conducted shortly after surgery and continued at regular intervals for one year, with patient attendances recorded as 52, 37, 36, 27, 13, and 12 at the subsequent assessments. Throughout the study, all participants maintained intraocular pressure levels below 21 mmHg. Moreover, the uncorrected distance visual acuity (UDVA) of the patients was consistently equal to or better than their preoperative BCVA at all assessment stages. There were no reports of intraoperative issues, nor was there a need for any ICL adjustments or replacements. Additionally, the study period was free from any serious ocular complications including but not limited to opacity, cataract formation, glaucoma, or blockages affecting the pupil, ensuring no adverse impacts on vision were observed.

When examining changes in anterior and middle SCD compared to baseline (877.06±159.0/mm² vs. 492.29±72.6/mm²), we observed a significant decrease postoperatively, reaching their lowest values at the postoperative month 3 time point (828.61±129.0/mm² vs. 451.78±67.1/mm²). Subsequently, there was a slight increase, which continued until the end of the follow-up period (864.24±133.6/mm² vs. 467.38±67.1/mm²). It's noteworthy that these values remained lower than the baseline measurements. In contrast, the lowest value observed for posterior SCD (391.53±74.8/mm²) occurred at the postoperative month 1 assessment, followed by an increase in subsequent assessments. However, it did not reach the baseline value (409.87±51.4/mm²) even at the postoperative month 12 assessment (391.59±41.7/mm²) (details in Table 1; Fig. 1A-C.).

A similar trend was observed in the changes in corneal central ECD and PHC. The endothelial cell loss rates at postoperative day 1, 1 week, 1 month, 3 months, 6 months, and 12 months were 0.63% ± 4.8%, 2.18% ± 6.6%, 1.20% ± 5.6%, 1.27% ± 7.0%, 0.83% ± 4.7%, and 0.21% ± 7.0%, respectively. In comparison to baseline values of ECD and PHC (2992.26±250.9/mm² vs. 64.22%±11.6%), there was a significant decrease in the early postoperative period. The lowest ECD value was observed at postoperative week 1 (2943.61±269.0/mm²), while the lowest PHC occurred at the postoperative month 3 assessment (58.47%±15.4%). These values increased subsequently, reaching their peak at the postoperative month 12 time point (3050.70±279.4/mm² vs. 61.93%±15.3%). When considering ECA and CoV, compared to baseline values (336.87±28.0 mm² vs. 64.22%±11.6%), there was a significant increase after surgery, peaking at the postoperative week 1 time point (342.19±31.6 mm² vs. 3.60%±3.1%), followed by a decrease until the end of the follow-up period (330.90±33.4 mm² vs. 2.91%±1.1%) (details in Table 2; Fig. 1D-G).

To conduct a thorough evaluation of surgical outcomes, we focused on two critical time intervals: from baseline to the first week post-operation and from the first month to the twelfth postoperative month. Analyzing the short-term effects between baseline and the first week post-operation, we utilized information gathered from 72 eyes of 37 patients who underwent both preoperative and first-week postoperative assessments. Employing a repeated measures mixed-effects model, our analysis unveiled a notable decline in stromal and endothelial cell densities, as well as PHC, coupled with an elevation in ECA and CoV. Conversely, examining the medium- to long-term effects between the first and twelfth postoperative months, we utilized information gathered from 20 eyes of 10 patients who underwent both assessments. Similar analytical methods revealed an increase in stromal and endothelial cell densities, alongside a reduction in ECA and CoV, suggesting a general improvement of these parameters over the medium to long term. A comprehensive summary of our findings can be found in Table 3.

