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Does a two-year period of orthokeratology lead to changes in the endothelial morphology of children?

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ABSTRACT

Purpose: To compare changes in endothelial morphology in the central and superior cornea in subjects wearing single-vision spectacles and orthokeratology lenses over two years.

Methods: Endothelial images of the two locations of 99 subjects (6–12 years) from completed myopia control studies were analysed. Endothelial cell density (ECD), coefficient of variation in cell size (CV), and hexagonality (HEX) before and two years after treatment were compared between the two groups of subjects.

Results: Baseline ECD and CV in the central cornea were slightly lower than those in the superior cornea, but no significant difference in HEX was found in the two corneal locations. After two years, reduction in ECD and increase in CV were only significant in the central cornea, but not in the superior cornea. Reduction in HEX was significant in both corneal locations. Subjects receiving orthokeratology had smaller reduction in ECD in the central cornea compared to the controls (orthokeratology: 56 ± 94 cells/mm^2; control: 98 ± 91 cells/mm^2, $p = 0.024$), otherwise, there were no significant differences in the changes in endothelial morphology in the two corneal locations between the two groups of subjects.

Conclusions: The current study confirmed that there were differences in endothelial morphology of central and superior cornea of Chinese children aged 6–12 years. The morphological response to normal ageing differed between the two corneal locations as reduction in cell density and polymegathism were found only in the central cornea whilst pleomorphism was found in both locations. Orthokeratology lens wear had minimal effect on the developmental changes in endothelial morphology.

1. Introduction

Clinical evidence has shown that orthokeratology (ortho-k) is an effective and safe treatment to slow axial elongation in children [1–9]. One of the indicators for safety in ortho-k lens wear is corneal endothelial morphology. A few longitudinal studies have evaluated the long term effects of ortho-k on the corneal endothelium [10–14]. Three of these studies [10–12] did not find any significant change in endothelial cell density (ECD), pleomorphism in terms of percentage of hexagonal cells (HEX), and polymegathism in terms of coefficient of variation in cell size (CV) 1–7 years after treatment. Hiraoka et al. [10] reported no changes in ECD, CV, and HEX in 52 eyes of 31 subjects aged 10–44 years (mean ± SD: 17 ± 9 years) before and after one year of ortho-k lens wear. Zhong et al. [11] conducted a cross-sectional study to compare corneal thickness and morphology in subjects after one night and five years of ortho-k lens wear (mean ± SD age: one night = 23 ± 4 years; 5 years = 19 ± 5 years). Data collected 8 h prior to lens wear from subjects on one-night treatment were used as control. The authors used data collected from the two eyes and reported no significant difference in ECD and HEX after either one night or 5 years of lens wear. In the retrospective study conducted by Guo and Xie [12], there were no significant changes in ECD, CV, and HEX in 30 subjects aged 8–20 years before and after seven years ortho-k lens wear. In contrast, Cheung and Cho [13] found a significant reduction in ECD without any changes in polymegathism or pleomorphism in children aged 7–17 years (median: 10 years) after two years of lens wear. On the other hand, Nieto-Bone et al. [14] observed an increase in polymegathism without any changes in ECD or pleomorphism after one year of lens wear in 15 adults aged 18–30 years. These studies varied with respect to study designs, age of subjects, duration of study, and lack of proper control subjects. Thus, there is a need to confirm if ortho-k leads to changes in corneal endothelium.

It is known that ECD reduces with age, starting at birth [15–22]. The rate of reduction is most rapid in the first five years of age, slows down during childhood and the adolescent period, and finally becomes stable in adulthood. Of the five studies on the effect of ortho-k on the

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Table 1
Demographic data and baseline ocular and corneal endothelial parameters of the subjects.

	All (N = 99)	Orthokeratology (N = 50)	Control (N = 49)	Between groups p-value[+]
Age (y), median (range)	9 (6–12)	9 (6–12)	9 (6–12)	0.367[a]
Gender, female	57%	56%	57%	0.909[b]
Sphere (D)	−2.13 ± 0.99	−2.25 ± 1.05	−2.00 ± 0.92	0.221
Cylinder (D)	−0.90 ± 0.93	−0.82 ± 0.88	−0.98 ± 0.98	0.437[a]
Spherical equivalent (D)	−2.58 ± 1.12	−2.66 ± 1.22	−2.49 ± 1.02	0.465
Axial length (mm)	24.3 ± 0.8	24.4 ± 0.7	24.2 ± 0.9	0.162
Central cornea				
ECD (cells/mm^2)[#]	3271 ± 215	3241 ± 178	3302 ± 246	0.160
CV (%)[#]	24.49 ± 1.92	24.67 ± 1.96	24.31 ± 1.88	0.346
HEX (%)	71.47 ± 7.11	71.06 ± 7.34	71.90 ± 6.92	0.560
Superior cornea				
ECD (cells/mm^2)	3475 ± 287	3461 ± 226	3488 ± 341	0.648
CV (%)	26.70 ± 3.26	26.95 ± 2.89	26.45 ± 3.62	0.451
HEX (%)	71.07 ± 7.45	69.24 ± 6.48	72.94 ± 7.97	**0.013**

