SAFETY OF LONG-TERM ORTHO-K (SOLO) STUDY: CORNEAL AND OCULAR MICROBIOME CHANGES IN CHILDREN UNDERGOING ORTHOKERATOLOGY TREATMENT AND CONCERNS OF CHILDREN, PARENTS AND PRACTITIONERS ABOUT THE TREATMENT

CHEUNG SIN WAN

PhD

The Hong Kong Polytechnic University

2018

The Hong Kong Polytechnic University
School of Optometry

Safety of Long-term Ortho-k (SOLO) Study: Corneal and Ocular Microbiome Changes in Children Undergoing Orthokeratology Treatment and Concerns of Children, Parents and Practitioners about the Treatment

Cheung Sin Wan

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

October 2017

CERTIFICATE OF ORIGINALITY

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

Signed: _____

Cheung Sin-Wan

ABSTRACT

Abstract of dissertation entitled:

Safety of long-term ortho-k (solo) study: corneal and ocular microbiome changes in children undergoing orthokeratology treatment and concerns of children, parents and practitioners about the treatment

Submitted by ***Cheung Sin Wan*** *for the degree of PhD at The Hong Kong Polytechnic University in October 2017*

Background

The rapidly increasing prevalence of myopia is an issue of global concern. Orthokeratology (Ortho-k) has been shown to be an effective myopia control treatment in children. Current evidence from clinical studies shows that the treatment is safe for children provided there is good compliance with proper usage and care by all parties involved in the treatment, i.e. children, parents and practitioners. Occurrence of microbial keratitis in the community implies a certain degree of non-compliance from the parties involved in the treatment. As microbial keratitis can have serious consequences, prevention of this and other possible adverse effects that may be associated with orthokeratology should be assessed to determine if they can be avoided or ameliorated. The following gaps in knowledge regarding the safety of ortho-k lens wear in children were investigated in this thesis:

1. Changes in corneal endothelium associated with ortho-k lens wear or normal ageing
2. Contamination is a risk for microbial keratitis, however, sources of contaminants have not been investigated

3. Compliance of patients is affected by the attitudes of users/parents towards the treatment, but little attention has been given to this area
4. Safe ortho-k practice for myopia control could be improved by careful patient selection; however, there are no guidelines for myopia control treatment for practitioners

Objectives

1. To investigate changes (if any) on the corneal endothelium of children wearing ortho-k lenses or spectacles for two years
2. To compare the ocular microbiota of children with and without ortho-k lens wear and to determine the sources of contaminations of their accessories
3. To evaluate the attitudes towards ortho-k of parents seeking myopia control treatment for their children
4. To develop a decision tree for patient selection for practitioners using ortho-k for myopia control

Methods

Four studies were conducted with different experimental designs to address the above objectives.

Study 1. A retrospective cohort study to compare the changes in corneal endothelial morphology in the central and superior segments of subjects

aged six to 12 before and two years after wearing orthokeratology and single-vision spectacles.

Study 2. A cross-sectional study to compare the carriage rates of ocular microbiota in the peri-orbital tissues and the accessories of two groups of subjects aged seven to less than 15, one group using orthokeratology over a year and the other single-vision spectacles. The associations between carriage of *Staphylococcus aureus* and Gram-negative bacteria and contamination of accessories were determined.

Study 3. A telephone interview with parents seeking myopia control for their myopic children was conducted to assess their attitudes towards the use of orthokeratology, the use of daily wear soft contact lenses, and spectacles for myopia control.

Study 4. A prospective study to investigate the effect of history of myopia progression and age on the efficacy of myopia control by comparing the rate of axial elongation after switching from seven months of spectacle-wear to another seven months of orthokeratology-wear in subjects aged six to less than 16. A protocol for myopia treatment based on the individual characteristics was derived from the results.

Results

Study 1. Ortho-k retarded reduction in endothelial cell density in the central cornea ($p = 0.024$). Otherwise, it had little influence on polymegathism and pleomorphism induced by normal ageing ($p > 0.105$).

Study 2. Bacterial count in the conjunctiva was reduced after orthokeratology ($p = 0.009$), but the diversity of the bacteria in other skin tissues was not

affected. The diversity of bacteria of the accessories was similar to that found in the skin ($p > 0.122$). However, association between carriage of bacteria in the skin tissues and in the accessories was only significant for *Staphylococcus aureus* in the control subjects ($p = 0.029$).

Studies 3. Parents were conservative about children using contact lenses for refractive correction, but were more receptive to use the lenses for myopia control. Confidence in the treatment was affected by prior knowledge of myopia control strategy.

Study 4. More significant reduction in axial elongation was observed in subjects with a history of rapid myopia progression, whereas no beneficial effect was observed in those displaying slow elongation. The history of progression was affected by age, thus, a decision tree for myopia control based on age and history of progression was proposed.

Conclusions

The safety of orthokeratology was confirmed as it exerted minimal stress on the cornea. Risk of contamination could be reduced by regular review of handling procedures to identify patients/parents with poor hygiene or poor handling habits, whereas compliance with correct ortho-k practice was affected by positive exposure to this treatment. Myopia control was not indicated for non-progressing myopic children. Safety of the treatment could be further improved with the aid of the proposed guideline for selecting candidates for whom the benefits outweigh the risks.

PUBLICATIONS ARISING FROM THE THESIS

Journal articles

1. Cheung, S. W., Boost, M. V., Cho, P. (2018) Pre-treatment observation of axial elongation for evidence-based selection of children in Hong Kong for myopia control. *Cont Lens Anterior Eye*. (in press).

2. Cheung, S. W., Cho, P. (2018). Does a two-year period of orthokeratology lead to changes in the endothelial morphology of children. *Cont Lens Anterior Eye*. 41:214-218.

3. Cheung, S. W., Boost, M. V, Shi, G. S., Cho, P. (2016). Microbial contamination of periorbital tissues and accessories of children. *Optom Vis Sci*. 93: 612-618.

4. Cheung, S. W., Lam, C., Cho, P. (2014). Parents' knowledge and perspective of optical methods for myopia control in children. *Optom Vis Sci.* 91: 634-641.

Conferences papers

1. Cheung, S. W., Boost, M., Cho, P. Decision tree for myopia control management. Paper presentation at *The 2018 BCLA Asia Conference.* 17-18 September 2018, Singapore, Singapore.
2. Cheung, S. W., Cho, P. Effect of two-year orthokeratology lens wear on the endothelial morphology of children. Paper presentation at *The 2016 BCLA Asia Conference*. 13-14 September 2016, Hong Kong, China.
3. Cheung, S. W., Boost, M. V., Shi, G. S., Cho, P. Normal flora in young children with and without orthokeratology treatment. Paper presentation at *The 2015 British Contact Lens Association Clinical Conference* 29-31 May 2015, Liverpool, UK.
4. Cheung, S. W., Shi, G. S., Boost, M. V., Cho, P. Normal flora in young children with and without orthokeratology treatment. Poster presentation at *The 4th Asia Orthokeratology and Specialty Lens Conference*, 13-14 December 2014, Taipei, Taiwan.
5. Cheung, S. W., Chan, Y. Y., Cho, P. Orthokeratology for myopia control in older and younger children: a short-term study. Poster presentation at *The 4th Asia Orthokeratology and Specialty Lens Conferenc*e, 13-14 December 2014, Taipei, Taiwan.
6. Cheung, S. W., Lam, C., Cho, P. Parent's perspective on myopia control treatment options. Poster presentation at *The 3rd Asia Orthokeratology and Specialty Lens Conference*, 30-31 March 2012, Hangzhou, China.

ACKNOWLEDGEMENTS

I would like to thank Prof Pauline Cho for her guidance and supports during my study and her patience while I was having difficulties. She was my lighthouse when I was stumbling in the dark.

I would also like to thank Dr Maureen Boost not only for her help with writing, but also her professional advice on microbiology.

I have to thank Dr Dede Chan, Ms Christie Lam, Mr Jason Lau, Ms Paggie Pang, Dr Guangsen Shi, and Ms Jianglan Wang for their assistance in the studies reported in this thesis, and to my colleagues at the ortho-k research team: Mr Ben Chan, Ms Cherie Chan, Ms Jessie Charm, Dr Connie Chen, Dr Shanica Hon and Dr Tsui Tsui Lee.

This work was supported by The Hong Kong Polytechnic University and Menicon Co. Ltd. (Collaborative Research Agreement: H-ZG35).

It has been a difficult path for me. Thank you to my friends Ms Sandy Chat, Ms Karen Leung, Ms Alice Lok, Dr Caren Sheng and Dr Wing Tang for your sharing thoughts and your encouragement.

Last, I would like to thank my family for their unfailing support during all these years!

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

BHI	Brain Heart Infusion
CFU	Colony-forming unit
CNC	Computer numeric-controlled
CNS	Coagulase-negative staphylococci
CV	Coefficient of variation in cell size
D	Diopter
Dk	Oxygen permeability
Dk/t	Oxygen transmissibility
DWSL	Daily wear soft lenses
ECD	Endothelium cell density
FDA	Food and Drug Administration
HEX	Hexagonality
HM-PRO	High myopia partial reduction ortho-k (study)
MPS	Multipurpose solutions
NNT	Number needed to treat
LORIC	Longitudinal orthokeratology research in children (study)
Ortho-k	Orthokeratology
PBS	Phosphate buffered saline
PMMA	Polymethylmethacrylate
RCT	Randomized controlled trial
RGL	Reverse geometry lens
ROMIO	Retardation of myopia in orthokeratology (study)
S. aureus	Staphylococcus aureus
SER	Spherical equivalent refraction
TLT	Tear lens thickness
TO-SEE	Toric orthokeratology slowing eyeball elongation (study)
VA	Visual acuity

CHAPTER 1

Literature Review

1.1 Introduction to orthokeratology: from refractive correction to myopia control

Conventional contact lenses correct refractive errors while in situ on the cornea in waking hours without altering corneal shape. In contrast, orthokeratology (ortho-k) corrects the refractive error of the eye by reshaping the cornea with lenses in situ during sleep, hence reducing the corneal refractive error. Although, the molding effecting is temporary and reversible; improved unaided vision is sustained throughout the day after lens removal after stabilization of the treatment and if the ortho-k lenses are applied regularly at night. Initial reports providing the clinical impression of retardation of myopia progression in children on ortho-k treatment (Cho *et al.*, 2002; Cho *et al.*, 2003; Cheung *et al.*, 2004), inspired researchers to conduct clinical trials to gain stronger clinical evidence for the effectiveness of ortho-k for myopia control. Moving from prospective cohort studies (Cho *et al.*, 2005, Walline *et al.*, 2009; Kakita *et al.*, 2011; Hiraoka *et al.*, 2012; Santodomingo-Rubino *et al.*, 2012; Chen *et al.*, 2013) to randomized clinical trials (Cho and Cheung, 2012; Charm and Cho, 2013) and meta-analyses (Si *et al.*, 2015; Sun *et al.*, 2015; Wen *et al.*, 2015; Huang *et al.*, 2016; Li *et al.*, 2016), ortho-k has been shown to be effective in slowing axial elongation by about 40% compared to myopic children receiving single-vision correction. This

treatment is considered to be the second best myopia control option compared to moderate-to-high concentration atropine (Huang *et al.*, 2016) but without the side effects of atropine, such as photophobia and blurred near vision (Chua *et al.*, 2006; Chia *et al.*, 2016).

The treatment rate of ortho-k in Hong Kong is one of the highest in the world (Morgan *et al.*, 2013) and has increased by three to four fold in the last decade (Yung *et al.*, 2005; Charm *et al.*, 2010; Morgan *et al.*, 2013). Over 75% of patients using this treatment to control myopia are children and its popularity is likely to be due to both the efficacy for myopia control and freedom from the need for vision correction aids in the daytime provided by the treatment (Cho *et al.*, 2002a; Cho *et al.*, 2003a; Lipson *et al.*, 2005). This chapter will review the use of ortho-k for refractive correction and evidence for myopia control.

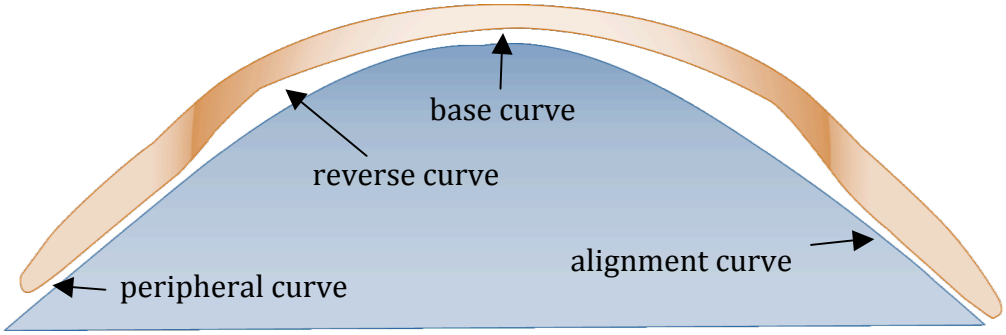
1.1.1 History of orthokeratology

Ortho-k, which is also known as corneal reshaping therapy, is a procedure which temporarily reduces refractive error. It developed from a technique which originated in the 1960s involving wearing a polymethylmethacrylate (PMMA) lens that was fitted flatter than the corneal curvature of the eyes (Kerns 1976, 1976a). The refractive outcome was limited to around -2.00 D and was unpredictable due to the poor centration of the lens (Kerns, 1976, 1976a). The treatment became popular in 1980s with the advancement in technology, including the introduction of reverse geometry lens (RGL) designs for better lens centration and refractive correction, improvement of

corneal topography for more accurate measurement of corneal contour for lens design, introduction of computer numeric-controlled (CNC) lathes for accurately fabricating the lenses, and the availability of high oxygen permeable (Dk) materials for overnight wear, improving compliance and refractive outcomes (Mountford, 2004).

The earliest RGL lens was a 3-curved design for daytime correction. However, it has been replaced by the 4- or 5-curved design to be worn during sleep for better comfort and predictability. The four curves in a typical 4-curved RGL design are base curve, reverse curve, alignment curve and peripheral curve (Figure 1.1). The base curve, which is flatter than the cornea, is used to control the refractive correction. The alignment curve is designed to fit on the cornea in the periphery to aid centration. The reverse curve joins the base curve to the alignment curve and the peripheral curve and provides edge lift for tear exchange. Because the reverse curve is steeper than the base curve and alignment curve, an RGL can be made easily by CNC lathes, but not by hand.

Overnight ortho-k not only reduces myopia by up to -6.00 D (Barr *et al.*, 2003; Mountford, 2004; Swarbrick, 2006), but also has the potential to correct astigmatism (Chen *et al.*, 2012; Luo *et al.*, 2014; Paune *et al.*, 2012), hyperopia (Gifford and Swarbrick, 2008), and presbyopia (Gifford and Swarbrick, 2013). Currently, the term ortho-k usually refers to the use of this overnight RGL which will be adopted in this dissertation.