Corneal endothelial deposits were detected in 49 out of 101 eyes on postoperative day 1. These deposits exhibited varying shapes and numbers, ranging from several small dots (showed in Fig. 2A-B) to single patches (showed in Fig. 2C-D). Among these 49 cases, 16 eyes showed no detectable deposits by the end of the first postoperative week, although it is important to note that week 1 assessments did not constitute the final evaluations. Additionally, in 9 of these 49 eyes, the deposits became undetectable by the end of the first postoperative month, recognizing that month 1 assessments were not the concluding evaluations. In a single case within this cohort of

Table 1 Comparisons of corneal stroma cell before and after the surgery

Density(cell/mm²)	Before Surgery	After Surgery						*P**
		Day 1	Week 1	Month 1	Month 3	Month 6	Month 12	
Eyes(n)	103(*n*=53)	101(*n*=52)	72(*n*=37)	51(*n*=36)	53(*n*=27)	25(*n*=13)	24(*n*=12)	
Anterior stroma cell	877.06±159.0	840.62±125.7	831.53±122.7	840.84±144.4	828.61±129.0	840.48±157.7	864.24±133.6	0.0026
Mid-stroma cell	492.29±72.6	467.24±69.0	467.57±56.8	456.99±65.2	451.78±67.1	463.08±48.9	467.38±61.7	0.0046
Posterior stroma cell	409.87±51.4	399.20±75.4	400.61±53.3	391.53±74.8	398.60±50.1	391.95±37.3	391.59±41.7	0.0004

* Analysis conducted using a repeated measures mixed-effects model, with statistical significance denoted as $P < 0.05$