ECD: endothelial cell density; CV: coefficient of variation in cell size; HEX: hexagonality.
[+] Probability values for between group differences using unpaired *t*-tests (unless otherwise specified); bold for p-values < 0.05.
[a] Mann-Whitney *U* tests.
[b] Pearson Chi-square.
[#] Significant differences in ECD and CV between the central and superior cornea using paired *t*-tests, p-values < 0.001.

endothelium, three were longitudinal studies. Hiraoka et al. [10] and Nieto-Bone et al. [14] included adults in their studies and they did not find significant change in ECD, whereas Cheung and Cho [13] reported ECD reduction in the children. As ECD can be affected by normal ageing in children, without information from control subjects, the change or lack of change in corneal endothelial morphology after ortho-k can be due to ageing or ortho-k lens wear, or both.

Previous studies mainly focused on changes in the central cornea, but little is known about the effects on the peripheral cornea. As the ortho-k lens is large and covers over 90% of the cornea, its use may lead to changes in the peripheral cornea which may differ from those observed in the central cornea. The primary objective of the current study was to compare the changes in endothelial morphology in the central and superior cornea over two years in children wearing ortho-k and controls wearing single-vision spectacles. The secondary objectives were to determine the morphological differences between the central and superior locations, and to determine factors affecting the endothelial morphology.

2. Methods

Endothelial images of subjects who had completed the Retardation Of Myopia In Orthokeratology (ROMIO) [4] and Toric Orthokeratology – Slowing Eye Elongation (TO-SEE) [7] studies were evaluated. These two studies investigated the effectiveness of orthokeratology for myopia control in children. The lenses and solutions used in these studies have been described elsewhere [4,7]. Endothelial images for the central and four peripheral corneal locations were captured using a specular microscope, TOPCON SP-2000P, but only images from the central and superior cornea were analysed. The superior cornea was selected as the peripheral site because pilot results showed a significantly highest ECD in this corneal location whereas there were no significant differences in ECD in the inferior, temporal and nasal cornea compared to the central cornea [13]. This may be related to the increased coverage of this part of the cornea by the upper eyelid in Asian eyes. For each subject, at least three images were captured for each corneal location and the clearest image was selected for analysis. The first image was selected if the image quality was similar for all the three captures [23]. Data from eyes with poor image quality or in which the cell count was less than 100 were excluded. Endothelial cells were manually retraced by one masked examiner (JW) trained to use the TOPCON IMAGEnet software (version 1.54). Endothelial cell morphology of the right eyes, including ECD, HEX, and CV, captured at baseline and after two years of the myopia control studies were compared between the ortho-k and control groups.

2.1. Statistical analysis

Commercially available software (SPSS 23.0; IBM Corp., Armonk, NY, USA) was used for statistical analysis. Paired *t*-tests were used to compare the baseline endothelial morphology in the two corneal locations for all subjects. Stepwise multiple linear regression was used to evaluate the association between baseline endothelium morphology and demographic data and baseline ocular parameters.

The baseline characteristics between the ortho-k and control subjects were evaluated to ensure that there was no between-group difference at the beginning of the study. The comparisons were performed using unpaired *t*-tests, Mann-Whitney *U* tests, or Pearson Chi-Squares, depending on the type of the data and the normality of the distribution of data. Repeated measures ANOVAs (or ANCOVAs) were used to compare the endothelial morphology at the baseline and the 24-month visits in the two study groups after controlling for covariates identified in the multiple linear regression models for the baseline characteristics. Unpaired *t*-test for between-group comparison of changes in the endothelial morphology was performed if significant interaction was found between time and study group. Factors affecting changes in the endothelial morphology were evaluated for the two study groups using stepwise multiple linear regression.

3. Results

Of the 136 participants who completed the two studies, data from 37 subjects were excluded (16 missing baseline; 21 poor image quality). For the remaining 99 subjects, approximately 50% had used ortho-k. There were no significant differences in the demographic data, including initial age and gender, and baseline ocular parameters, including refractive error and axial length, between the two groups of subjects (Table 1).