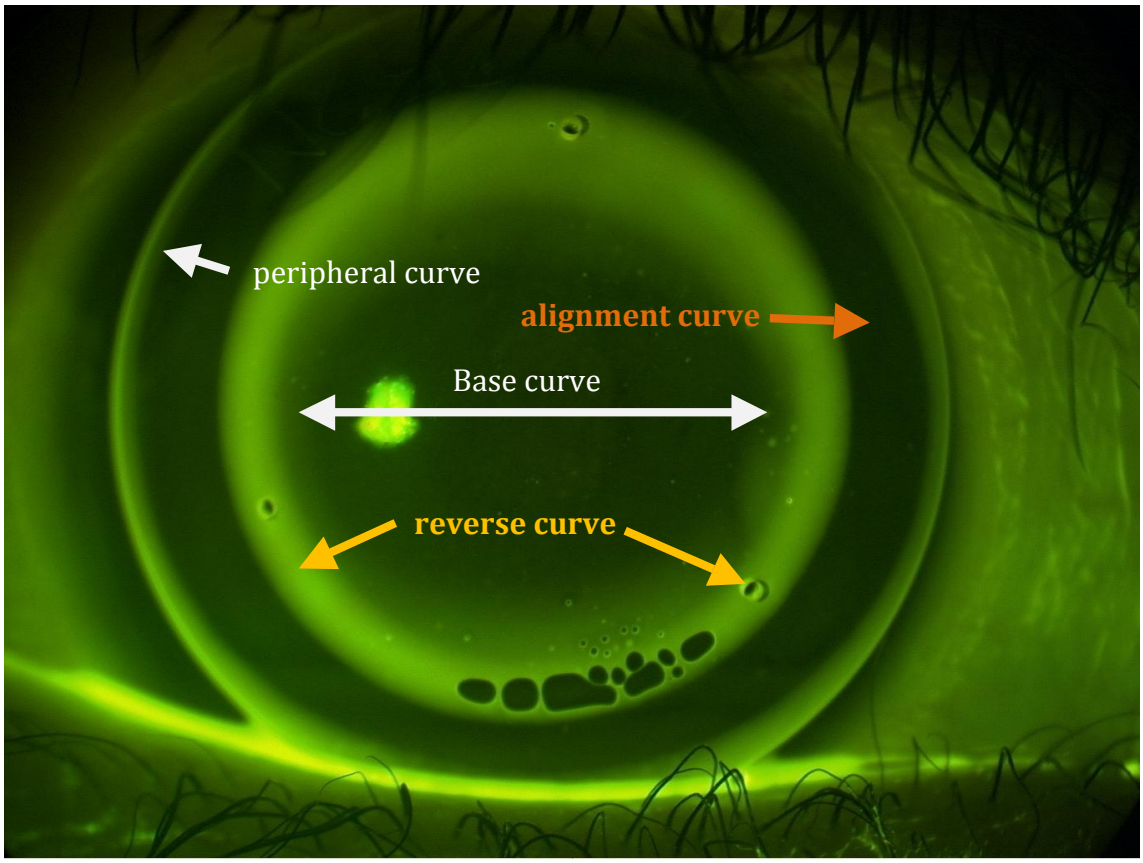


Figure 1.1 The lens design (above) and the fluorescein pattern (below) of a 4-curved orthokeratology lens

1.1.2 Orthokeratology for refractive correction

Since its introduction in the new millennium, overnight ortho-k has been shown to be effective in correcting myopia with minimal regression in the daytime, such that successful patients can have stable vision throughout the day by using the lenses nightly or every other night. Paragon CRT lens made in HDS and HDS 100 materials (Paragon Vision Science, AZ, US) was the first overnight RGL lens to receive premarket approval for corneal refractive therapy from the United States Food and Drug Administration (FDA) in 2002 (Paragon Vision Science, 2002). It is indicated for temporary reduction of myopia up to 6.00 D in eyes with astigmatism of not more than 1.75 D. The other lens that has received similar premarket approval is the Boston VST made in Equalens II material (Bausch & Lomb, NY, US) which was granted in 2004 for temporary reduction of myopia up to 5.00D in eyes with astigmatism not more than 1.50 D (Bausch & Lomb, 2004). That is, the effectiveness and safety of these lenses for refractive correction have been established by international standard.

An effective ortho-k treatment for refractive correction should include these elements: high success rate, predictable outcomes, short treatment course for good unaided vision, sustained refractive correction throughout the day, and, lastly, minimal threats to vision and ocular health and safety in use. The effect on predictable refractive changes and safety at the end of the study period (9 months of lens wear) was presented in the two premarket applications. However, the time course of changes and daytime regression were not reported.

Although the early research by Mountford (1997) and Fan and co-workers (1999) on refractive correction of overnight ortho-k could not establish the safety and effectiveness (e.g. success rate, predictability of visual outcomes and daytime regression) of the treatment as the results merely illustrated trends without any statistical descriptions nor any statistical analyses, their results did provide a frame work for the following research. The first reported systematic evaluation of the performance of overnight RGL was performed by Nichols and co-workers in 2000. They studied the changes in refractive error, visual acuity (VA), corneal topography, and corneal thickness in 10 adults with refractive sphere and cylinder not more than -3.50 D and -1.00 D, respectively, wearing ortho-k lenses made in fluorosilicone acrylate material with oxygen permeability of 88 for 60 days. The success rate was 80% as two subjects were excluded after 1 and 14 days of lens wear due to significant corneal staining and lens intolerance respectively. Good unaided vision and full correction were achieved at 7 and 60 days, respectively, after lens wear. Unaided high contrast vision (mean logMAR VA: -0.03 ± 0.16) was good and sustained at the end of the study for 8 hours after lens removal. Central corneal flattening and thinning were observed throughout the study. The majority of changes occurred in the first week of lens wear and the changes slowed down and stabilized after this time. Except for the excluded subject with significant corneal staining, no other significant adverse event was reported.

The authors evaluated the success rate, predictability of refractive correction, and safety of ortho-k. However, due to the wide gap between visits and the

lack of appropriate statistical analysis, the time course for refractive change as well as the daytime regression in refraction and vision were either unknown or inconclusive. Generalization of results was also limited by the small sample size. Following this study, research conducted in different parts of the world has provided answers to fill this knowledge gap (Rah *et al.*, 2002; Alharbi and Swarbrick, 2003; Soni *et al.*, 2003; Tahhan *et al.*, 2003; Walline *et al.*, 2004a; Sorbara *et al.*, 2005; Chan *et al.*, 2008). They showed that the success rate of ortho-k fitting is high (over 80%) and the success rate can be improved by prior training for eye care providers (Rah *et al.*, 2002). For low to moderate level of myopia, about 50% correction can be achieved after one night of lens wear and full correction achieved within 2 to 4 weeks of treatment (Alharbi and Swarbrick, 2003; Soni *et al.*, 2003; Tahhan *et al.*, 2003; Chan *et al.*, 2008). Regular use of the lenses can provide good whole day vision which can last for six to eight hours (Rah *et al.*, 2002; Walline *et al.*, 2004a; Chan *et al.*, 2008) without causing significant adverse effects on the eyes (Rah *et al.*, 2002; Soni *et al.*, 2003; Tahhan *et al.*, 2003; Walline *et al.*, 2004a; Sorbara *et al.*, 2005).

1.1.2.1 Mechanism of refractive correction in orthokeratology

An RGL is supported by the alignment curve and the reverse curve, which as it is much steeper than the curvature of the cornea, does not come into contact with the cornea but creates a tear reservoir. The optimal tear lens thickness (TLT) at the central cornea is about 10 μm. This thin TLT will give an impression of central corneal touch in normal fluorescein assessment

(Figure 1.1). Tears in the tear reservoir are sealed off, between the back optic zone curve and the alignment curve. The presence of the minimal TLT behind the back optic zone and the thick TLT in the tear reservoir creates a positive compressive force in the back optic zone and negative suction force in the reserve zone (Mountford, 2004b). Corneal flattening is achieved by the resultant force of the gradient difference in the hydraulic pressures within the alignment zone. The hydraulic pressures alter not only the corneal curvature, but also the corneal thickness. In myopic correction with ortho-k, central corneal flattening is accompanied by central corneal thinning (Swarbrick *et al.*, 1998; Alharbi and Swarbrick, 2003; Wang *et al.*, 2003).

It has been proposed that corneal flattening is mainly due to central cell compression (change in cell height) followed by redistribution of corneal tissue (changes in number of cell layers) resulting in central corneal thinning (Swarbrick *et al.*, 1998; Swarbrick, 2006). The variation in corneal sagittal height alters the anterior contour of the cornea (corneal flattening), thus reducing the power of both the cornea and the eye (refractive correction).

The process of cell compression and redistribution of corneal tissue in the central cornea has been demonstrated by histological examination of the cornea in animal studies by evaluating the changes in number of layers and shape of the corneas of rabbits (Matsubara *et al.*, 2004), primates (Cheah *et al.*, 2008) and cats (Choo *et al.*, 2008). Central corneal compression is the initial response to the pressure created underneath the lens, causing a downward displacement of the epithelial cells in the central cornea. Cells are squeezed together in the vertical direction resulting in central corneal

thinning. This process usually happens within the first 8 hours of lens wear without affecting the number of cell layers (cell compression) (Cheah *et al.*, 2008; Choo *et al.*, 2008). The change in mid-peripheral cornea is minimal at that period of time (Cheah *et al.*, 2008; Choo *et al.*, 2008). With continuous application of the lenses and pressure exerted underneath the lens, redistribution of corneal tissues occurs such that cells start to displace from the center towards the mid-peripheral area causing a reduction in the number of epithelial cell layers centrally and an increase in the number of cell layers mid-peripherally (Matsubara *et al.*, 2004; Cheah *et al.*, 2008; Choo *et al.*, 2008). These changes cause further changes in the anterior corneal contour thus further corneal flattening.

The rate of corneal changes varied from study to study which may be due to the species of the animals used and the wearing modality of the ortho-k lenses, but the trends observed were pretty similar. Slowest changes were observed in the rabbit model in which ortho-k lenses were used 8 hours a day for 28 days (Matsubara et al, 2004). The lenses were used on a daily basis, but the authors did not mention how they ensured that the eyes were closed during use of ortho-k lenses. The lens wear duration in primates (Cheah *et al.*, 2008) was longer (started from 4 hours up to 24 hours), whereas the lens wear duration in cats (Choo *et al.*, 2008) was the longest (started from 4 hours and up to 14 days). Therefore, changes observed in the cats' eyes were the most significant.

The cell compression and redistribution model in human eyes was evaluated by the changes in the thickness of the individual layers in human cornea

(Swarbrick *et al.*, 1998; Alharbi and Swarbrick, 2003; Wang *et al.*, 2003; Haque *et al.*, 2007; Zhong *et al.*, 2009; Nieto-Bona *et al.*, 2011, 2011a). Although ortho-k lenses were used in the daytime instead of during sleep, when Swarbrick and co-workers (1998) proposed this model, the daily wear of RGL lenses will correct myopia in a similar manner as overnight ortho-k, just take a longer time to achieve the target correction. Results showed that the changes in the central cornea were epithelial in origin (Alharbi and Swarbrick, 2003; Zhong *et al.*, 2009; Nieto-Bona *et al.*, 2011) with reduced response in older adults (Jayakumar and Swarbrick, 2005).

The findings of the effects of ortho-k on the stroma and on mid-peripheral corneal thickness are not in good agreement. Alharbi and Swarbrick (2003) reported mid-peripheral corneal thickening was stromal in origin, whereas Nieto-Bona and co-workers (2011) did not find significant change in mid-peripheral corneal thickness. In animal studies, mid-peripheral epithelial thickening observed in cat's eyes (Choo *et al.*, 2008) was not observed in monkey's eyes (Cheah *et al.*, 2008), in which mid-peripheral stromal thickening was reported otherwise. Stromal response is complicated by the oedematous response in overnight wear (Alharbi *et al.*, 2005). The differences can also be related to the study designs, methods of measurements and location of examination which indicates a need for further investigation of the stromal involvement in the central and mid-peripheral cornea. However, it does not affect the current model for refractive correction in ortho-k. That is, alteration of the corneal curvature is achieved by redistribution of epithelial tissues driven by the hydraulic pressures created

underneath the lenses in the central cornea resulting in central corneal thinning as well as central corneal flattening. The actual refractive changes can be predicted by the changes in central corneal thickness (corneal sag) and central corneal curvature.

Corneal bending to account for refractive correction in ortho-k has been rejected. Swarbrick and co-workers (1998) applied Munnerlyn's formula to demonstrate that the refractive changes could be sufficiently explained by the change in the anterior surface without the involvement of the posterior surface. Although there are two papers showing posterior corneal changes (Owens *et al.*, 2004; Gonzalez-Mesa *et al.*, 2013), the majority of the studies did not find any significant change in posterior curvature (Stillitano *et al.*, 2007; Tsukiyama *et al.*, 2008; Chen *et al.*, 2010; Yoon and Swarbrick, 2013) nor change in anterior chamber depth (Tsukiyama *et al.*, 2008; Chen *et al.*, 2010; Cheung and Cho, 2013) to support the corneal bending theory.

Most, but not all, of the refractive correction in ortho-k can be accounted by the change in corneal curvature. The residual may be attributable to the change in corneal thickness and refractive index of the cornea. Recently, it has been proposed that choroidal thickening may shorten the axial length thus, reducing the overall myopia of the eye. It is reported that choroidal thickness increases with age in children and myopic children have a thinner choroidal layer than non-myopic children (Ruiz-Medrano *et al.*, 2014; Read *et al.*, 2015; Xiong *et al.*, 2017). The effect of ortho-k on choroidal thickness and axial length is still unclear as there are only two reports published. In a short term study (Chen *et al.*, 2016), choroidal thickness was found to be

increased in subjects wearing ortho-k and remained unchanged in subjects wearing spectacles after one month of treatment. A significant association was found between the increase in choroidal thickness and reduction in axial length in both groups of subjects. On the other hand, results from a longer term study (Gardner *et al.*, 2015) showed no significant changes in either axial length or choroidal thickness in nine subjects after wearing ortho-k for nine months. The research on the effect of ortho-k on the choroid is still ongoing. Further work is warranted to confirm the role of choroid on refractive error after ortho-k as well as its role in myopia development.

In summary, overnight ortho-k has been demonstrated to be able to effectively correct low to moderate myopia in both adults and children within one week (Soni *et al.*, 2003; Tahhan *et al.*, 2003; Sorbara *et al.*, 2005) to one month (Rah *et al.*, 2002; Chan *et al.*, 2008) of lens wear without causing any significant complications. Daytime regression in refractive error and unaided VA was minimal (Nichols *et al.*, 2000; Rah *et al.*, 2002; Walline *et al.*, 2004a; Sorbara *et al.*, 2005). Influence of variation in lens design in 4-curved RGL on efficacy of refractive correction is minimal (Tahhan *et al.*, 2003), but the effectiveness of refractive correction can be reduced by wearing lenses made in low Dk materials (Dk < 100) (Lum and Swarbrick, 2011). The Dk of materials used in modern overnight lenses is usually not less than 100, which is sufficient to provide good clinical performance as well as having a minimal effect on corneal physiology. Lack of severe adverse events reported in these clinical studies suggested that, with good care provided by the

practitioners and good compliance from subjects, the risk involved in this treatment can be minimized.

1.1.3 Orthokeratology for myopia control

Myopia is a refractive error of the eye in which images of far distance objects focus in front of the retina causing blurry distance vision. It cannot be merely regarded as an optical disorder, which can be simply rectified by optical methods (e.g. glasses and contact lenses) or surgical methods (e.g. laser refractive surgery). Myopic eye is usually longer than average and high myopia can become a pathological condition which can be associated with various ocular disorders such as cataract, glaucoma, macular degeneration and retinal detachment (Saw *et al.*, 2005; Hayashi, *et al*., 2010; Marcus *et al*., 2011; Pan *et al.,* 2013). It raises concerns in public health as these problems can be sight-threatening and affect the quality of life, especially with early onset of these pathological disorders (Holden *et al.*, 2014; Wong and Saw, 2016).

The optical imperfection in myopia is a mismatch of the refractive power of the eye and the axial length, that is, either axial length is too long or refractive power of the eye is too strong. Infants are born to be hyperopic, as they have small eyeballs. Emmetropization occurs when the growth in axial length is complemented by the internal refractive power of the eye. It usually stabilizes between 6 to 9 years of age. Juvenile myopia describes the continued increase in axial length beyond emmetropization, which is attributed to genetic and environmental factors. Children with two myopic

parents are 5-6 times more likely to become myopic than those with one or no myopic parent (Gifford and Gifford 2016). The odds of becoming myopic in children aged 11-15 years of South Asian and African in origin are 8 times and 3 times, respectively, higher than those of Caucasian in origin (Ip *et al.*, 2008; Rudnicka *et al.*, 2010). Outdoor activity is proven to be able to reduce the risk of myopia development (Rose *et al.*, 2008; Guggenheim *et al.*, 2012; Sherwin *et al.* 2012; He *et al.*, 2015; Li *et al.*, 2015; Guo *et al.*, 2017; Xiong *et al.*, 2017). This exposure can retard the onset of myopia in non-myopic children, but not in myopic children (He *et al.*, 2015; Li *et al.*, 2015; Guo *et al.*, 2017; Xiong *et al.*, 2017). Early work shows that near work was associated myopia progression (Mutti *et al.*, 2002), but recent studies suggested that near work is not directly associated with myopia progression (Ip *et al.*, 2008; Jones-Jordan *et al.*, 2012). The weighting of genetics and environment on myopia progression varies with age. For preschool children, myopia is primarily driven by genetics (Low *et al.*, 2010). As the demand for near work increases and the time for outdoor activities decreases with the implementation of formal education, the role of environmental factors on myopia progression increases among school children.