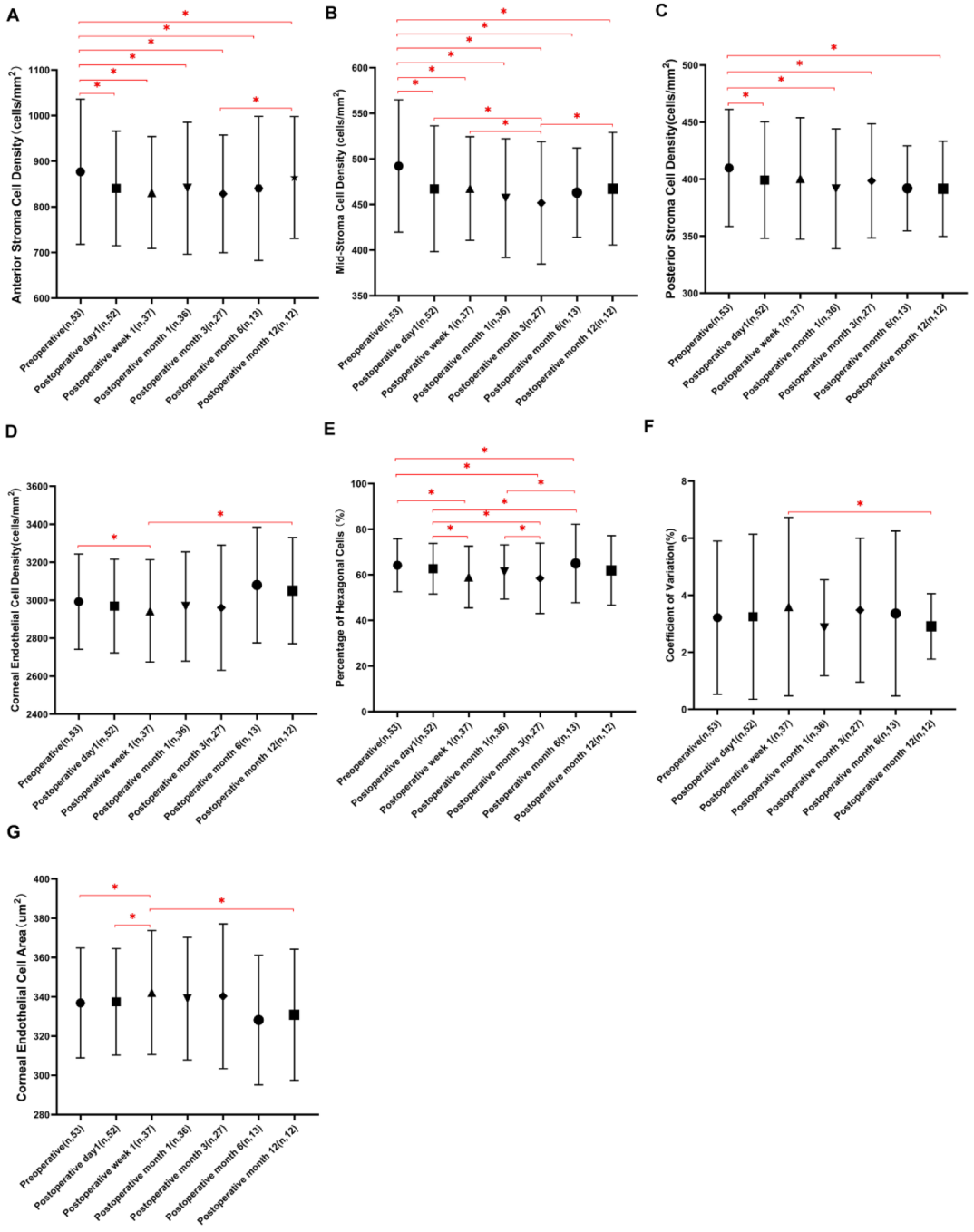

Fig. 1 Postoperative Changes in Corneal Stromal and Endothelial Parameters. (**A**), (**B**), and (**C**): The measurements of anterior, mid-, and posterior stroma cell densities (SCD) demonstrated a significant postoperative decrease in all three SCD parameters. Anterior and mid-SCD reached their lowest values at the postoperative month 3 time point, while posterior SCD reached its lowest value at the postoperative month 1 assessment. Subsequently, there was a slight increase after their respective lowest points, which continued until the end of the follow-up period. Importantly, these values remained lower than the baseline measurements. (**D**): The values of endothelial cell density (ECD) exhibited a significant decrease at the postoperative week 1 time point when compared to baseline and the postoperative month 12 time point. (**E**): The measurements of the Percentage of Hexagonal Cells (PHC) showed a significant decrease during the early follow-up period until the postoperative month 3 time point, followed by a slight increase without statistical significance. (**F**): The measurements of corneal endothelial cell area (ECA) significantly increased and reached their peak value at the postoperative week 1 time point, followed by a slight decrease afterward until the end of the follow-up period. (**G**): The coefficient of variation values (CoV) showed a slight increase during the early postoperative period, followed by a decrease after the postoperative week 1 time point. A statistical difference was observed at the postoperative week 1 time point when compared to the postoperative month 12 time point.Statistical significance ($p < 0.05$) was assessed by a mixed-effects model

Table 2 Comparisons of corneal endothelial cell before and after the surgery

	Before Surgery	After Surgery						*P*
		Day 1	Week 1	Month 1	Month 3	Month 6	Month 12	
Eyes(n)	103(*n*=53)	101(*n*=52)	72(*n*=37)	51(*n*=36)	53(*n*=27)	25(*n*=13)	24(*n*=12)	
Central cell density (cell/mm^2)	2992.26±250.9	2969.14±246.6	2943.61±269.0	2967.11±287.8	2960.65±329.6	3080.15±304.4	3050.70±279.4	0.0007
Hexagonal Cells Percentage (%)	64.22%±11.6	62.71%±11.1	59.07%±13.6	61.28%±11.9	58.47%±15.4	65.00%±17.2	61.93%±15.3	0.0476
Cellular Area(um^2)	336.87±28.0	337.42±27.1	342.19±31.6	339.08±31.2	340.31±36.8	328.20±33.0	330.90±33.4	0.0002
Coefficient of Variation (%)	3.22%±2.7	3.20%±2.9	3.60%±3.1	2.86%±1.7	3.48%±2.5	3.36%±2.9	2.91%±1.1	0.0378

* Statistical analysis was performed using a repeated measures mixed-effects model, with significance indicated as $P < 0.05$

Table 3 Comparison of stromal and endothelial parameters at different time points