3.1. Baseline endothelial morphology

Baseline endothelial morphology for all subjects showed a higher ECD and CV in the superior cornea compared to the central cornea (unpaired *t*-tests, $p < 0.001$), but no significant difference in HEX

Table 2
Factors affecting baseline endothelial morphology.

	Standardized beta			Adjusted R^2	F	p-value[+]
Factors	Age	Gender	Axial length			
Central cornea						
ECD	−0.246	0.245	–	0.109	6.988	**0.001**
CV	–	–	–	–	–	–
HEX	–	–0.238	–	0.047	5.816	**0.018**
Superior cornea						
ECD	–	0.308	–	0.086	10.187	**0.002**
CV	–	–	–0.231	0.044	5.474	**0.021**
HEX	–	–	–	–	–	–

ECD: endothelial cell density; CV: coefficient of variation in cell size; HEX: hexagonality.
[+] Probability values for the multiple linear regression; other excluded variables: initial sphere, initial cylinder; bold for p-values < 0.05.

between the two locations (unpaired *t*-tests, p = 0.647) (Table 1). The baseline morphology was weakly associated with age, gender, and initial axial length at both corneal locations (Table 2). For instance, 11% of variance in central ECD could be explained by age and gender. In this model, ECD reduces by 42 cells/mm^2 for each year increase in age after controlling for gender. ECD in females was 106 cells/mm^2 higher than males after controlling for age. The mean ± SD ECD in the central cornea was 3208 ± 209 cells/mm^2 and 3320 ± 209 cells/mm^2 for male and female subjects, respectively, and this difference was statistically significant (unpaired *t*-test, p = 0.010). Baseline endothelial morphology in the central cornea was not associated with initial sphere or cylinder or axial length.

3.2. Effect of time and use of orthokeratology on endothelial morphology

At the beginning of the study, except for a significantly lower HEX in the superior cornea in ortho-k subjects (unpaired *t*-test, p = 0.013), there were no differences in the baseline endothelial morphology between the ortho-k and control subjects at the two corneal locations (unpaired *t*-tests, p > 0.160) (Table 1). Changes in the endothelial morphology over two years at the central and superior cornea in the two groups were shown in Table 3. Time was shown to have significant effect on ECD and CV at the central cornea and HEX at both corneal locations (repeated measures, p < 0.044), but not on ECD and CV at the superior cornea (repeated measures, p > 0.489). That is, for the central cornea, all the three parameters were significantly changed over time; but for the superior cornea, only HEX changed with time. Except for the central ECD with significant interaction (repeated measures, p = 0.032), there were no significant interactions found between time and study groups in the endothelial morphological parameters (repeated measures, p > 0.105). That is, except for the central ECD, the change or lack of change in endothelial morphology was not significantly different between the two groups. For the central ECD, *post-hoc* analysis comparing the changes between the two study groups showed that the reduction in central ECD was significantly greater in the control group (mean ± SD: 98 ± 91 cells/mm^2) than in the ortho-k group (mean ± SD: 56 ± 94 cells/mm^2) (unpaired *t*-test: p = 0.024) (Table 3).

At the central cornea, changes in ECD were not associated with the demographic data, baseline ocular parameters, or the baseline values in both groups of subjects (multiple linear regressions, p > 0.05). Changes in CV and HEX were weakly and negatively associated with their baseline values (CV controls: adjusted R^2 = 0.079, p = 0.028; CV ortho-k: adjusted R^2 = 0.100, p = 0.015; HEX controls: adjusted R^2 = 0.094, p = 0.018; HEX ortho-k: adjusted R^2 = 0.134, p = 0.006). At the superior cornea, changes in ECD and CV were not associated with the demographic data, baseline ocular parameters or its baseline value in both groups of subjects (multiple linear regressions, p > 0.05). Changes in HEX were negatively associated with the baseline value in the control subjects (adjusted R^2 = 0.240; p < 0.001) and associated with both gender (standardized beta = −0.287) and the baseline value (standardized beta = −0.279) in the ortho-k subjects (adjusted R^2 = 0.140; p = 0.012).