Hong Kong is an area with one of the world's highest prevalences of myopia (Lam and Goh, 1991; Matsumura and Hirai, 1999; Zhao *et al.*, 2000; Lin *et al.*, 2004; Saw *et al.*, 2006; Vitale *et al.*, 2009; Yoon *et al.*, 2011; William *et al.*, 2013; McCullough *et al.*, 2016). In Hong Kong, the prevalence of juvenile-onset myopia is less than 5% in preschool children (Fan *et al.*, 2004), but rapidly increases by about 10% per year during first 4 years in primary

education (from 17.6% at the age of 6 years to 57.6% at the age of 10 years (Edwards, 1999; Lam *et al.*, 2012), then gradually increases to 74% at the age of 17 (Lam and Goh, 1991). The prevalence of myopia among school children is similar in other Asian countries including China, Japan, Korea, Singapore, and Taiwan (Matsumura and Hirai, 1999; Zhao *et al.*, 2000; Lin *et al.*, 2004; Lim *et al.*, 2012). There is no significant change in the prevalence in schoolchildren in Hong Kong since 1991 (Lam *et al.*, 2012). The situation is better than other developed countries including Singapore, Taiwan, UK and US where the prevalence has increased over the last two decades (Lin *et al.*, 2004; Vitale *et al.*, 2009; Koh *et al.*, 2014; McCullough *et al.*, 2016). Any intervention for arrest or slowing the progression during school age could relieve the burden on public health in the future. Optical and pharmaceutical methods are currently the most promising interventions for myopia control in children.

1.1.3.1 Effectiveness for myopia control of orthokeratology

In the previous section, studies showing safe and effective ortho-k for correction of myopia up to -6.00 D are based on good compliance from the users in following instructions from their practitioners and wearing lenses regularly on a nightly basis. It has been approved for adults as well as children for myopia correction (Paragon Vision Science, 2002; Bausch & Lomb, 2004). However, the lenses have not been approved for myopia control. In the early 2000s, clinical impressions (Cho *et al.*, 2002; Cho *et al.*, 2003, 2003a) and case reports (Cheung *et al.*, 2004; Chan *et al.*, 2014)

showed that refractive error and axial elongation were slowed in children receiving ortho-k treatment for refractive correction.

Retrospective studies on ortho-k for myopia control demonstrated that ortho-k can slow myopia progression (Mok and Chung, 2011; Downie and Lowe, 2013; Zhu *et al.*, 2014; He *et al.*, 2016) ranging from 30% (He *et al.*, 2014) to 80% (Mok and Chung, 2011). However, results from case reports and retrospective studies suffer from a major problem: bias in favor of positive outcomes. They tend to over-estimate the effectiveness for myopia control as the effects on dropout subjects were not determined and their characteristics not evaluated.

The first prospective cohort study on ortho-k for myopia control was 'The Longitudinal Orthokeratology Research in Children (LORIC) in Hong Kong' reported in 2005 (Cho *et al.*, 2005). It showed that myopic Chinese children aged 7 to 12 years receiving ortho-k treatment for two years had 46% slower axial elongation compared to the control subjects wearing single-vision spectacles. However, the major limitations of the LORIC study are the lack of randomization and the use of historical control subjects. More clinical evidence has emerged since LORIC from a range of other studies (Walline *et al.*, 2009; Kakita *et al.*, 2011; Hiraoka *et al.*, 2012; Santodomingo-Rubido *et al.*, 2012; Paune et al, 2015). The results from these non-randomized cohort studies supported the claim for a positive myopia control effect using ortho-k in myopic children aged 6 to 16 years as axial elongation was slowed by 32-38% in European children (Santodomingo-Rubido *et al.*, 2012; Paune *et al.*, 2015), 36% in Japanese children (Kakita *et al.*, 2011; Hiraoka *et al.*, 2012),

and 56% in American children (Walline *et al.*, 2009). It was shown to be more effective in reducing the prevalence of children with rapid axial elongation (annual progression more than 0.36 mm) especially in younger children below 9 years (Cho and Cheung 2012; Cho and Cheung 2017). Ortho-k can prevent the development of rapid progression (annual increase in myopia more than 1.00 D) by treating one out of two children aged 6-8 years compared to one out of 12 children aged 9-12 years (Cho and Cheung 2017).

To date, there are only two published randomized controlled trials (RCTs) on ortho-k for myopia control. The first one is the 'Retardation of Myopia in Orthokeratology (ROMIO) Study'. It showed that low myopic subjects (myopia not more than -4.50 D) aged 7-10 years on ortho-k demonstrated 43% slower axial elongation than those on single-vision spectacles. The second is the 'High Myopia – Partial Reduction Ortho-k' (HM-PRO) study. Axial elongation was slowed by 63% in high myopic subjects (myopia between -5.00 D to -8.00 D) aged 8-11 years using ortho-k compared to those using single-vision spectacles. In the last decade, ortho-k for myopia control in children has received support from studies ranging from low level clinical evidence (e.g. case reports and retrospective studies) to high level clinical evidence (e.g. well-designed prospective cohort studies and RCTs). Two meta-analyses have shown that the average of retardation in myopic progression is about 41% (Wen *et al.*, 2015) to 45% (Sun *et al.*, 2015). The meta-analysis performed by Huang and co-workers (2016) determined that

the myopia control effect of ortho-k is the best among other optical interventions and it is second compared to moderate to high dose atropine. Whereas there is no restriction on the refractive error for myopia control studies using therapeutic eyedrops (e.g. atropine), myopia control studies using optical intervention usually target children with myopia not more than -6.00 D, with a low to moderate amount of refractive astigmatism. In addition to HM-PRO study (Charm and Cho, 2013), four non-randomized studies on the effect of ortho-k on axial elongation included subjects from low to high myopia: one prospective cohort study (Kakita *et al.*, 2011), two retrospective studies with control subjects (Zhu *et al.*, 2014; He *et al.*, 2016) and one clinical study on factors affecting axial elongation in ortho-k subjects (Fu *et al.*, 2016). It has been shown that high myopes demonstrated slower progression than low myopes after ortho-k treatment (Kakita *et al.*, 2011; Fu *et al.*, 2016; He *et al.*, 2016). However, Zhu and co-workers (2014) reported that the myopia control effect was highest among moderately myopic subjects (spherical equivalent refraction (SER) not more than -3.00 D: 58%) compared to the low myopic (SER between -3.00 D and -6D: 47%) and high myopic subjects (SER more than -6.00 D: 44%). The RCT study on ortho-k for myopia control in high myopes, the HM-PRO study, showed that high myopic subjects (myopia between -5.00 D to -8.00 D) aged 8-11 years on partial correction of -4.00 D, with overnight ortho-k in combination of single vision glasses in the daytime correcting the residual refractive error, demonstrated 63% slower axial elongation compared to the control subjects using single-vision spectacles. The concept of partial correction is to provide

peripheral defocus with the application of ortho-k without creating excessive pressure on the cornea, thus lowering the risk of complications as observed in cornea with full correction (Liu *et al.*, 2016). Although there is no direct comparison with previous myopia control studies on low myopic children, the effectiveness of myopia control of HM-PRO is the highest among all the prospective cohort studies and ROMIO. This may support the claim for better myopia control effect in high myopic children (Kakita *et al.*, 2011; Fu *et al.*, 2016; He *et al.*, 2016).

In addition to high myopia, ortho-k has the potential to slow axial elongation in myopic astigmats. Studies have shown that toric ortho-k can correct myopia as well as astigmatism (Chen *et al.*, 2012; Paune *et al.*, 2012; Luo *et al.*, 2014; Lyu *et al.*, 2016). To date, there is only one published study on toric ortho-k for myopia control in astigmatic children: the Toric Orthokeratology – Slowing Eyeball Elongation (TO-SEE) study. It is a prospective cohort study with self-selected treatment assignment. It shows that astigmatic children (myopia not more than -5.00 D; astigmatism between 1.25 D to 3.00 D), aged 6-12 years, receiving ortho-k demonstrated 52% slower axial elongation than control subjects with single-vision spectacles. Study of ortho-k for myopia control in high myopes and high astigmats are still relatively new and scarce. Further investigation is warranted to collect stronger clinical evidence on the application of ortho-k for myopia control in children with higher degree of astigmatism and myopia.

It has taken over a decade to slowly but definitely build up evidence of effectiveness of ortho-k for myopia control. Although some eye care practitioners and researchers are still conservative regarding the application of ortho-k on children, its effectiveness for myopia control is now widely accepted, in contrast to the skeptical criticism received when the pilot study, the LORIC study, was published in 2005. The following sections will explore factors affecting the effectiveness and underlying mechanism of myopia control in ortho-k.

1.1.3.2 Factors affecting effectiveness of myopia control in orthokeratology

Researchers have investigated the associations between individual ocular characteristics (such as age, gender, refractive errors, corneal shape and power, intraocular pressure and corneal biomechanical properties) and axial elongation after ortho-k. Different studies have adopted different approaches in these statistical analyses to evaluate different ocular characteristics. As a result, there may be some variations in the results as reported by different research teams.

Some studies have shown that after ortho-k treatment, axial elongation was slower in higher myopic subjects (Cho *et al.*, 2005; Kakita *et al.* 2011; Fu *et al.*, 2016). These studies used univariate tests without considering more than one explanatory variable. For example, Cho and Cho (2005) considered refractive error and corneal curvature only and Kakita and co-workers (2011) considered age only. Fu and co-workers (2016) confirmed the association between axial elongation and refractive error using trend analysis adjusted

for age, gender, initial axial length, and duration of ortho-k lens wear. By using a multivariate model to investigate the relationship between various demographic data and axial elongation, axial elongation was shown to be negatively associated with age, i.e. younger children have faster progression and children on ortho-k have slower elongation than those on single-vision correction (Cho and Cheung, 2012; Chen *et al.*, 2013; Zhong *et al.*, 2015; Wang *et al.*, 2017). Cho and co-workers showed that axial elongation was not related to gender, corneal curvature or refractive error. It was related to age and use of ortho-k (Cho and Cheung, 2012; Chen *et al.*, 2013) such that slower elongation was associated with older subjects and in subjects with ortho-k treatment. Cho and Cheung (2017) assessed the benefit of myopia control in children using a different perspective. They determined the number (of subjects) needed to treat (NNT) to prevent one subject from experiencing rapid axial elongation (equivalent to myopia progression for more than 1.00 D per year). By reanalyzing the data obtained from ROMIO and TO-SEE studies (Cho and Cheung, 2012; Chen *et al.*, 2013), the 2-year NTT for subjects aged 6 to 8 years and 9 to 11 years was 2 and 12, respectively. That is, by treating two children aged 6 to 8 years, one of them can be prevented from becoming a fast progressor. On the contrary, ortho-k can only help one out of 12 children aged 9 to 11 years. The results support the suggestion for early initiation of myopia control treatment for younger children.

In addition to age and refractive error, Zhong and co-workers (2015) included the change in corneal power as an independent variable in their regression

model. They reported that slow axial elongation was associated with older age and greater change in corneal power, but not with refractive error. Wang and co-workers (2017) demonstrated that axial elongation was associated with age, refractive error, and duration of treatment in their multivariate model. However, there are two queries on their model. First, since ortho-k just slows, not totally arrests myopia, axial elongation is expected to increase with time. Hence, the positive association of greater elongation with longer duration of treatment is anticipated. Second, younger children tend to have lower refractive error. The authors did not comment on the effect of this collinearity between age and refractive error which may affect the accuracy of their regression model. In general, axial elongation is less likely to be associated with gender, refractive error, and corneal shape (Cho and Cheung, 2012; Chen *et al.*, 2013; Wang *et al.*, 2017).

1.1.3.3 Mechanism for myopia control in orthokeratology

Peripheral defocus has been proposed to account for myopia control in ortho-k (Charman *et al.*, 2006; Queiros *et al.*, 2010; Kang and Swarbrick, 2011). It has been suggested that the mechanism of ortho-k for myopia control is due to optical defocus created in the area with the most abrupt change in corneal curvature, i.e. area under the reverse curve or the area around red ring shown on the topographic map. The peripheral defocus of a myopic eye is changed from peripheral hyperopia to peripheral myopia (Charman *et al.*, 2006; Queiros *et al.*, 2010; Kang and Swarbrick, 2011) after ortho-k treatment.

Peripheral defocus is commonly described by peripheral refraction. Although a causal relationship has been shown in animal models (Smith *et al.*, 2005; Tse *et al.*, 2007; Smith *et al.*, 2009; Tepelus *et al.*, 2012; Benavente-Perez *et al.*, 2014), the association between peripheral refraction and myopia control of ortho-k in human is not conclusive. Cross-sectional studies have shown that peripheral refraction changes with refractive error such that it changes from peripheral myopia in emmetropes and hyperopes to peripheral hyperopia in myopes (Chen *et al.*, 2010; Sng *et al.*, 2011a; Lee and Cho, 2013; Chen *et al.*, 2016), but the degree of peripheral refraction and its change cannot predict myopia progression (Mutti *et al.*, 2011; Sng *et al.*, 2011b; Lee and Cho 2013; Atchison *et al.*, 2015). Atchison and co-workers (2015) suggested that myopia progression was regulated by the retina and peripheral refraction was merely 'an accompaniment, rather than a cause, of myopia'. That is, other defocus signals are involved for the regulatory pathway.

Aberrations have also been proposed to account for the myopia control in ortho-k. Despite numerous reports showing an increase in higher order aberrations, particularly spherical aberration and total higher order aberration after ortho-k, few studies have attempted to establish the association between axial elongation and aberrations. Hiraoka and co-workers (2015) and Santodomingo-Rubido and co-workers (2015) observed negative association between axial elongation and change in coma-like aberration using multivariate modeling. The lack of association between the initial aberration and axial elongation suggests that aberration is not a predicting

factor for myopia control, whereas the significant association between axial elongation and the changes in coma-like aberration supports the theory of regulatory channel via peripheral defocus.

Peripheral defocus has been also shown to vary with pupil size (Faria-Ribeiro *et al.*, 2016). Chen and co-workers (2012) reported slower axial elongation in ortho-k subjects with larger pupil size. The lack of association between pupil size and axial elongation in control subjects gives some support to the theory of peripheral defocus theory introduced by ortho-k for myopia control. However, the authors reported the scotopic pupil size, which did not account for the size of natural pupil in normal daily activities. They also did not consider the treatment zone size in relation to the pupil size, but their results did show a possibility of manipulation of axial elongation via alteration of visual signal (i.e. degree of defocus). Zhong and co-workers (2015) showed that axial elongation was associated with age and greater change in corneal power, but not with refractive error. They did not measure the pupil size nor the aberration, but demonstrated that the changes in corneal power varied with the chord diameters. In ortho-k, patients are targeted for full correction and the refractive change is highly correlated with the change in corneal power. The contribution of change in corneal power on axial elongation was unlikely to be related to the effect of refractive error. It was more likely to be related to peripheral defocus in relation to the treatment zone, which was out of scope of that study. Further investigation is warranted to understand the contribution of the effect of pupil size and treatment zone and other changes involved in ortho-k on axial elongation.

In conclusion, ortho-k can slow axial elongation by 40% in myopic children with and without astigmatism. High myopes can achieve myopia control through partial correction with no increased risk compared to low myopes. The exact mechanism for myopia control in ortho-k remains unclear, and the association between peripheral defocus of the eyes and myopia control is still under investigation. The best predictive factor in using ortho-k for myopia control is the initial age of the child. The treatment is most effective on younger children aged 6 to 8 years who have a history of fast progression, suggesting initiation of myopia control treatment for susceptible children at the onset of myopia.

1.2 Safety in orthokeratology

No treatment is risk-free, either physiologically or psychologically. As for other contact lens wear, ortho-k lens wear can be associated with complications, from mild problems, such as epithelial corneal staining to severe conditions, such as microbial keratitis. Most problems do not interfere with ortho-k lens wear, but severe conditions can be sight threatening. In the following sections, the effect of ortho-k on the health status of the cornea and risk involved in ortho-k will be reviewed. Corneal health conditions will be reviewed at the cellular level by exploring the morphological changes at each corneal layer. The risk, risk factors, and preventive measures in minimizing the risks involved in ortho-k will follow after the review of the corneal health.