	Preoperative vs. post-week 1	*P**	Post-month 1 vs. post-month 12	*P**
Eyes(n)	72(*N*=37)		20 (*n*=10)	
Anterior stroma cell density (cell/mm^2)	868.98±162.1 vs. 831.53±122.7	<0.0001	902.55±136.6 vs. 881.56±123.7	<0.0001
Mid-stroma cell density (cell/mm^2)	498.67±65.9 vs. 467.57±56.8	<0.0001	443.96±91.0 vs. 468.20±64.8	<0.0001
Posterior stroma cell density (cell/mm^2)	415.44±50.6 vs. 400.61±53.3	<0.0001	371.85±99.4 vs. 387.88±43.7	<0.0001
Central corneal endothelial cell density (cell/mm^2)	3013.21±244.7 vs. 2943.61±269.0	<0.0001	3041.58±336.1 vs. 3050.23±293.7	<0.0001
Hexagonal Cells Percentage (%)	62.75%±11.7% vs. 59.07%±13.6%	<0.0001	60.16%±17.3% vs. 60.04%±16.1%	<0.0001
Cellular Area(um^2)	334.54±27.3 vs. 342.19±31.6	<0.0001	332.99±37.5 vs. 331.26±35.4	<0.0001
Coefficient of Variation (%)	3.15%±2.6% vs. 3.60%±3.1%	<0.0001	3.21%±2.0% vs. 2.98%±1.1%	<0.0001

* Analysis was conducted using a repeated measures mixed-effects model, with statistical significance set at $P < 0.05$

49 eyes, the deposits could not be detected at the time point of postoperative month 3, with the understanding that the third-month assessment was not the final evaluation. However, in 9 of these 49 eyes, deposits were still evident at the time point of the third postoperative month, which marked the final assessment for this subgroup of patients. Furthermore, deposits were detected in 1 eye among these 49 at the time point of the sixth postoperative month and in 2 eyes at the time point of the twelfth postoperative month, both of which represented their respective final assessments.

It is noteworthy that among these 49 eyes, 12 exhibited deposits in the form of a single patch. Of these 12 eyes, 1 remained detectable at the time point of post-month 3 (which served as the final assessment). Six eyes were no longer detectable at the time point of postoperative week 1, while 2 eyes exhibited a reduction in deposit size at the time point of postoperative week 1 and were no longer detectable by the time of postoperative month 1(as dedicated in Fig. 3A-F). The remaining 3 cases were lost to follow-up after the time point of postoperative day 1.

A mixed-effects model was employed to investigate the correlations among the parameters before and after surgery. The results revealed that AL exhibited a negative relationship with changes in posterior SCD (F = -17.0374, p=0.0268) and a positive relationship with the CoV (F=0.007609, p=0.0101). Additionally, LT was negatively associated with changes in ECD (F = -276.15, p=0.0391), while spherical equivalent refraction (SER) showed a positive relationship with changes in the CoV (F=0.003102, p=0.0242).

Discussion

In this prospective study, we delved into the comprehensive evaluation of microstructural alterations in the cornea following V4c ICL implantation, shedding light on the factors influencing these corneal changes. Utilizing IVCM, we captured high-resolution images that allowed us to observe the detailed cellular interactions and structural changes at various depths of the cornea. This technique proved invaluable in discerning the nuanced microstructural alterations that are not apparent with other imaging modalities.

The transition from the earlier V1 model to the V4c model marked a significant advancement in the field, focusing on enhancing visual quality while mitigating complications. While corneal endothelial loss has been a concern post-ICL surgery, our research aimed to bridge the gap in understanding the nuanced microstructural alterations occurring in the cornea.

Through IVCM, our investigation into corneal microstructure changes post-ICL surgery yielded several noteworthy findings. Initially, there was a significant decrease in anterior, middle, and posterior SCD, followed by a gradual increase. Importantly, even after a 12-month post-surgery period, none of the SCD values returned to baseline. This observation aligns with previous studies that reported reduced SCD in cases of keratoconus, post-photorefractive keratectomy, and post-LASIK procedures