4. Discussion

The corneal endothelium has limited regenerating capacity. It consists of a single layer of cells which regulates ion transport of the cornea to maintain corneal health and transparency [15,16]. Most cells are in the shape of a hexagon and this structure is disturbed in the presence of cell loss or chronic stress [15]. The human cornea has up to 500,000 cells, with ECD up to 7500 cells/mm^2 at birth [15]. ECD reduction is most rapid in the first five years of age, dropping from about 4000 cells/mm^2 at the age of one year to 3500 cells/mm^2 at the age five years, and 3000 cells/mm^2 by age 20 [17–21]. The reduction in ECD before adolescence is mainly due to hypertrophy, as there are no remarkable changes in total cell counts [24]. ECD was negatively correlated with corneal diameter, but the association is significant only before the age of two, as the size of the cornea stabilizes in children aged between five and 14 years [17,21]. The current results agree with previous studies which reported that age-related reduction in ECD is accompanied by a reduction in HEX and an increase in CV in both normal children and adults [19,20,24,25]. As central ECD, CV, and HEX change with time in the control group (3.0% reduction in ECD, 2.2% increase in pleomorphism, and 0.4% increase in polymegathism), it was expected to find an association between these parameters with time. However, the changes in ECD were not associated with demographic or ocular parameters identified in the current study (*i.e.* age, refractive error, axial length and baseline ECD) in both corneal locations. This may suggest that the reduction of ECD in normal children is a natural process not influenced by external factors.

In addition to reduced ECD in older children, the current study also found that girls had higher ECD than boys. A few studies have reported

Table 3
Mean changes in the endothelial morphology over two years in the two groups of subjects.

	Effect of time[#]	Time*Group[#]	Orthokeratology (N = 50)	Control (N = 49)	Between groups p-value[+]
Central cornea					
ECD (cells/mm^2)	**0.017**	*0.032*	−55.5 ± 94.0	−98.4 ± 91.5	**0.024**
CV (%)	**0.030**	0.105	0.69 ± 1.76	0.10 ± 1.84	ns
HEX (%)	**0.044**	0.949	−1.52 ± 6.06	−1.59 ± 6.18	ns
Superior cornea					
ECD (cells/mm^2)	0.803	0.998	−13.7 ± 169.4	−13.8 ± 151.0	ns
CV (%)	0.489	0.247	1.24 ± 2.72	0.54 ± 2.81	ns
HEX (%)	**0.001**	0.183	−1.58 ± 7.77	−3.71 ± 8.06	ns

ECD: endothelial cell density; CV: coefficient of variation in cell size; HEX: hexagonality.
[#] Probability values for the repeated measures ANOVA/ANCOVA for the within subject effect and interaction; bold for p-values < 0.05.
[+] Probability value of the unpaired *t*-test to compare changes in central ECD between the ortho-k and control subjects.

no differences in ECD between males and females in young adolescents and young adults [20,25,27] but it is known that ocular parameters change before adulthood. In children, girls have smaller corneas than boys [26]. If the corneal diameter plays a role in ECD, then higher ECD would be expected in girls than in boys and this was observed in the current study. However, corneal diameter was not measured and could not be used to explore the effect of gender and corneal diameter on ECD.

Some researchers have found significant association between ECD and refractive error, that is, high myopia is associated with lower ECD [25,27,28]. Again, age and gender were not considered in their analyses. Sheng and Bullimore [29] investigated the effects of age, refractive error, ethnicity, and years of contact lens wear on ECD, CV, and HEX in adults aged between 19 and 71 years using multiple linear regression model. They found that ECD was negatively associated with age and positively associated with Asian ethnicity, whereas CV was positively associated with age and contact lens wear, and HEX was negatively associated with age and myopia. The current results agreed with their findings with respect to ECD not being associated with refractive error. However, the current study did not find any significant effect of age on CV and HEX, or any association between HEX and refractive error. In view of the limited number of reports, further study is warranted to investigate the effect of age, gender, and refractive error on endothelial morphology.

The current study shows that endothelial morphological changes were not associated with ortho-k lens wear. One of the concerns in ortho-k lens wear is hypoxia due to the overnight wear modality. Direct injury or chronic disorder of the corneal endothelium can lead to cell loss resulting in reduction in ECD, and increases in pleomorphism and polymegathism. Physiologically, the cornea suffers from hypoxia during sleep as eye closure reduces the oxygen supply to the eyes. Corneal oedema induced during sleep dissipates within minutes of waking up. The level of oedema during sleep and recovery upon awakening is affected by the presence of a contact lens. Sleeping with contact lenses made from low oxygen permeable material thus retards the recovery of hypoxia, resulting in chronic corneal oedema when the lenses are worn every night. Lens materials with oxygen transmissibility (Dk/t) of 87 and 125 units are recommended to avoid 3% and 4% oedema, respectively [30,31].