1.2.1 Morphological changes in the cornea related to orthokeratology

Corneal health is maintained by an intact epithelium, for oxygen supply as well as being a barrier to environment challenges, and a good endothelial pump, for nutrition supply. Good contact lens wear should have minimal effect on these two layers. With the advancement in technology, in vivo examination of the morphology of corneal layers can be made using specular microscopy for the endothelium or confocal microscopy of different layers of the cornea. Theoretically, confocal microscopy has a wider clinical application than specular microscopy, but as the latter is more commonly used in routine examination, more results have been published on the effect of ortho-k on the corneal endothelium.

1.2.1.1 Microscopic examination of the cornea

The avascular and transparent structure of the cornea is a challenge in anterior ocular examination. Magnification of the standard clinical examination of cornea with slit lamp biomicroscopy is limited up to 40 times. While specular microscopy allows the examination of the corneal endothelium, the introduction of a commercially available confocal microscope in early 2000 allowed the examination of the cornea from epithelium to the endothelium at a magnification of up to 800 times, in clinical practice and for research studies.

In addition to the morphological changes in corneal endothelium, confocal microscopy has been used to demonstrate the following changes after contact lens wear:

1. Increase in superficial cell size. The increase in cell size is related to the delay in desquamation of epithelial cells due to the presence of contact lenses. Ex vivo studies show that lenses with higher oxygen transmissibility have the least effect on the epithelium. The greater increase in cell size with rigid lens wear than soft lens despite good oxygen permeability of the materials shows that the mechanical contact of rigid lens has an enhanced effect on the turnover of the superficial cells.
2. Thinning of corneal epithelium. Again, rigid lenses have greater effect on epithelial thinning than soft lenses. The epithelial thinning in soft lens wear is inversely related to the oxygen permeability of the soft lenses. That is, epithelial thinning is affected by both mechanical factors and hypoxia in contact lens wear.
3. Higher Langerhan's cell density in sub-basal layer without significant change in nerve fiber density. Langerhan's cells are activated during the inflammatory process and their density is regarded as an indicator of the immune status of the cornea. Increase in Langerhan's cell density in contact lens wear is an indication for a chronic hypoxia effect on the cornea.
4. Increase in stromal thickness, folds and microdots. The changes are related to hypoxia and they are more prominent in soft lenses with lower oxygen permeability than in rigid lenses.

Most studies have focused on population of interest, such as patients wearing contact lenses or diabetic patients. Normative data of epithelial and

stromal morphology are less well studied. Gambato and co-workers (2015) evaluated the effect of age on corneal morphology in 108 subjects aged 11 to 74 years. They found that the superficial cell size increased, while keratocyte density reduced with age. Otherwise, the effect of age was insignificant on basal cell density, number and density of nerve fibers, and endothelial morphology.

1.2.1.2 Microscopic changes in corneal epithelium and stroma after orthokeratology

The earliest report of application of confocal microscopy in ortho-k is for the differential diagnosis in corneal infections, especially fungal keratitis and *Acanthamoeba* keratitis (Sun *et al.*, 2003; Robertson *et al.*, 2007), and measurement of corneal thickness (El Hage *et al.*, 2007). To date, there are three reports by two research groups using confocal microscopy of the microstructural changes in the cornea associated with ortho-k lens wear. These studies also investigated the effect of ortho-k on corneal thickness and the results have been presented in *Section 1.1.2*.

Zhong and co-workers (2009) performed a cross-sectional study to investigate corneal thickness and corneal morphology in eyes with no experience in ortho-k lens wear, and eyes with one night and 5 years of ortho-k experience. Density of the basal cells, keratocytes in anterior and posterior stroma and endothelial cells were determined and compared. They found that there was no significant changes in endothelial morphology after ortho-k, but the density of basal cells and keratocytes were significantly

reduced after 5 years of lens wear. The authors commented that the "cell activity in the posterior stroma appeared to increase as evidenced by the spiky and elongated keratocytes". Otherwise, little was discussed on the differences in the epithelial and stromal morphology.

A research team in Spain led by Nieto-Bona have conducted two prospective studies on effect of ortho-k lens wear on epithelial, stromal, and endothelial morphology after one month of lens wear (Nieto-Bona *et al.*, 2011a) and after one year of lens wear (Nieto-Bona *et al.*, 2011). Changes in epithelial cell density (superficial cells, wing cells, and basal cells), keratocyte density (in anterior, middle, and posterior stroma), and endothelial cell density and morphology were determined before and after lens wear.

In the short term study, the authors did not find any significant changes in the endothelial cell density and keratocyte density, percentage of microdots, Langerhan's cells and number of nerve fibers after ortho-k. However, the basal cell density was reduced, whilst superficial cells were found to have become wider after one month of lens wear (Nieto-Bona *et al.*, 2011a). Qualitatively, visibility of superficial and wings cells increased from 39-48% before lens wear to 75-79% one month after lens wear, whereas the visibility of basal cells reduced from 100% to 85%. It was proposed that the reduction in basal cell density was due to 'compression of the epithelial layers causing thinning, which impairs the visibility of these cells'. However, the change in visibility in basal cells was small and there is little theoretical background to associate cell thinning and reduction in visibility. The authors also discussed the suppression in basal cell proliferation resulting in cell enlargement. This

may be a probable explanation for the reduction in basal cell density. The increase in width of the superficial cells after ortho-k is in agreement with the change after conventional rigid lens wear as a results of change in surface exfoliation of epithelial cells. The persistent epithelial changes observed in this one-month study indicates a need for further investigation for the possibility of slowing or plateau effect after ortho-k treatment.

In the long term study, Nieto-Bona and co-workers (2011) investigated the effect of ortho-k on cell morphology and its recovery after one year of lens wear and one month after cessation of lens wear. The significant reduction in basal cell density, increased visibility of superficial and wing cells, reduction in visibility of basal cells, and increase in width of the superficial cells reported were in agreement with previous studies (Zhong *et al.*, 2009; Nieto-Bona *et al.*, 2011a). They showed that the effect on basal cell density and visibility of the epithelial cells were reversible upon cessation of lens wear for one month. However, this study suffered from a major limitation of small sample size. Six of the 21 subjects (29%) did not complete this one-year study and good images were not obtained for some subjects. For instance, good epithelial images were captured for only seven of the 15 subjects after lens cessation. There are some inconsistencies in findings with previous studies:

1. Polymegathism did not vary before and within 6 months of lens wear, but it was significantly increased at the one year and one month of lens cessation visits

2. Keratocyte activation gradually increased from 5 units before lens wear to 11 units six months after lens wear, but it dropped 3-4 units at one year and one month of lens cessation visits
3. Statistically insignificant reduction in stromal keratocytes density

Despite the limitations and inconsistencies of these three studies, they provide insights into the influence of ortho-k on cell morphology at epithelial, stromal, and endothelial level, indicating that ortho-k slows the turn-over of the epithelial cells and increases the cellular activity in the stroma. The long term effect of the changes in cellular activity on the corneal health is unknown. Further investigation with better study design is warranted to confirm the changes and to explore the effect of changes on corneal health.

1.2.1.3 Sub-basal nerve plexus and corneal sensation

Lum and co-workers (2012) showed that ortho-k altered the orientation of the sub-basal nerve plexus from a whorl-like pattern in normal eyes to “a tortuous network of less dense and fewer interconnected nerve fibers centrally, and curvilinear fibers mid-peripherally’ in post-ortho-k treated eyes. They superimposed the sub-basal nerve plexus pattern with the corneal topographical map and found that the curvilinear fibers in the mid-periphery became more apparent (thicker nerve fibers) and appeared underneath the reverse curve in subjects with ortho-k experience for over 9 years. The re-arrangement of the nerve fibers indicates that during the process of corneal flattening in refractive correction of ortho-k, the positive and negative pressure created underneath an ortho-k lens causes redistribution of the

epithelial cells as well as the sub-basal nerve fibers. The redistribution of epithelial cells results in central thinning and flattening and mid-peripheral thickening (*Section 1.1.2*) whereas the redistribution of sub-basal nerve plexus accounts for the fibrillary lines (Cheung *et al.*, 2006; Lum and Swarbrick 2007) and white lesion (Cheung *et al.*, 2005) observed in ortho-k subjects.

Corneal sensation is reduced after ortho-k (Hiraoka *et al.*, 2009; Nombela-Palmo *et al.*, 2016; Lum *et al.*, 2017, 2017a, 2017b; Nombela-Palmo *et al.*, 2017) and the reduction is associated with the change in the sub-basal nerve plexus (Lum *et al.*, 2017a; Nombela-Palmo *et al.*, 2017). However, these changes are not in good accordance as the measurement is influenced by corneal hypoxia and the method of measuring corneal sensitivity (Lum *et al.*, 2017a). The reduction in corneal sensitivity in ortho-k can be due to the mechanical force caused by the rigidity of the ortho-k lens, and hypoxia in ortho-k lens wear as well as the reduction in nerve fiber density in the central cornea. Despite the significant change in nerve fibers in the ortho-k treated eye and the lack of change in rigid lens treated eyes, the reduction in corneal sensitivity in the ortho-k treated eyes is not significantly different from the rigid lens treated eyes after 3 months of lens wear (Lum et al, 2017a). Corneal sensitivity can recover after one month of cessation of lens wear, but the recovery in the orientation of nerve fibers lags behind the recovery of corneal sensitivity upon lens wear cessation (Lum *et al.*, 2017b). These findings suggest that rearrangement of the corneal nerve bundle after ortho-k

does affect corneal sensitivity, but its role in corneal sensation is not as important as the mechanical stress and hypoxia.

1.2.1.4 Effect of ortho-k on Descemet's membrane

Descemet's membrane is the basement membrane of the endothelium. Wide-spaced collagen is secreted during fetal life and it reaches full thickness of about 3 μm by birth, forming the anterior banded pattern between the stroma and the non-banded layer of Descemet's membrane. The thickness of this membrane increases to 13 μm by the age of 70 years. The collagen secreted after birth forms the non-banded zone which consists of an amorphous conglomeration of extracellular matrix. The non-banded layer is secreted by the endothelium when under stress (e.g. damage or disease). If the endothelial pump fails (e.g. Fuchs dystrophy), it secretes abnormal (wide-spaced) collagen resulting in a vicious cycle: thickening of the Descemet's membrane and increasing the difficulty to remove water from the stroma causing further damage to the endothelial pump (Levy *et al.*, 1996; Bourne, 2004). To date, there is no documentation about the effect of ortho-k nor contact lens wear on Descemet's membrane.

1.2.1.5 Factors affecting corneal endothelium

The corneal endothelium consists of a single layer of cells, with limited regenerating power, which regulates fluid, ions, and other materials in and out of the cornea (Tuft and Coster, 1990; Joyce, 2003). The human cornea has up to 500,000 cells at birth (Tuft and Coster, 1990). As the cornea grows

and corneal diameter increases after birth, it is not surprising that endothelial cell density (ECD) decreases with age. The reduction is the most rapid in the first three years of life, in which ECD reduces from up to 7500 cells/mm^2 at birth to about 4000 cells/mm^2 at the age of one and 3500 cells/mm^2 at the age of five (Nucci *et al.,* 1990). The change in ECD becomes more gradual and stabilizes in adulthood with an average density of 3000 cells/mm^2 (Hillingsworth *et al.,* 2001; Shao *et al.,* 2007). It may reduce again after middle age (Abib *et al.,* 2001).

Reduction in ECD before adulthood is mainly due to hypertrophy after loss of endothelial cells, as the corneal endothelium has limited regenerating capacity (Tsukahara and Yamamoto, 1989). Hypertrophy of corneal endothelial cells is demonstrated as studies have shown that the diameter of the cornea increases between the age of five to 14 (Nucci et al., 1990; Elbaz *et al.,* 2017), without significant changes in total endothelial cell counts (Tsukahara and Yamamoto, 1989) resulting in reduction in ECD.

Most of the cells are in the shape of a hexagon, as honeycomb geometry is the most stable structure in nature for distributing stress and load when nested. Endothelial morphology of the cornea can be affected by direct injury to the endothelium (e.g. corneal transplant), diseased or compromised cornea (e.g. Fuchs' dystrophy), and hypoxia (e.g. contact lens wear). Thus, it reflects the status of corneal health. Direct injury can cause cell loss resulting in reduction in ECD, whilst chronic conditions can cause increase in pleomorphism (increase in variation of cell shape) and increase in

polymegathism (increase in variation of cell size) (Tsukahara and Yamamoto, 1989; Chang *et al.*, 2001; Hollingsworth *et al.*, 2001; Shao *et al.*, 2007).

Peripheral cornea is considered as a 'physiologic reserve and storage region,' especially in wound healing (Amann *et al.*, 2003). However, published reports on the endothelial morphology of this area is inconclusive because of the differences in study designs, subjects, and the locations of peripheral cornea being examined (Cheung and Cho, 2000; Wiffen *et al.*, 2000; Amann *et al.*, 2003; Doughty and Aakre, 2007; Zheng *et al.*, 2016; Tanaka *et al.*, 2017). Most studies reported lower ECD in the central cornea in both children (Cheung and Cho, 2000) and adults (Amann *et al.*, 2003; Doughty and Aakre, 2007; Tanaka *et al.*, 2017). One study reported no significant difference in ECD in the central and inferior cornea (Zheng *et al.*, 2016), whereas one study reported higher ECD in the central cornea in the non-contact lens wearers (Wiffen et al, 2000) (*Section 1.2.1.5*).

1.2.1.6 Effect of orthokeratology on endothelium

A few studies have evaluated the long term effects of ortho-k on corneal endothelium and the general findings show minimal disturbance to endothelial cell morphology. Hiraoka and co-workers (2004) and Zhong and co-workers (2009) did not find any change in ECD, polymegathism, or pleomorphism. Cheung and Cho (2005) found a significant reduction in ECD without any changes in polymegathism and pleomorphism. On the other hand, Nieto-Bone and co-workers (2011) observed an increase in polymegathism without any changes in ECD or pleomorphism. One of the

limitations of these studies is the lack of control subjects. As ECD can be affected by normal aging in children, without a control group, it cannot be concluded whether the change or lack of change in ECD reported by previous studies is due to ortho-k or not. Further investigation with control subjects is warranted to evaluate the effect of ortho-k and aging on endothelial morphology.

1.2.1.7 Oxygen supply to the cornea in orthokeratology lens wear

One of the concerns of ortho-k lens wear is hypoxia caused by sleeping with lenses on overnight. Physiologically, the cornea suffers from mild hypoxia during sleep as eye closure cuts off most of the oxygen supply to the eyes. Corneal edema induced during sleep, about 3% (Harvitt *et al.*, 1999), dissipates within minutes after waking. This recovery can be affected by contact lens wear and hypoxia induced by extended wear soft lens made in materials with Dk/t of 125 is less than 3% (Harvitt *et al.*, 1999).

Most of the current ortho-k lenses are made of highly oxygen permeable materials with Dk 100 or above. With lens thickness ranging from 0.15 to 0.20mm, the oxygen transmissibility (Dk/t) of ortho-k lenses varies from around 60 to 108. Although Dk/t for most of the ortho-k is lower than the recommended number to avoid corneal edema during sleep, these recommended values are more meaningful for extended wear modality in which the lenses will remain on the eyes after eye opening such that lower Dk/t lenses will have slower corneal recovery. Unlike the conventional contact lenses which correct vision with lenses in situ, ortho-k corrects vision

during sleep. Patients are required to remove lenses after waking and thus, this modality will have minimal effect on the recovery of corneal edema. The recommended Dk/t values for avoiding edema, derived in vitro conditions, serves better as a guideline for lens selection.

In summary, current findings suggest that ortho-k slows the turnover of corneal epithelial cells and temporary reduction in corneal sensation as observed in other rigid lens wear, increases cellular activity of keratocytes in the stroma and has minimal effects on corneal endothelium. Although there are some inconsistencies in findings due to limited reports which indicates a need for further investigation, the current findings only show changes not harm after ortho-k. That is, the cornea is not compromised after the treatment. Microbial keratitis or other complications associated in ortho-k lens wear may be related to other extrinsic factors.