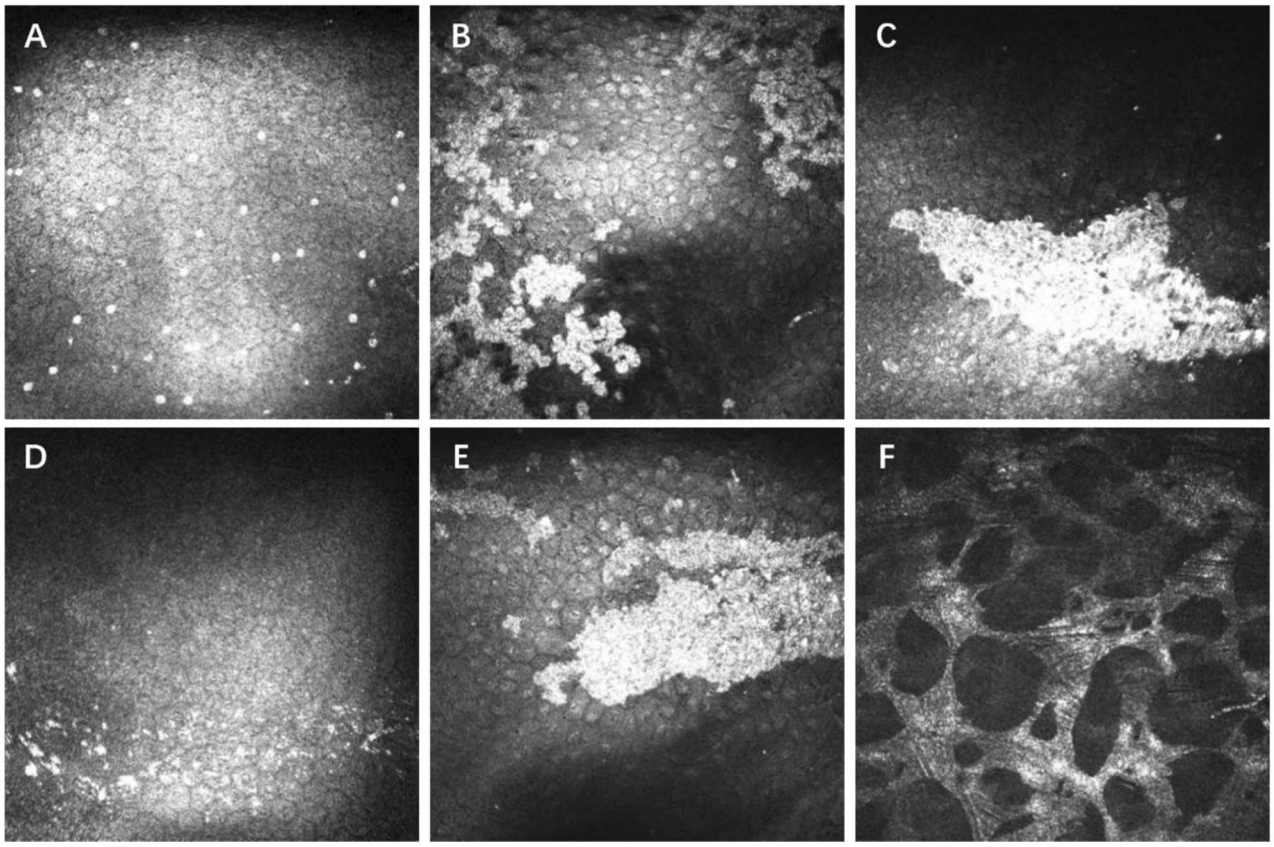

Fig. 2 Corneal Deposits Detected Post ICL Implantation Surgery. (**A**) IVCM endothelial image from the right eye of a 24-year-old female with a spherical equivalent refraction (SER) of -9.5 D and an axial length of 27.45 mm. Numerous hyperreflective corneal deposits in a round dot pattern were observed on the first postoperative day but were no longer detectable by the end of the first postoperative week. (**B**) IVCM endothelial image from the left eye of a 17-year-old female with a SER of -11.875 D and an axial length of 28.07 mm. Dense, round, hyperreflective corneal deposits and nucleated endothelial cells were evident on the first postoperative day but were absent at the 3-month postoperative examination. (**C**) and (**D**) IVCM endothelial images from the left eye of a 24-year-old female with a SER of -9.875 D and an axial length of 27.51 mm. (**C**) A dense, patchy hyperreflective corneal deposit pattern and nucleated endothelial cells were observed on the first postoperative day. (**D**) These deposits were still detectable at the 3-month postoperative examination, which served as the final assessment for this patient. (**E**) and (**F**) IVCM endothelial images from the right eye of a 20-year-old female with a SER of -6.0 D and an axial length of 25.89 mm. (**E**) A dense, patchy hyperreflective corneal deposit pattern and nucleated endothelial cells were observed on the first postoperative day. (**F**) The cytoplasmic reflection of stromal cells in the corresponding area is enhanced, and these changes persisted until the 3-month postoperative assessment, which marked the final evaluation for this patient