Most current ortho-k lenses are made of highly oxygen permeable materials with Dk 100 or above. With lens thickness ranging from 0.15 to 0.20 mm, the Dk/t of ortho-k lenses varies from around 60 to 79. Unlike soft lenses covering both cornea and limbus, the lens diameter of an ortho-k lens is smaller than the corneal diameter, allowing some degree of oxygen supply in the limbal area. Although Dk/t for most of the ortho-k lenses is lower than the recommended value to avoid corneal oedema during sleep, these recommended values are more meaningful for extended wear modality of soft lenses. In extended wear, the lenses cover both the cornea and limbus and remain on the eyes after eye opening, such that lower Dk/t lenses will have slower corneal recovery. Unlike conventional contact lenses that correct vision with lenses *in situ*, ortho-k corrects vision by molding the cornea during sleep. Patients are required to remove their lenses after waking and thus, ortho-k will have minimal effect on recovery from hypoxia. The recommended Dk/t value for avoiding oedema, derived from *in vitro* conditions, is less crucial and serves better as a guideline for lens selection. Dk/t of the ortho-k lenses used in this study was 79 and results show that it exerted minimal corneal stress to the endothelium after two years of lens wear resulting in insignificant effects on polymegathism and pleomorphism, as there were no differences between the two groups of subjects. One issue left unanswered is the slower reduction in ECD in ortho-k subjects compared to the controls. Further study is warranted to confirm this observation.

Because of the limited regenerating power, endothelial cells in the peripheral cornea serve as a 'physiologic reserve and storage region' especially in wound healing [32]. Although the limbus is spared in ortho-k lens wear, little is known about the physiological response towards ortho-k in the peripheral cornea, as previous studies have mainly focused on the effect in the central region [10–14]. Results from *in vivo* studies on endothelial morphology in humans vary with respect to characteristics of the subjects (*e.g.* age, contact lens experience *etc.*), instrumentation and method of cell analysis, and locations of peripheral cornea examined [22,23,32–35]. Wiffen et al. [33] reported a higher ECD in the central cornea compared to the temporal location in 84 non-contact lens wearers, aged 46 ± 18 years, but observed no difference in ECD in 43 contact lens wearers, aged 35 ± 9 years, who had been using contact lenses for over five years. Zheng et al. [22] did not find any difference in ECD and HEX between the central and inferior cornea in 80 normal Chinese aged 0 to 79 years. However, despite the differences in the methodology, most studies reported an increase in ECD in para-central and peripheral cornea either in children [23] or in adults [32,34,35]. Only two *in vivo* studies examined topographic difference in ECD [23,32], both reporting highest ECD in the superior cornea compared to the other three peripheral locations, but reasons for the differences were not suggested. The ECD in the inferior, nasal, and temporal cornea was similar. The current study selected the superior cornea for the investigation of changes in the peripheral cornea, because the results of pilot study on ortho-k showed highest ECD in the superior cornea [13]. Eyelid position may be a possible reason accounting for the difference. Whilst the cornea in the nasal, temporal, and inferior regions is usually exposed to the atmosphere, the superior cornea is usually covered by the upper eyelids, especially in Asian eyes, with small vertical palpebral aperture height, causing chronic hypoxia in the superior cornea, resulting in lower HEX and higher CV. If upper eyelids do play a role, it is expected that morphology in the nasal, temporal, and inferior cornea would be similar, thus, these areas were not investigated in the current study.

Topographic *in vivo* assessment of polymegathism and pleomorphism are less well studied. If the upper lid does affect endothelial morphology, the superior cornea would be expected to have higher CV and lower HEX than the central cornea. This hypothesis is supported by a histological study by Holley et al. [36] but no conclusion can be drawn from *in vivo* studies [22,32,33]. Wiffen et al. [33] found lower HEX in the central cornea with no significant difference in CV. Amann et al. [32] did not find any significant difference in HEX and CV between central and peripheral locations. Zheng et al. [22] found lower HEX in the peripheral cornea. In the current study, higher CV in the superior cornea was observed, but no significant difference in HEX was found. The honeycomb geometry of the endothelium is the most stable structure in nature for distributing stress. High HEX is essential to maintain endothelial function and the lack of difference in central and peripheral locations in the current study may suggest that homeostasis of the endothelium is maintained in the healthy cornea in children aged 6–12 years. Change in HEX in the superior cornea was similar to that observed in the central area, but the changes in ECD and CV in the superior cornea did not reach statistically significance. Considering the variability of the endothelial cell evaluation of CV and HEX, the effects of normal ageing on polymegathism and pleomorphism in the central and superior cornea indicate a need for further investigation of these parameters in children.

In summary, ortho-k had a minimal effect on the endothelial morphology of children after two years of lens wear. The changes in endothelial morphology observed over the two years period were primarily driven by the normal ageing process.

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