1.2.2 Overview of ocular infection and microbial keratitis in children

There are three conditions for infection to occur: an open wound, presence of pathogens (small quantity for virulent strains (e.g. *Acanthamoeba*) or a large quantity for non-virulent strains (e.g. coagulase negative staphylococcus (CNS)), and a compromised immune systems (e.g. sickness in elderly people or under-developed immune system in young children). The severity of the infection is dependent on the virulence of the pathogens, the timeliness of treatment and the treatment prescribed.

Children are more susceptible to illness for a number of reasons. Their immunological defense system is developing, but they may not be able to practice good hygiene to protect themselves and they spend a lot of time in environments (e.g. schools and playground) in which they may be more exposed to transmissible contagious pathogens. Noncompliance in hygiene makes these young children not only susceptible to infection, but also to be potential carriers of diseases. Common childhood diseases, such as hand-foot-mount disease, influenza, and pinkeye are transmitted by direct or indirect contact or respiratory droplet transmission (Currie and Brewster 2001; Atler *et al.*, 2011; Al-Otaibi, 2012). Pinkeye is one of the most common diseases in children. This contagious form of acute conjunctivitis can be caused by bacteria or virus and is transmitted by direct and indirect contact with the infected person or by air droplets in coughing and sneezing. The incubation period is short and the prognosis of this disease is usually good because of early detection of the problems as parents can easily detect symptoms of redness and pain/discomfort without any professional training. The problems usually resolve without affecting vision and ocular health if the cornea is spared.

The prevalence of microbial keratitis in young children is much lower than that of pinkeye, but it is a more severe ocular infection. In the case of mild ocular inflammation such as allergic conjunctivitis, most children will recover without treatment. However, in the case of microbial keratitis, because of the short incubation period and the virulence of the pathogens, the condition can get worse within days. It is considered a medical emergency, as the potential

development of corneal scarring can cause permanent damage to vision if appropriate treatment is not implemented in time. Microbial culture is essential in diagnosis and prescription of the right treatment. Untimely treatment, such as delay in referral or misuse of topical steroid, is a risk factor leading to penetrating keratoplasty (Miedziak *et al.*, 1999).

Like pink eye, microbial keratitis in children is related to the environment or personal hygiene. The causes of microbial keratitis in developing and developed countries are different – the predisposing factor is corneal trauma (e.g. injury caused by agricultural accident) in developing countries and contact lens wear in developed countries. In UK, 65% of microbial keratitis was attributed to contact lens wear in the general population and the risk of infection was increased with extended wear soft lenses (Dart *et al.*, 1991). In pediatric microbial keratitis, ortho-k has been reported to be the leading cause in Taiwan and Hong Kong (Young *et al.,* 2013; Chan *et al.*, 2014; Lee *et al.*, 2014).

In the last few years, a lot of concerns have been raised on the safety of ortho-k. Ongoing research is being conducted with the objective to minimize the risk in ortho-k: by understanding the severity of the situation and associated problems, exploring possible remedies. Current literature on microbial keratitis in ortho-k, organisms involved in the infected eyes, noncompliance and contamination in ortho-k followed by changes in cellular level will be presented in the following sections.

1.2.3 Microbial keratitis in orthokeratology

The incidence of microbial keratitis in normal eyes is low but the risk is increased with the use of contact lenses. Stapleton and Carnt (2012) have reviewed contact lens-related microbial keratitis. The annual incidence of microbial keratitis in developed countries increases from 2.7-4.1 per 10000 in daily wear soft lenses to 19.5-20.0 per 10000 in extended wear soft lenses. Daily disposable soft lenses and the use of silicone hydrogel material allowing higher oxygen permeability have had limited effects on the incidence in daily wear and extended wear soft lenses, respectively. However, severity of infection was reduced with daily disposable modality. Young and co-workers (2013) identified 18 pediatric microbial keratitis between 2001 and 2010 in a government hospital in Hong Kong. Only three cases were non-contact lens related (16.7%). For the other 15 cases, 7 (38.9%) were related to ortho-k. However, incidence of microbial keratitis associated with ortho-k cannot be determined because of the small number of cases.

In a post market surveillance of Paragon CRT and Boston VST lenses, Bullimore and co-workers (2013) reported higher incidence of microbial keratitis in children compared to adults. Among the 1317 ortho-k wearers investigated, the average ortho-k treatment duration was two years. Almost half of the ortho-k wearers were children and the only two incidents of microbial keratitis identified were in children. The risk of microbial keratitis was estimated to be 13.9 per 10,000 patient-year in children compared to 7.7 per 10,000 patient-year in all patients. Again, the authors also commented

that the study power was low because of the few cases identified with problems.

The risk of microbial keratitis in ortho-k has been assessed by different perspectives. Watt and Swarbrick have conducted two reviews of microbial keratitis in ortho-k (2005, 2007) to identify their profile and the risk factors involved. The report in 2007 is an extension of their report in 2005. In the latter report (Watt and Swarbrick 2007), they identified 123 cases of microbial keratitis reported between 2001 and 2007. Sixty-four cases were reported in 2001 and accounted for 52% cases found over the seven years. All the cases reported in 2001 were from China (73.4%), Taiwan (17.2%) and Hong Kong (9.4%) and the major pathogens involved were *Acanthamoeba* and *Pseudomonas*, suggesting use of tap water and poor hand hygiene. Watt and Swarbrick (2007) observed a reduction in microbial keratitis after 2001 in China after the intervention of the Chinese government who tightened the regulation of ortho-k practice such that only ophthalmologists with prior training could prescribe ortho-k in fully equipped clinics. The ortho-k lenses and solutions must have had prior clearance from the authority or they could not be prescribed for ortho-k practice.

Van Meter and co-workers (2008) assessed the risk of microbial keratitis in ortho-k according to the level of evidence. Quality of evidence is qualified by the study designs and ranges from good (Level I) to bad (Level III). Level I evidence refers to evidence obtained from properly designed randomized controlled trials. Level II evidence refers to evidence obtained from cohort studies and level III evidence refers to evidence obtained from case reports,

case series, and case-control studies. In 2008, there were 38 level III reports, but only two level II grade evidence and no level I grade evidence (Van Meter *et al.*, 2008). The only two level II grade evidence available were the two premarket approval applications for overnight ortho-k from Paragon CRT and Bausch and Lomb Boston Ortho-k lenses. Ten percent (12 out of 121) of the subjects using the CRT application were adolescent while all subjects were adults for the other lenses. No microbial keratitis was observed in these two reports. The level III evidence from case reports and case series could not provide sufficient data for the analysis of risk for the identification of population at risk. Thus, they recommended the highest level of vigilance in compliance from practitioners and patients to reduce the potential risk of complication in ortho-k lens wear.

Level I evidence and more level II evidence on safety in ortho-k lens wear emerged after 2011 with the release of results from long term prospective cohort studies (Kakita *et al.*, 2011; Hiraoka *et al.*, 2012; Santodomingo-Rubido *et al.*, 2012; Chen *et al.*, 2013; Zhu *et al.*, 2014), including two randomized clinical trials, on efficacy and safety in ortho-k lens wear (Cho and Cheung *et al.*, 2012; Charm and Cho, 2013). No severe adverse event was reported in any of these clinical studies.

Recently, there have been three systematic reviews and/ meta-analysis on safety of ortho-k which can be classified as level II evidence. Liu and Xie (2016) excluded 208 papers and reviewed safety in ortho-k based on 170 papers on ortho-k and adverse events in ortho-k. Most of the publications were in Chinese. They concluded that the risk of microbial keratitis in ortho-k

was comparable to other contact lenses using an overnight modality. The treatment was safe if associated with good compliance from patients and practitioners. Li and co-workers (2016) performed meta-analysis on adverse events in ortho-k. They reported that the odds ratio of encountering an adverse event was 8.87 times higher in subjects on ortho-k treatment compared to the control subjects wearing single-vision spectacles. However, they also commented that none of the adverse events were significant and all patients recovered quickly after treatment or discontinuation of ortho-k use.

Kam and co-workers (2017) investigated microbial keratitis in ortho-k from another perspective. In addition to the general analysis on age, gender, and pathogens involved, they also evaluated the clinical time course in terms of time line for hospitalization, and medical and surgical treatment. They identified 173 eyes in 164 subjects from 29 of 172 published reports. Subject numbers in the reports ranged from 1 to 28. The majority of subjects were female below the age of 18 years at the time of onset of disease. The duration of ortho-k treatment ranged from 5 days to 156 months before onset of disease. Hospitalization was reported in three studies and the duration of hospitalization ranged from 3 to 15 days. The antimicrobial treatment could last from 3 days to 20 months. It should be noted that no prospective cohort studies were included in their review, suggesting that the severe adverse events in ortho-k is related to non-compliance in non-research setting.

Preventive measures in minimizing complications is the key to success for any prospective cohort study. Proper education, review of compliance and re-education, and regular follow up visits are essential elements in well-

designed clinical studies. Subjects may be excluded in case of compliance not meeting the requirements. In contrast, the requirements for compliance may not be as high in general clinical practice. The lack of microbial keratitis in level I and level II grade evidence in clinical research and abundant level III evidence in clinical practice may indicate poor compliance in patients and practitioners in general practice.

In Hong Kong, the prescription of ortho-k is not regulated. All optometrists with license to practice contact lens are allowed to prescribe ortho-k, which results in variation in quality of ortho-k practice. As microbial keratitis may be related to poor compliance of patients and practitioners, a guideline on good clinical practice in ortho-k was proposed in 2008 (Cho *et al.*, 2008). It covers various areas including the requirement for proper education on ortho-k fitting, essential equipment, and specification of the equipment, and follow up schedule as well as legal aspects of ortho-k practice. These guidelines aim at promoting ortho-k practice to the highest professional standard, thus minimizing complications and other problems associated with the treatment.

1.2.3.1 Pathogens and pre-disposing factors in microbial keratitis in orthokeratology

Watt and Swarbrick (2007) reported that the major infectious organisms identified in the 123 cases with microbial keratitis were *Pseudomonas* (38%) and *Acanthamoeba* (33%). Similar results were reported by Kam and co-workers (2017) as they used similar criteria in selecting reports with six additional reports between 2009 to 2014 for 40 additional subjects. The

major pathogens identified were *Pseudomonas* (36%), *Acanthamoeba* (34%), and CNS (7%). The cultural and socioeconomic differences, and the difference in ortho-k practice in these three regions may account for the variability in organisms identified. The major pathogens identified in China are *Acanthamoeba* (46%) followed by *Pseudomonas* (29%) and fungi (7%) (Sun *et al.*, 2006) whereas the major pathogens identified in Taiwan are *Pseudomonas* (31-45%) followed by fungi (6-14%) and *Acanthamoeba* (2-6%) (Lee *et al.*, 2014). In Hong Kong, the major pathogens involved are *Pseudomonas* (39-71%) followed by *Acanthamoeba* (0-13%) (Young *et al.*, 2013; Chan *et al.*, 2014). The differences in pathogens involved in different areas suggest that proper education on hygiene and lens usage may be able to reduce the contamination of virulent pathogens such as *Acanthamoeba*.

In summary, microbial keratitis associated with ortho-k most affects children who are the primary users of this treatment. The lack of severe adverse events in research setting and the presence of reports in the community may imply poor compliance in the general population, either by the children (non-compliance in lens handling), patients (delayed seeking for treatment) or the practitioners (non-compliance with good clinical practice). *Acanthamoeba* infection is associated with the use of tap water, whereas *Pseudomonas* and CNS infection are related to poor hygiene and improper hand drying. Researchers worldwide are advising good hand hygiene, against the use of tap water, and more stringent avoidance of unclean water in contact lens wear e.g. no swimming with contact lenses, including disposable soft lenses.

Practitioners should be vigilant in enforcing good clinical practice in their practice.

1.2.4 Non severe adverse events in orthokeratology

Despite the lack of severe adverse events, non-significant complications were reported even in well-designed prospective cohort studies. The first two systematic reports on ortho-k safety are the two pre-market applications for Paragon CRT and Euclid ortho-k lenses (Paragon Vision Science, 2002; Bausch & Lomb, 2004). Most of the subjects in these two reports were adults as ortho-k was intended for refractive correction, when they were introduced to the market. In the premarket application for Paragon CRT ortho-k lens for overnight refractive correction, there was no severe adverse event observed in 122 out of the 205 subjects who completed the nine-month monitoring period. The 18 cases of grade 3 corneal edema observed in the adult subjects were found to be related to a lower percentage of oxygen due to living at high altitude. Nine cases of grade 3 corneal staining were observed all in the adult subjects. No grade 2 or higher level of corneal staining was observed in the adolescent subjects. In the premarket application for Euclid ortho-k lenses, no severe adverse event was reported for their subjects aged 17 to 64 years. There were five incidents of grade 3 and above corneal staining, one incident of grade 3 corneal infiltrative response, and a bilateral iritis observed in one subject. All problems resolved without complications after cessation of lens wear.

Results of prospective cohort studies published between 2011 and 2014 show no severe adverse events (Kakita *et al.*, 2011; Hiraoka *et al.*, 2012; Santodomingo-Rubido *et al.*, 2012; Chen *et al.*, 2013; Zhu *et al.*, 2014). The reported adverse events were all non-significant, ranging from mild corneal staining (Cho and Cheung 2012; Santodomingo-Rubido *et al.*, 2012; Charm and Cho, 2013; Chen *et al.*, 2013; Zhu *et al.*, 2014), mild conjunctival hyperemia (Cho and Cheung 2012), mild corneal erosions (Kakita *et al.*, 2011; Hiraoka *et al.*, 2012; Santodomingo-Rubido *et al.*, 2012), to papillary conjunctivitis (Santodomingo-Rubido *et al.*, 2012). These problems can happen in any kind of contact lens wear and they all resolved after cessation of lens wear with or without antibiotic treatment, without affecting vision.

1.2.5 Contamination and non-compliance in orthokeratology

The presence of *Pseudomonas* and *Acanthamoeba* in ortho-k lens wear (*Section 1.2.2*) suggests contamination with tap water and poor hand hygiene. Cho and Boost have conducted a series of studies to investigate contamination in ortho-k lens wear and corrective measures for the situation. In an early study, they investigated the effect of ortho-k on the ocular microbiome by following the change in ocular microbiome before and 1, 2, 3, 4, 8 and 12 weeks of ortho-k lens wear in 41 children aged 14 ± 8 years (Boost *et al.*, 2005). It was found that the majority of the subjects (68%) had good resistance to harbouring pathogens such that no pathogen was identified before and after lens wear whilst a small proportion of subjects (7%) were constantly exposed to pathogens before and after lens wear. For

the remaining 25% of subjects, the contamination was transient in which pathogens were isolated in one occasion (15%) or more than one occasion (10%). Transient contamination suggests that subjects might not practice safe lens handling resulting in variation in level of noncompliance.

In a later study, they investigated the effect of active intervention on non-compliance and contamination rate in ortho-k (Cho *et al.*, 2009). The intervention adopted was re-education on lens care procedures and replacement schedule at the aftercare visit. Contamination rate before and after intervention was reviewed for subjects aged 12 ± 3 years with ortho-k experience for over six months were invited. The most contaminated accessories identified were the suction holders and the tweezers. Active intervention with re-education significantly reduced the contamination rate from 60% to 30% for the suction holder, from 50% to 10% for the tweezers, and from 29% to 8% for the ortho-k lens. However, re-education had little effect on the contamination rate of the lens cases which remained at around 30% before and after intervention. In this study, the most common pathogens identified were *Staphylococcus aureus* (*S. aureus*) (36–52%), *Acinetobacter* (10–11%) and *Pseudomonas* (2–6%). In a subsequent study, the authors failed to improve contamination of the lens cases by the use of cylindrical lens case (Boost *et al.*, 2012). Both the flat and cylindrical cases demonstrated similar rate of contamination.

Fang and co-workers (2017) used a more aggressive approach in re-education. The level of contamination of the lens cases were determined for 31 existing ortho-k subjects (aged 8–18 years). The report was presented to

the subjects and the level of contamination of lens cases was determined after forewarning. The authors reported that forewarning could effectively improve compliance by reducing the level of bioburden of lens cases.