[15–21]. These studies collectively indicate that SCD relies on a delicate balance between stimulated keratocyte apoptosis and reactive cell proliferation and migration. Various factors, including components of the ocular surface and underlying corneal layers, as well as neural elements, can influence stromal keratocyte homeostasis through both biochemical and biomechanical pathways. Biochemical factors, such as the secretion of cytokines and decreased levels of ascorbate, are believed to play roles in keratocyte apoptosis. Biomechanical elements, encompassing changes in stress-strain patterns subsequent to surgical incisions and energy applications, within the front layers of the cornea, along with interlamellar displacement in the rear layers, may lead to keratocyte depletion. The depth-specific analysis enabled by IVCM was critical in mapping these changes accurately over the duration of the study.

To our awareness, this study represents the initial investigation to document alterations in SCD subsequent to ICL surgery. In cases where patients exhibited dense patches of corneal deposits, not only did the endothelial cells exhibit a nucleated status, but their corresponding posterior stromal cells also displayed an active status with hyperreflective cytoplasm. This observation suggests that a microenvironmental stimulation, likely induced by intraocular surgery (see Fig. 2), is at play. Therefore, we hypothesize that microenvironmental changes, including biochemical stimulation resulting from intraocular surgery and mechanical damage caused by corneal incisions, may be the primary mechanisms behind the observed reduction in SCD in our experiments. Further studies are warranted to explore this intriguing phenomenon in greater detail.