It is noticed that the hand hygiene was good in these three clinical studies on ortho-k contamination as no *Acanthamoeba* was identified. The negative culture for *Pseudomonas* in 2012 also indicates that compliance in hand hygiene improved from 2005 to 2012. On the other hand, the presence of *S. aureus* and *Acinetobacter* suggests that the ortho-k wearers may touch their faces and other surfaces before handling lenses after hand cleaning. It indicates room for improvement for re-education on compliance.

1.2.6 Summary

Ortho-k causes changes in the cornea at the cellular level. However, there is no evidence to suggest that the changes involved in ortho-k will compromise corneal health, making it more vulnerable to infection. Children are susceptible to infection and the risk of microbial keratitis in ortho-k is higher in children than in adults, but noncompliance is the key problem in ortho-k related microbial keratitis. The lack of reports of severe adverse events in well-designed prospective cohort studies suggests that good compliance from patients and practitioners can enhance safety in ortho-k wear. The lower incidence of *Pseudomonas* identified in clinical studies compared to the incidence identified in case reports and case series of infectious keratitis also support the claim for better compliance from subjects in clinical studies than in general population. Mild and non-significant adverse events can

happen, but these problems can be resolved by lens cessation and/ timely medical treatment. Contamination in ortho-k is a concern regarding safety in the lens wear and it may be related to poor hygiene. Although *Pseudomonas* and *Acanthamoeba* infection can be prevented with good hand hygiene, presence of organisms in the ocular microbiome such as *S. aureus* indicates a need to understanding the mode of transmission of these microorganisms to the eyes in order to further minimize risk infection in ortho-k.

CHAPTER 2

Knowledge Gaps and Objectives

2.1 Effect of orthokeratology on corneal endothelium

The results for changes in the corneal endothelium are not in good agreement (*Section 1.2.1.7*). Since endothelial cell density reduces with age, a reduction in cell density in children and no change in cell density in adults could be anticipated. Studies vary with respect to study designs, instrumentation, subject population and study duration. Therefore, the change or lack of change in these studies without control subjects has provided little information regarding the effect of ortho-k on corneal endothelial function.

2.2 Ocular microbiome and source of contamination

Poor compliance with ortho-k protocols by both practitioners and patients may cause damage to the eye (e.g. improperly fitted lenses or eye rubbing) or introduce pathogens (e.g. poor hygiene). It has been demonstrated that most pathogens isolated from cases of ortho-k related microbial keratitis are either from tap water (i.e. *Acanthamoeba* and *Pseudomonas*) or transferred from other body sites (e.g. *S. aureus*, *Acinetobacter,* and CNS). Identification of the sources of contamination may help the practitioners to take appropriate action, however, sources of contamination have received little attention in studies of contact lens associated infection (*Section 1.2.5*).

2.3 Attitudes of parents towards orthokeratology for myopia control

The importance of good compliance with care routines in any contact lens wear cannot be under-estimated. There are number of methods to improve compliance such as regular replacement of lenses, solutions and accessories (*Section 1.2.5*). The end users (i.e. children and parents) and their care taker (i.e. eye care practitioners) must all ensure there is no deviation from the standard of care. Ortho-k for myopia control is usually initiated by parents, rather than the children. Parents are also responsible for supervising the children to use the lenses at home. Their attitudes towards the treatment are therefore key issues to ensure good compliance. However, little is known about the attitudes, knowledge and behavior of parents towards ortho-k. It is important to have a better understanding of these parameters to improve the practice of ortho-k. Over-expectation of the effect of the treatment or under-estimation of the risks may affect attitudes and compliance with the treatment.

2.4 Guideline on myopia control using orthokeratology

Ortho-k is intended for children who need the intervention for myopia control. The best way to reduce risk of microbial keratitis will be to offer the treatment to only those in need. Those without indication for myopia control should not be considered for the treatment. Unfortunately, no guidelines or protocols for myopia control have been proposed for individual children (*Section 1.1.3*). A well-designed guideline customized for individual needs and conditions can

help the practitioners to select the appropriate children for the treatment, not just offer the treatment for any myopic child with or without the requirement for myopia control.

2.5 Objectives

Ortho-k for myopia control is popular in Hong Kong for concerns of myopia progression even though there is awareness of a possible risk of sight-threatening complications associated with the treatment. Current evidence shows that the treatment can be considered to be safe for slowing axial elongation in children provided that good compliance is followed by all parties involved in the treatment, i.e. practitioners, children and parents. The current study aims at filling the research gaps described in *Sections 2.1–2.4*, thus improving the practice of ortho-k for myopia control. The objectives of study are as follows:

1. To investigate the effect of ortho-k and normal ageing on corneal endothelium in children
2. To investigate the ocular microbiome in children, and its association with contamination of lenses and accessories
3. To evaluate the perspectives of parents and children towards ortho-k before and after using the treatment
4. To develop a guideline for patient selection for myopia control using ortho-k

2.6 Experimental design

This thesis consists of four related studies of different designs to address the above research objectives. Methodology and results for each study are presented in *Chapters 3 to 6*.

Chapter 3 involves a cohort study to investigate the 2-year changes in endothelial morphology in children wearing ortho-k and controls wearing single-vision spectacles. The aim was to determine whether the use of ortho-k affected the endothelial function of children.

Chapter 4 describes a is a cross-sectional study to evaluate the ocular microbiome in children using ortho-k or single-vision spectacles, and the contamination levels of the ortho-k lenses and lens care accessories for ortho-k subjects and spectacles for the control subjects. The association between carriage of *S. aureus* and Gram-negative bacteria with contamination of accessories was determined.

Chapter 5 describes a survey of the parents who are considering myopia control treatment for their children, regarding the use of ortho-k for visual correction and myopia control.

Chapter 6 describes a prospective cross-over study to investigate the effect of history of myopia progression and initial age on the myopia control effects. The results were used to develop a protocol for myopia control for patient selection and monitoring.

Chapter 7 is a summary of all the research findings, significance and limitation of these studies.

CHAPTER 3

Effect of Normal Ageing and Orthokeratology Lens Wear on the Corneal Endothelium

3.1 Introduction

Corneal endothelial morphology is one of the indicators for safety in ortho-k lens wear (*Section 1.2.1.5*). Papers reporting the long term effects of ortho-k on the corneal endothelium are scarce and the results are inconclusive (*Section 1.2.1.6*). Contradictory results may be related to the differences in study designs (longitudinal versus cross-sectional) and age of the subjects recruited (adults versus children) (*Section 1.2.1.6*).

Without the results from control subjects, it is unknown whether the changes or lack of changes in corneal endothelium after ortho-k were due to the use of ortho-k, normal ageing, or both. The primary objective of this study was to evaluate and compare changes in corneal morphology in children wearing ortho-k and spectacles. The second objective of this study was to evaluate the morphological changes in the both central and the peripheral cornea as ortho-k covers at least 90% of the cornea, but previous studies mainly investigated the endothelial changes in the central cornea. In this study, the superior cornea is selected for comparison, as this area has the highest ECD compared to other peripheral locations (Cheung and Cho, 2005).

3.2 Methods

In this retrospective study, endothelium images from the central and the superior cornea of the right eyes captured at the baseline and 24-month visits for 136 subjects (72 ortho-k; 64 controls (single-vision glasses)), of age 6–12 who completed two myopia control studies (Cho and Cheung, 2012; Chen *et al.*, 2013) were retrieved (Figure 3.1). All images were captured by the TOPCON SP-2000P specular microscope and analyzed using the TOPCON IMAGEnet software (version 1.54). Three images were captured for each corneal location and cell analysis was performed on the clearest image by a masked examiner, using the manual retracing method as described by Cheung and Cho (2000). Images from eyes with poor image quality resulting in cell count less than 100 were excluded. Changes in endothelial parameters, including ECD, hexagonality (HEX), and coefficient of variation in cell size (CV), over two years were compared between the ortho-k and control groups.

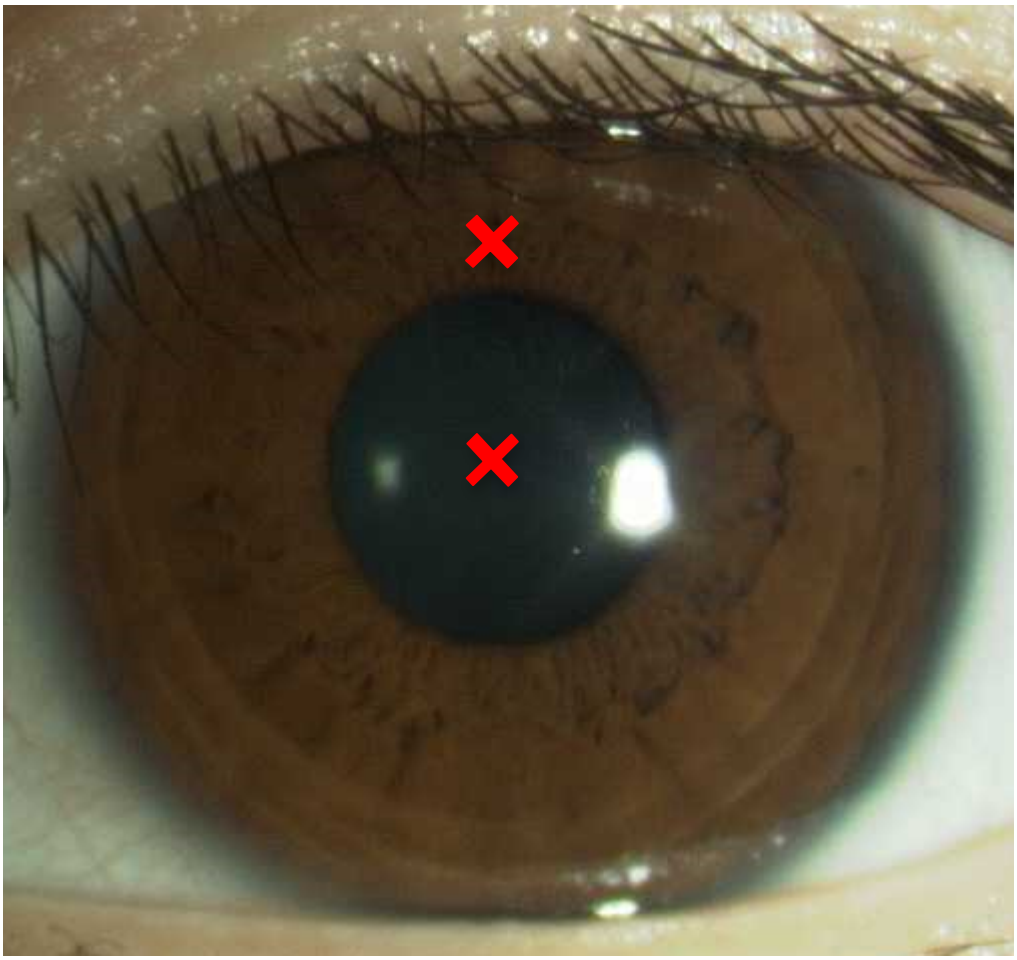


Figure 3.1 The central and superior corneal locations for the endothelial cell analysis

3.2.1 Statistical analysis

Parametric tests were used for data that followed a Gaussian distribution while non parametric tests were used for data that followed a non-Gaussian distribution. Baseline characteristics between the two groups of subjects were compared using unpaired t tests (refractive sphere, spherical equivalent, initial axial length and endothelial parameters), Mann-Whitney U tests (initial age and refractive cylinder) and Pearson Chi-square test (gender). Baseline endothelial parameters in the central and superior corneal locations were compared using paired t tests. Factors associated with the baseline endothelial morphology were determined by stepwise multiple linear regression for all subjects. Effect of time and intervention on endothelial parameters were determined using repeated measures analysis of variance (ANOVA), with time as the within subjects factor and intervention as the between subjects factor. Repeated measures analysis of covariance (ANCOVA) was performed with factors affecting the endothelial parameters determined in the linear regression model controlled as the covariates. If significant interactions were found in the repeated measures ANOVA / ANCOVA, unpaired t tests were performed to compare the changes in endothelial parameters between the two groups. Factors affecting the changes in endothelial parameters were determined for the two group using stepwise multiple linear regression.

3.3 Results

Data from 37 subjects were excluded, 16 due to missing baseline values and 21 due to poor image quality. Table 3.1 shows the demographic data and baseline ocular parameters for the remaining 99 subjects. There were no significant differences in initial age, gender, refractive error and axial length between the 50 ortho-k subjects and 49 control subjects ($p > 0.162$).

Table 3.1 Demographic data and baseline ocular parameters of the 99 subjects

	All (N=99)	Orthokeratology (N=50)	Control (N=49)	p-value
Age (y), median (range)	9 (6–12)	9 (6–12)	9 (6–12)	0.367[#]
Gender, female	57%	56%	57%	0.909[^]
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[#]
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

p-value: probability values for unpaired t tests used to compare for between group differences (unless otherwise specified)

[#] Mann-Whitney U tests

[^] Pearson Chi-square

3.3.1 Baseline endothelial morphology in the central and superior cornea

Table 3.2 shows the baseline endothelial parameters in the central and superior corneal locations. Higher ECD and CV were observed in the superior cornea than in the central cornea for all 99 subjects (paired t tests, $p < 0.001$), whereas no significant difference in HEX was observed in the two corneal locations (paired t test, $p = 0.647$).

Table 3.2 Baseline endothelial parameters in the central and superior cornea for the 99 subjects

	ECD (cells/mm^2)	CV (%)	HEX (%)
Central cornea	3271 ± 215	24.49 ± 1.92	71.47 ± 7.11
Superior cornea	3475 ± 287	26.70 ± 3.26	71.07 ± 7.45
p-value	**< 0.001**	**< 0.001**	0.647

ECD: endothelial cell density; CV: coefficient of variation in cell size; HEX: hexagonality

p-value: probability values for paired t test comparing the endothelial parameters in the two cornea locations; bold for p-values < 0.05

3.3.2 Factors affecting baseline endothelial morphology

For the central cornea, ECD was associated with age and gender (adjusted R^2 = 0.109, p = 0.001) whereas HEX was associated with gender only (adjusted R^2 = 0.047, p = 0.018) (Table 3.3). Central endothelial parameters were not associated with refractive errors or axial length (p > 0.05). For the superior cornea, ECD was associated with gender (adjusted R^2 = 0.086, p = 0.002), whereas CV was associated with initial axial length (adjusted R^2 = 0.044, p = 0.021) (Table 3.3). Superior endothelial parameters were not associated with age or refractive errors (p > 0.05).

Table 3.3 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

p-value: probability values for multiple linear regression for the prediction of the baseline endothelial morphology; other excluded variables: initial sphere, initial cylinder; bold for p-values < 0.05

The coefficients of determination of these models were so weak that only 11% of the variance was explained even in the best model for the central ECD. In this model, central ECD reduced by 42 cells/mm^2 for every year increase in age after controlling for gender, and was 106 cells/mm^2 higher in females than males after controlling for age. The difference in central ECD between male (mean ± SD: 3208 ± 209 cells/mm^2) and female (mean ± SD: 3320 ± 209 cells/mm^2) subjects was statistically significant (unpaired t test, $p = 0.010$).

3.3.3 Effect of time and use of orthokeratology on endothelial morphology

The endothelial parameters of the ortho-k and control subjects before and after two years are shown in Table 3.4. At the beginning of the study, except for a significantly lower HEX in the superior cornea in the ortho-k subjects (unpaired t test, $p = 0.013$), there were no significant differences in the baseline endothelial morphology between the ortho-k and control subjects in the two corneal locations (unpaired t tests, $p > 0.160$) (Table 3.4). At the end of the two-year study period, there were no significant changes in superior ECD (repeated measures ANCOVA, $p = 0.803$) and superior CV (repeated measures ANCOVA, $p = 0.489$). However, central ECD, central HEX, and superior HEX were significantly reduced (repeated measures ANCOVAs, $0.001 < p < 0.044$) whereas central CV was significantly increased (repeated measures ANOVA, $p = 0.030$). The reduction in the central ECD was significantly affected by the study group ($p = 0.032$) such that the reduction in

the ortho-k subjects (mean ± SD: -56 ± 94 cells/mm^2) was significantly less than that in the control subjects (mean ± SD: -98 ± 92 cells/mm^2) (unpaired t test, p = 0.024). The mean ± SD changes in the central HEX, superior HEX, and central CV are shown in Table 3.4 and the changes were not significantly affected by the use of ortho-k (0.998 > p > 0.105).