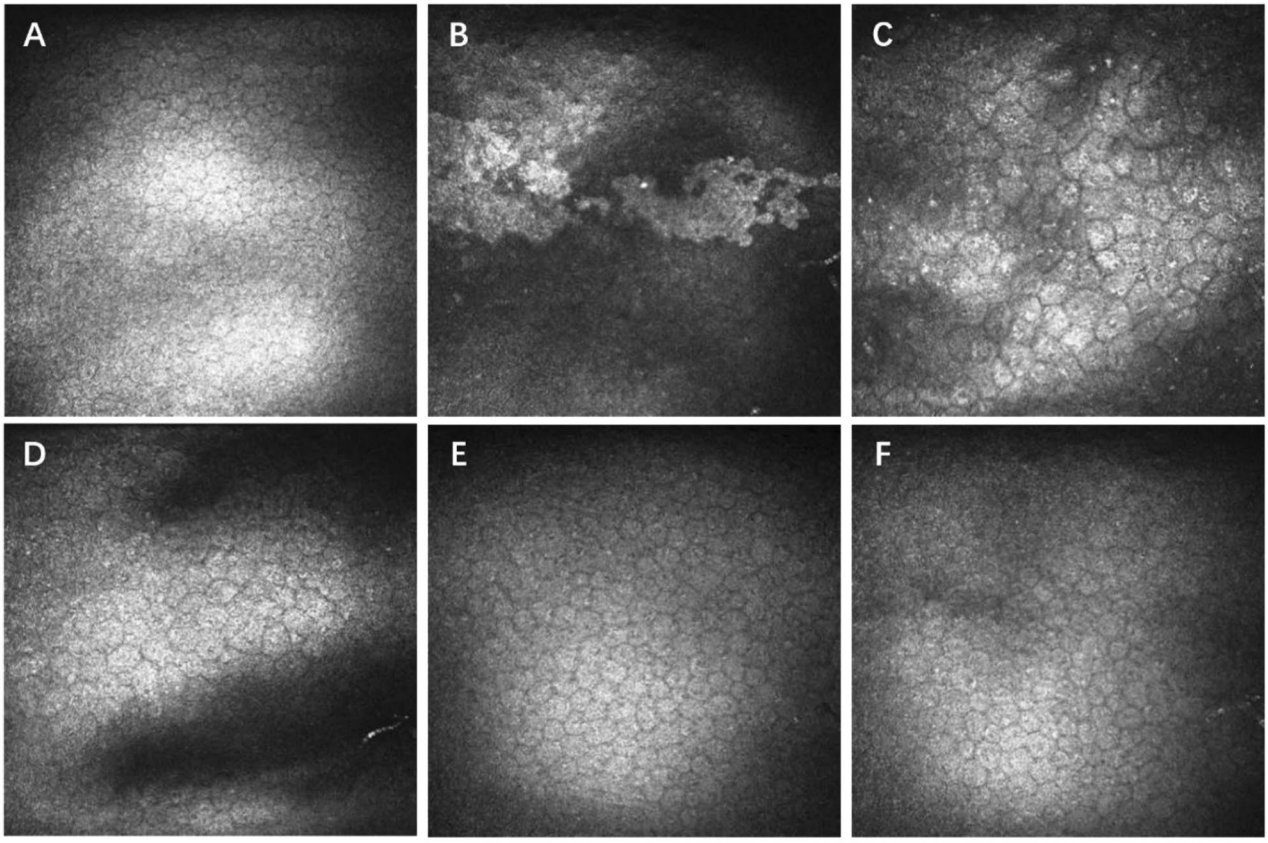

Fig. 3 IVCM Endothelial Images from the Left Eye of a 25-Year-Old Male Following ICL Implantation Surgery for Corrected −8.0 D Myopia with an Axial Length of 26.19 mm. (**A**) Pre-surgery endothelial cell image displaying an endothelial cell density (ECD) value of 2778 cells/mm^2. (**B**) Endothelial cell image captured 1 day after surgery. Notable features include a prominent thick patch deposit at the corneal center, with a few endothelial cells slightly protruding from the surface and exhibiting blurred cell boundaries. The ECD value measures 2050 cells/mm^2. (**C**) Image acquired 1-week post-surgery, showcasing the transformation of the central patch deposit into small, highly reflective dots. Endothelial cells display irregular morphology, with a decreased proportion of hexagonal cells, deepened borders, and increased cell area. The ECD measures 1231 cells/mm^2. (**D**) Image obtained 1-month post-surgery, indicating the absence of hyperreflective material in the endothelial layer. Endothelial cells exhibit a smaller area compared to the 1-week post-surgery image, and cell boundaries become shallower. The ECD is 1700 cells/mm^2. (**E**) and (**F**) represent images captured at postoperative month 3 and month 12, respectively, with corresponding ECD values of 2130 cells/mm^2 and 2428 cells/mm^2

The results regarding ECD and its percentage loss post-ICL surgery vary among published studies, influenced by patient characteristics and follow-up durations. For example, Alfonso et al. reported a 0.43% ECD loss at 12 months postoperatively [22], whereas Bhandari et al. reported a 6.1% loss at 9 months post-surgery [23]. In contrast, Wei et al. found no significant ECD difference at the 6-month mark [24], while Niu et al. reported that individuals presenting with anterior chamber depths below 2.8 mm displayed an 8.38±0.06% decline relative to preoperative measures during the 12-month follow-up [25]. In our study, ECD showed significant declines during the early postoperative period, reaching its lowest point at 1-week post-surgery, followed by a gradual recovery, eventually peaking at 12 months post-surgery. These findings align with previous studies [5], emphasizing that the most substantial endothelial cell loss occurs early postoperatively, as confirmed by the comparison between preoperative and 1-week postoperative measurements (detailed in Table 3).