3.3.4 Effect of changes in corneal endothelial morphology

At the central cornea, the reduction in ECD was not affected by age, gender, refractive error, axial length, changes in axial length, or the baseline value in both groups of subjects (multiple linear regression, p > 0.05). The changes in CV and HEX were associated with their baseline values in both the ortho-k (CV: adjusted R^2 = 0.100, p = 0.015; HEX: adjusted R^2 = 0.134, p = 0.006) and control subjects (CV: adjusted R^2 = 0.079, p = 0.028; HEX: adjusted R^2 = 0.094, p = 0.018). At the superior cornea, the changes in ECD and CV were not affected by age, gender, refractive error, axial length, changes in axial length, or the baseline value in both groups of subjects (multiple linear regression, p > 0.05). For the control subjects, change in HEX was associated with the baseline value (adjusted R^2 = 0.240, p < 0.001); whereas for the ortho-k subjects, change in HEX was associated with both the baseline value (standardized beta = -0.279) and gender (standardized beta = -0.287) (adjusted R^2 = 0.140, p = 0.012).

Table 3.4. Endothelial morphology of the two groups of subjects before and after the 2-year study in the central and superior cornea

	Orthokeratology		**Control**		**p-value**	
Central cornea	Baseline	2 years	Baseline	2 years	Time	Time*Group
ECD (cells/mm^2)	3241 ± 178	3185 ± 207	3302 ± 246	3204 ± 237	**0.017**	**0.032**
CV (%)	24.7 ± 2.0	25.37 ± 2.2	24.31 ± 1.9	24.41 ± 2.2	**0.030**	0.105
HEX (%)	71.1 ± 7.3	69.54 ± 7.5	71.90 ± 6.9	70.31 ± 7.6	**0.044**	0.949
	Changes		**Changes**			
ECD (cells/mm^2)	-55.5 ± 94.0		-98.4 ± 91.5			
CV (%)	0.69 ±1.76		0.10 ± 1.84			
HEX (%)	-1.52 ± 6.06		-1.59 ± 6.18			
	Orthokeratology		**Control**		**p-value**	
Superior cornea	Baseline	2 years	Baseline	2 years	Time	Time*Group
ECD (cells/mm^2)	3461 ± 226	3448 ± 262	3488 ± 341	3474 ± 338	0.803	0.998
CV (%)	27.0 ± 2.9	28.19 ± 3.9	26.45 ± 3.6	26.99 ± 4.1	0.489	0.247
HEX (%)	69.2 ± 6.5	67.7 ± 8.5	72.94 ± 8.0	69.22 ± 8.0	**0.001**	0.183
	Changes		**Changes**			
ECD (cells/mm^2)	-13.7 ± 169.4		-13.8 ± 151.0			
CV (%)	1.24 ± 2.72		0.54 ± 2.81			
HEX (%)	-1.58 ± 7.77		-3.71 ± 8.06			

ECD: endothelial cell density; CV: coefficient of variation in cell size; HEX: hexagonality

p-value: probability values for repeated measures ANOVA/ANCOVA for within subject effect (Time) and interaction (Time*Group); bold for p-values < 0.05

3.4 Discussion

The current study shows that reduction in ECD and increase in polymegathism and pleomorphism in the central cornea in children after two years of treatment with single-vision spectacles or ortho-k were due to normal ageing. Ortho-k did not affect polymegathism and pleomorphism but modified the reduction in ECD in the central cornea.

3.4.1 Endothelial morphology in the central cornea

Contact lens wear on overnight modality is associated with increased risk of hypoxia, thus increasing risk of inflammation or even infection (*Section 1.2.3*). Theoretically, hypoxia induced by ortho-k is minimal compared to extended wear soft lenses for the higher oxygen permeability of the lens material and smaller corneal coverage sparing the limbal area (*Section 1.2.1.7*). It also has minimal effect on the retardation of recovery of hypoxia because of lens removal upon waking (*Section 1.2.1.7*). Although the recommended Dk/t values for avoiding oedema, derived from in vitro conditions, is less crucial in ortho-k lens wear than in extended soft lens wear (*Section 1.2.1.7*), Dk/t is always preferred to be as high as possible to reduce the risk of hypoxia. Dk/t of the lenses used in the current study was 68. The results show that the effect of ortho-k on endothelium was minimal as its effect on polymegathism and pleomorphism in the central cornea was comparable to those who did not wear contact lenses. Subjects on both ortho-k and spectacles showed reduction in ECD, however, the reason for

slower reduction in ECD observed in the ortho-k subjects is not clear and further investigation is warranted.

The current results also agreed with previous finding that the reduction in ECD is accompanied by polymegathism and pleomorphism in the central cornea for normal people (*Section 1.2.1.5*). The two-year changes in ECD, polymegathism, and pleomorphism in the central cornea found in the current study was 3.0%, 2.2%, and 0.4%, respectively. Although central ECD was associated with age and gender, its changes were not associated with any parameter identified in the current study. The changes in central CV and central HEX were also not associated with the demographic or ocular parameters measured, but were associated with their baseline values. These results may indicate that changes in the endothelial parameters are a natural process which is not influenced by external factors and may vary between individuals.

Early studies show that long term wear of PMMA or hydrogel lens is associated with polymegathism and pleomorphism but endothelial cell reduction (Holden *et al,,* 1985; Nieuwendaal et al., 1994; Bourne 2001). The reported polymegathism varied from 10% after 10 years of PMMA/hydrogel contact lens wear (Nieuwendaal *et al.,* 1994) to 22% after 3 years of hydrogel lens wear (Holden *et al,,* 1985). Most of these studies were cross-sectional with poor control of the demographic data of subjects (e.g. age and gender), refractive status of the eyes and the usage of contact lenses (e.g. materials of lenses, duration of lens wear and modality of lens wear). The current study also reported changes in polymegathism, pleomorphism and ECD, however,

the reported changes (≤ 3%) were much smaller than those reported in other contact lens studies in adults, and the changes were observed in both contact lens and non-contact lens wearing eyes. Further study is warranted to explore and differentiate effect of longer term ortho-k and ageing effect on corneal endothelium.

3.4.2 Other factors affecting the corneal endothelial morphology

The current study shows that girls have higher central ECD than boys. It has been documented that girls have smaller corneas than boys (Chan *et al.*, 2012), hence it is reasonable to hypothesize that girls will have higher ECD than boys. However, corneal diameter was not determined in this study to account for the differences. Previous studies have shown insignificant differences in ECD between males and females in adults (Chang *et al.*, 2001; Urban *et al.*, 2002; Shao *et al.*, 2007), however, the effect of corneal diameter and other ocular parameters were not considered in their analyses. Further investigation is warranted to explore the factors affecting central ECD.

Some studies reported that the reduction in ECD was associated with an increase in myopia (Chang *et al.*, 2001; Urban *et al.*, 2002; Delshad and Chun, 2013). However, these studies performed univariate analyses and did not consider other potential confounding factors such as age and gender. Sheng and Bullimore (2007) have conducted a comprehensive investigation on factors affecting corneal endothelial morphology in subjects aged between 19 to 71 years using multiple regression analyses. Their results showed that

ECD was associated with age and ethnicity. CV was associated with age and contact lens wear, whereas HEX was associated with age and myopia. The current findings agreed with their findings on ECD, that is, ECD was associated with age but not with refractive error. However, the associations between CV and HEX and age and between HEX and myopia reported by Sheng and Bullimore (2007) were not observed in the current study (*Section 1.2.1.5*).

3.4.3 Endothelial morphology in the peripheral cornea

Superior cornea was selected as the peripheral location for the investigation of the effect of ortho-k on central and peripheral cornea as this corneal location has been shown to have the highest ECD compared to other corneal locations whereas ECD was comparable in the other three peripheral corneal locations (*Section 1.2.1.5*). Asians have different eye shapes and tend to have smaller vertical palpebral aperture height than the Caucasians. Increased coverage of the superior cornea by the upper eyelid is common in Asians. This may be associated with chronic hypoxia, thus there may be an increase in polymegathism and pleomorphism in this location. The higher ECD and CV in the superior cornea observed in the current study supported the hypotheses about peripheral cornea being cell reservoir (stem cells at limbus) of the corneal endothelium and increased polymegathism in the superior cornea.

However, increased pleomorphism was not observed in the superior cornea. This may be due to the high population of hexagonal cells in this location,

suggesting that the honeycomb geometry of the corneal endothelium is stable in the superior cornea and has a higher resistance to stress. The reduction of HEX in the central and peripheral locations in both ortho-k and control subjects suggested that the homeostasis of the corneal endothelium is affected by age.

3.5 Conclusion

In conclusion, ortho-k exerted minimal stress on the corneal endothelium. The changes observed in the current study were mainly due to normal ageing of the cornea in children.

Publication: Cheung S. W., Cho P. (2017). Does a two-year period of orthokeratology lead to changes in the endothelial morphology of children. *Cont Lens Anterior Eye*. 42; 214-218.

CHAPTER 4

Effects on the Ocular Microbiome of Children Wearing Orthokeratology Lenses and Single-Vision Glasses

4.1 Introduction

In Hong Kong, the most commonly isolated pathogens from ortho-k related microbial keratitis are *Pseudomonas* (39–71%) and *Acanthamoeba* (0–13%) in (*Section 1.2.3.1*), and *S. aureus* and *Acinetobacter* in contamination studies following ortho-k wear (*Section 1.2.5*). The former two organisms are related to the use of tap water, whilst the latter two are either microbiome from other parts of the body, or contaminants from fomites transferred to the eyes (*Section 1.2.3*–*1.2.5*). Boost and Cho (2005) showed no significant changes in the ocular microbiome before and after 12 weeks ortho-k treatment, but they did isolate pathogenic organisms from the lenses and accessories. However, no attempt was performed to determine the source of contamination and no comparison was obtained from control subjects (*Section 1.2.5*). The study also only examined effects after three months use of ortho-k and changes may take longer to become apparent. The two main objectives of the current study aimed to bridge the knowledge gaps with respect to the long term effects of ortho-k on the ocular microbiome and the sources of contamination in ortho-k lens wear. The first objective was

determined by comparing the ocular microbiome in children with and without ortho-k wear. The second one was determined by investigating the odds of having positive cultures of *S. aureus* and Gram-negative bacteria in the eyes and positive cultures in the accessories, respectively.

4.2 Methods

In this cross-sectional study, myopic subjects of age 7 to 14 were recruited from the Optometry Clinic of The Hong Kong Polytechnic University. Ortho-k subjects had been receiving the treatment for over one year and were using the MeniCare Plus (Menicon Co, Ltd, Nagoya, Japan) multipurpose solution (MPS) to disinfect their lenses. The control subjects all used spectacles for vision correction and had no prior experience of contact lens wear. The ocular health of these subjects was unremarkable and none were receiving any regular medication at the time of the study. Informed consent was obtained at the commencement of the study from both the subjects and their parents. The study followed the tenets of the Declaration of Helsinki and was approved by the Human Subject Ethics Committee of The Hong Kong Polytechnic University.

For the control subjects, samples from the peri-orbital tissues and conjunctiva as well as from their spectacles were collected on the day of consent. For the ortho-k subjects, they were required to replace their MPS solutions with a complimentary bottle and lens case on the day of consent, and were scheduled to return for sample collection one month afterwards. They were required to follow the instruction for cleaning their lenses with

daily cleaner (Menicon Spray and Clean; Menicon Co, Ltd, Nagoya, Japan), rinsing lenses with normal saline before soaking in the MPS solution. They also needed to follow procedures to care for the lens cases by disposing of the MPS solution in the case after lens insertion, rinsing with saline and allowing it to air dry on a facial tissue overnight. On the day of examination, subjects were required to put the lenses in a disinfected lens case (transport lens case), which was provided within seven days before the scheduled visit, for temporary storage. After removal on waking, the lenses without cleaning were soaked in unidose normal in the transport lens case. The MPS bottle (for the contamination of MPS bottle) and lens case (for the contamination of lens case) used for one month, and the transport lens case containing the lenses were returned for culture. Eye swabs were collected from the peri-orbital tissues and conjunctiva, as described below. The lenses were returned to the subjects after being transferred from the transport lens case to a new regular lens case with a pair of sterile forceps. Contamination of the ortho-k lens was determined from the solution in the transport lens case. Sample collection and laboratory tests were performed by another research staff trained for these procedures.

4.2.1 Samples from the peri-orbital tissues, lower conjunctiva and spectacles

Samples collection was performed in the examination room. Four eye swabs were collected from the left eyes for all subjects. One swab was collected for each of the four locations: three eye swabs from the upper eyelid, the lower

eyelids and the eyelashes with eye closed, and one swab from the lower palpebral conjunctiva. Two swabs were collected from the spectacles for the control subjects, one from the spectacle arm and the other from the nose pad. All swabs were pre-moistened in sterile phosphate buffered saline (PBS) before sample collection and were broken off into universal bottles containing 10 ml of sterile brain heart infusion (BHI) broth after sample collection. All samples were carefully labelled after collection.

4.2.2 Samples from orthokeratology lenses, lens cases and multipurpose solution

Sample collection was performed in the laboratory. One sample was collected from the lenses, two samples were collected from the lens cases and two samples were collected from the MPS solution. Sample from the lenses was obtained from the saline extracts in the transport lens cases. Samples of the lens cases were collected using two sterile cotton swabs, one for the inner surface and the other for the screw tops of the lens case. Two samples were collected for the MPS solution, one from the bottle tip using a sterile cotton swab and the other from the MPS extracted from the bottle using a syringe. An aliquot of 2 ml of MPS was withdrawn 2 cm from the bottom of the bottle and transferred to a universal bottle containing 8 ml of Dey-Engley neutralizing broth with EDTA. The swabs for lens cases and bottle tips were pre-soaked in Dey-Engley neutralizing broth with EDTA (2mM) before sample collection and were broken off into individual universal

bottles containing 10 ml of sterile BHI broth. All samples were carefully labelled after collection.

4.2.3 Microbial Assessment

All bottles with BHI containing samples taken with swabs (i.e. from peri-orbital tissues, conjunctiva, spectacles, lens cases and MPS bottle tip) were vortexed for one minute to release microbial organisms adhering to the swabs. All samples were then plated out from BHI or Dey-Engley broth within 30 minutes of collection. Total bacterial count was performed by culturing a 100µl aliquot of each sample on Nutrient agar incubated at 37°C for 24 hours. The colonial morphology for each plate was recorded if positive cultures were observed and the number of colonies were counted using an automated colony counter (aCOLyte 3, Synbiosis, Frederick, MD, USA). For the identification of individual microbial organisms, the broths were incubated for 24 hour for enrichment before subculture onto *SaSelect* agar for *S. aureus* and CNS, cetrimide agar for *Pseudomonas*, MacConkey agar for *Enterobacteriaceae* and other Gram-negative rods and *Acinetobacter* select agar. All agar plates were incubated at 37°C for 24 hours then examine for the presence of colonies with typical morphology. All culture media were purchased from Thermo-Fisher Scientific Inc. (Waltham, MA, USA).

4.2.4 Statistical analysis

Since the total number of isolates demonstrated non-Gaussian distributions, non-parametric tests were used for the statistical analyses (SPSS 18.0,

SPSS, Chicago, IL). Differences in the total number of isolates between the two study groups were determined using Mann-Whitney U Tests. Differences in carriage rates of the individual isolated organisms between ortho-k and control subjects, and the associations between positive culture in eyes and positive culture in the accessories for each group of subjects were determined using Chi-square and Fisher's Exact Tests. The odds of having specific microbial organisms in subjects with and without ortho-k treatment, and the odds of having positive culture in eyes and in the accessories were also determined.

4.3 Results

Forty-three subjects were recruited, 23 wearing ortho-k (13 female; 10 male) and 20 wearing single-vision spectacles (11 female; 9 male). There were no significant differences in age and gender between the two groups of subjects ($p > 0.07$). The median (range) age was 12.0 (7–13) years for the ortho-k subjects and 11.0 (8–14) years for the control subjects. *Pseudomonas* was not identified in any of the samples collected from the peri-orbital tissues and accessories. *Acinetobacter* was only isolated from two samples from the peri-orbital tissues, one in the conjunctiva and one in the eyelashes of two ortho-k subjects. It was not found in the samples from the accessories.