Parameters such as the PHC, ECA, and CoV are critical for assessing the structural integrity and function of the corneal endothelium. Alterations in these parameters may signify corneal diseases, surgical effects, or other factors affecting corneal health. In our study, PHC exhibited significant declines during the early postoperative period, followed by a gradual recovery, ultimately reaching peak values at 12 months post-surgery. Conversely, changes in corneal ECA and CoV followed an opposing trajectory, with significant increases post-surgery, peaking at the 1-week postoperative assessment, followed by gradual decreases until the study's conclusion. While numerous studies have already established the safety of V4c implantation for the corneal endothelium, our study adds valuable insights by providing a more extensive

evaluation through spans over 12 months. This strengthens the existing evidence supporting the safety of this procedure.

The surgical procedure itself constitutes a primary cause of endothelial cell loss, with variability depending on the surgeon, particularly in the early postoperative period. Our study, firstly utilizing IVCM, observed corneal deposits detectable in 49 out of 101 eyes. These deposits, resembling viscoelastic residue, were visible in the very early postoperative period, and approximately half of them were absorbed within 3 months post-surgery. Importantly, these deposits significantly affected the structural integrity of the endothelium, with larger deposits necessitating more time for absorption and leading to significant changes in the corneal posterior stroma and endothelial cells. These findings suggest that residual viscoelastic agent may contribute to endothelial cell loss or damage in the early postoperative period. Nevertheless, additional investigation is imperative to validate these findings and acquire a more profound comprehension of their implications.

While our study offers critical insights into the microstructural changes in the cornea following ICL implantation, we recognize several limitations that could affect the repeatability and sustainability of our findings. Firstly, the follow-up duration of 12 months, while adequate for short-term assessments, may not be sufficient to evaluate the long-term effects of ICL on corneal microstructure. Extended follow-up studies are necessary to fully elucidate these enduring impacts. Additionally, the lack of an external control group in our study design limits our ability to attribute the observed changes exclusively to the ICL implantation. Without a control group undergoing natural aging or receiving different interventions, it remains challenging to isolate the specific effects of the ICL. Secondly, our study's focus on a relatively young patient population with a narrow age range may limit the generalizability of our findings to older individuals or those with different demographic characteristics. To address potential repeatability concerns, we employed IVCM at multiple predetermined time points throughout the 12-month follow-up. This methodological choice aimed to provide a consistent framework for observing corneal microstructure changes systematically. Furthermore, we used a repeated measures mixed-effects model to analyze our data. This statistical approach is advantageous in longitudinal studies like ours, effectively accounting for correlations in repeated measurements from the same subjects over time. Such analysis enhances the reliability of our findings by providing a robust framework for detecting changes within individuals, thereby mitigating potential biases and variability from external factors.

Despite these limitations, our study holds several notable advantages. It offers a comprehensive evaluation of corneal microstructure changes following ICL implantation, contributing to the existing body of knowledge on the safety and effectiveness of this refractive procedure. The use of IVCM enabled us to find out SCD decreased in the first years after V4c implantation and remain the surgeon that residual viscoelastic agent may contribute to endothelial cell loss or damage in the early postoperative period.

In summary, our prospective study employing IVCM revealed significant corneal microstructural alterations following V4c ICL implantation, shedding light on the mechanisms behind these changes, including a noteworthy reduction in stromal cell density and the impact of residual viscoelastic agent. These findings contribute valuable insights to the safety and efficacy profile of V4c ICL implantation, with implications for future patient care and research.

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Author contributions
Qiaoling Wei, Xingtao Zhou, and Zhiqiang Yu conceptualized the study; Qiaoling Wei and Weiteng Chang curated the data; Zhiqiang Yu managed the project; Rui Jiang, Xingtao Zhou, and Zhiqiang Yu provided resources; Xingtao Zhou supervised the study; Qiaoling Wei drafted the manuscript; and Qiaoling Wei, Rui Jiang, Xingtao Zhou, and Zhiqiang Yu reviewed and edited the manuscript. All authors have read and consented to the published version of the manuscript.

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Data availability
The data supporting the results of the current study can be found within the article.

Declarations

Ethics approval and consent to participate
An approval from the Ethics Committee of the Institutional Review Board of the EYE & ENT Hospital was obtained (no. 2023-YS-016). The study adhered to the ethical principles outlined in the Declaration of Helsinki. Written informed consent has been obtained from the patients to publish this paper.

Competing interests
The authors declare no competing interests.

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