4.3.1 Ocular microbiome in the peri-orbital tissues and accessories in ortho-k and control subjects

The numbers of micro-organisms in the periorbital tissues were high in both ortho-k and control subjects (Table 4.1). Organisms were found in the lower eyelids in all subjects (Table 4.1). The percentage of subjects carrying organisms on their upper eyelids was slightly reduced from 100% in the ortho-k controls to 95% in the control subjects 95%. The percentage of colonized subjects ranged from 85 to 96% for the conjunctiva and was the lowest for the eyelashes (78–80%). The carriage rates of pathogens (including *S. aureus*, *Acinetobacter* and other Gram-negative bacteria with or without the presence of CNS) varied from 35–39%, 45–52%, 39–55% and 25–48% in the lower eyelids, upper eyelids, conjunctiva and eyelashes, respectively, in the two groups of subjects (Table 4.1) and the between-group differences in the carriage rates were statistically insignificant ($p > 0.122$).

Figure 4.1 shows the carriage rates of individual organisms isolated at the four peri-orbital locations. There were also no significant differences in the carriage rates of individual organisms in the peri-orbital tissues between the two groups of subjects ($p > 0.187$) (Table 4.2). The most commonly isolated organisms in the peri-orbital tissues were CNS being presented in 65–91% of subjects, followed by *S. aureus* being present in 22–40% of subjects. Other Gram-negative bacteria were present in 17–35% in the upper and lower eyelids and conjunctiva in all subjects. Gram-negative bacteria were only identified in ortho-k subjects but not in the control subjects.

Table 4.1 Carriage rates for pathogenic (*S. aureus, Acinetobacter* and other Gram-negative bacteria; alone or in the presence of CNS) and opportunistic organisms (CNS only) isolated at the four peri-orbital locations

	Orthokeratology (N=23)	Control (N=20)	Odds ratios	95% CI	p-value
Lower eyelid					
Pathogens present	39%	35%	1.19	0.34–4.14	0.780
CNS only	61%	65%			
Upper eyelid					
Pathogens present	52%	45%	1.33	0.40–4.44	0.639
CNS only	48%	50%			
Conjunctiva					
Pathogens present	39%	55%	0.53	0.16–1.77	0.298
CNS only	57%	30%			
Eyelashes					
Pathogens present	48%	25%	2.75	0.75–10.11	0.122
CNS only	30%	55%			

95% CI: 95% confidence interval

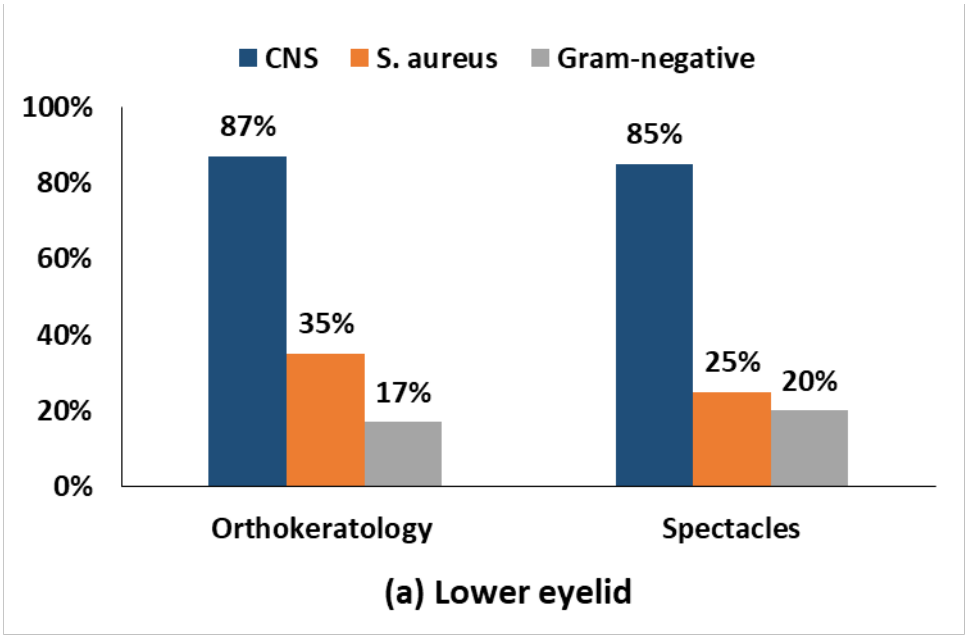
p-value: probability values of the significance of the odds ratio

Table 4.2 Odds of having positive culture of individual organisms in eyes with ortho-k treatment

	Orthokeratology (N=23)	Control (N=20)	Odds ratios	95% CI	p-value
Lower eyelid					
CNS	87%	85%	1.18	0.21–6.61	1.000
S. aureus	35%	25%	1.60	0.42–6.03	0.486
Gram-negative	17%	20%	0.84	0.18–3.92	1.000
Upper eyelid					
CNS	91%	90%	1.17	0.15–9.14	1.000
S. aureus	39%	30%	1.50	0.42–5.35	0.531
Gram-negative	35%	20%	2.13	0.53–8.58	0.281
Conjunctiva					
CNS	83%	65%	2.56	0.62–10.55	0.187
S. aureus	22%	40%	0.42	0.11–1.58	0.193
Gram-negative	22%	20%	1.11	0.25–4.87	1.000[#]
Eyelashes					
CNS	83%	75%	1.58	0.36–6.95	0.711
S. aureus	35%	25%	1.60	0.42–6.03	0.260
Gram-negative	17%	0	--	--	--

95% CI: 95% confidence interval

p-value: probability values of the significance of the odds ratio



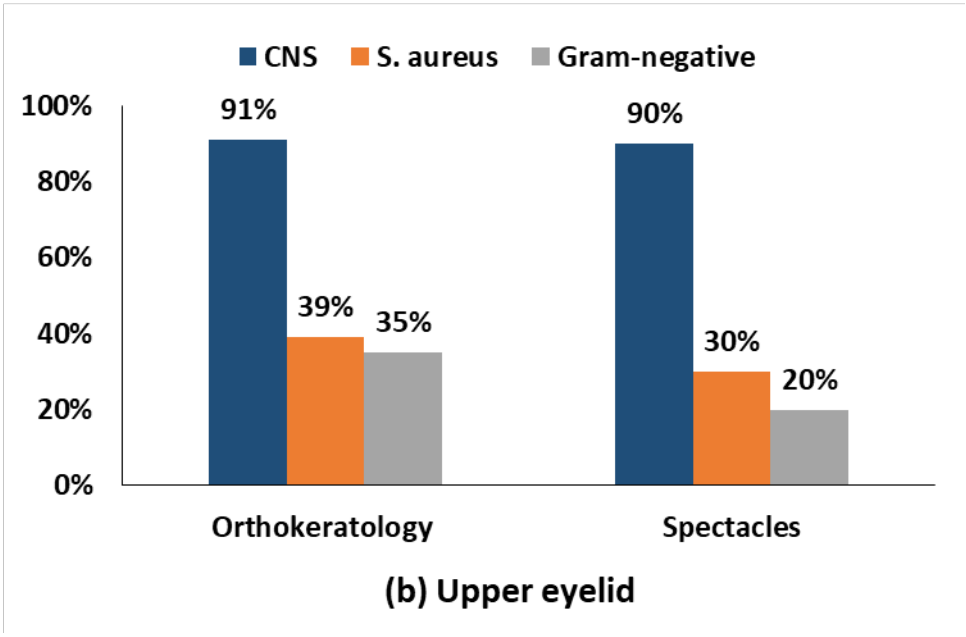
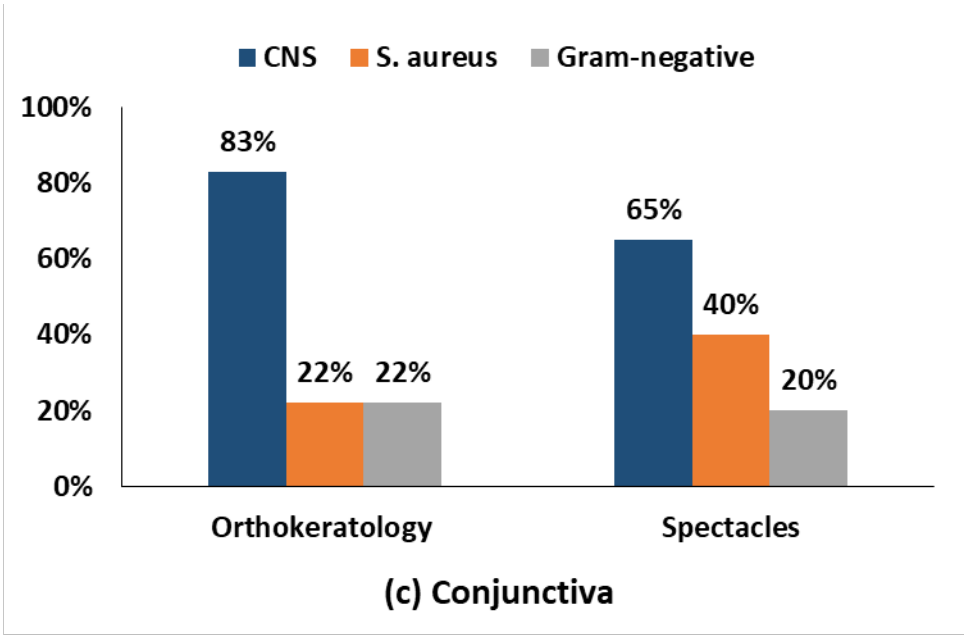


Figure 4.1 Carriage rates of individual organisms isolated at the four peri-orbital locations (a) lower eyelid (b) upper eyelid



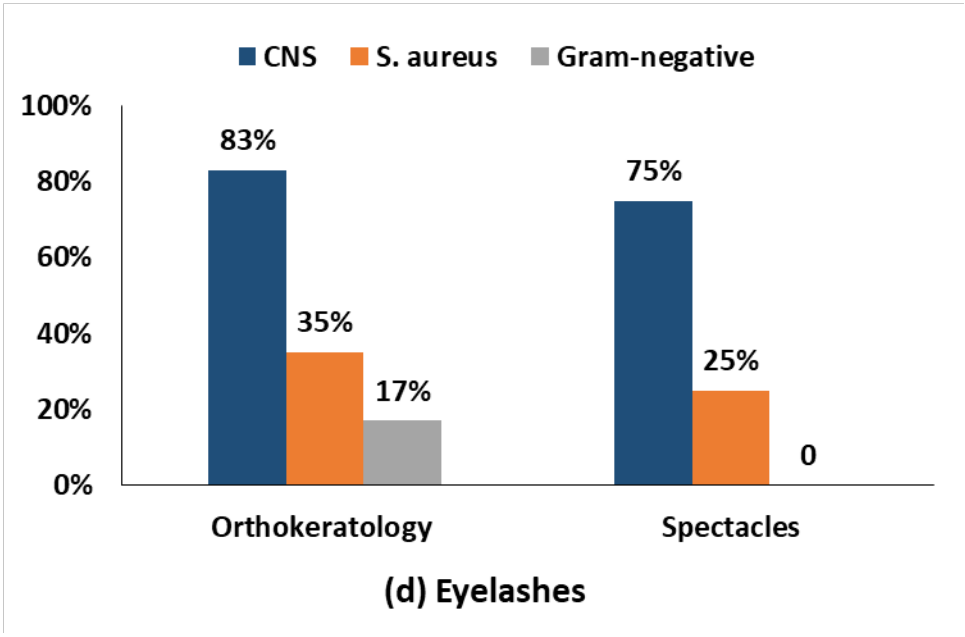


Figure 4.1 (continue) Carriage rates of individual organisms isolated at the four peri-orbital locations (c) conjunctiva (d) eyelashes

Table 4.3 shows that total number of isolates from the four peri-orbital tissues, contact lenses, contact lens accessories and spectacle glasses. Fewer isolates were obtained in the conjunctival cultures of the ortho-k subjects compared to the controls ($p = 0.009$). Otherwise, no significant between group differences in total number of isolates were observed from in the upper and lower eyelids, or the eyelashes ($p > 0.386$). In the ortho-k subjects, fewer isolates were found in the cultures from the lenses and accessories compared to the level in the peri-orbital tissues. In contrast, the amount of isolates found in the cultures from spectacles were comparable to those in the peri-orbital tissues of the control subjects.

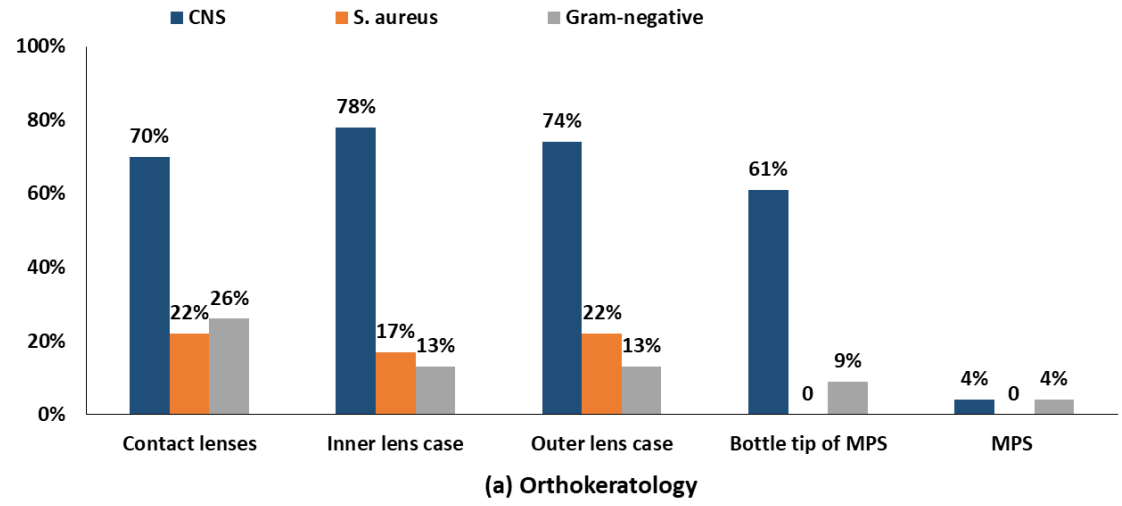
Table 4.3 Median and interquartile range (IQR) of total number of isolates (x10^2 CFU) in the four locations of the peri-orbital tissues and accessories

	Orthokeratology (N=23)		**Control (N=20)**		**p-value**
	Median	IQR	Median	IQR	
Lower eyelid	3	8.5	4.5	9.75	0.386
Upper eyelid	3	10.5	3	3.25	0.638
Conjunctiva	0	1	1.5	2.25	**0.009**
Eyelashes	5	9	2	4.75	0.776
Contact lenses	0	1.5	--	--	--
Inner lens case	0	1.5	--	--	--
Outer lens case	1	4	--	--	--
Bottle tip of MPS	1	5	--	--	--
MPS	0	0	--	--	--
Nose pad	--	--	3.5	11.5	--
Spectacles arm	--	--	2	4.25	--

p-value: probability values of Mann Whitney U Tests for between groups differences; **bold** indicates $p < 0.05$

The contamination rates of the lenses and accessories for the ortho-k and control subjects are shown in Table 4.4 and Figure 4.2. For all samples collected from items with direct contact with skin (i.e. lenses, lens cases and spectacles), CNS was the most commonly isolated micro-organisms on 70–78% of ortho-k lenses and lens cases and 80–85% of spectacles (Figure 4.2). The levels of *S. aureus* in these samples (17–35% (Figure 4.2)) were comparable to those in the peri-orbital tissues (22–40% (Figure 4.1) in both groups of subjects. The levels of Gram-negative rods found in the accessories were comparable to those found in the peri-orbital tissues in the ortho-k subjects (skin: 17–35% (Figure 4.1); accessories: 13–26% (Figure 4.2a)) but were slightly reduced in the control subjects (skin: 0–20% (Figure 4.1); spectacle: 5–10% (Figure 4.2b)).

Trace amounts of bacteria were found in two samples of the MPS extracts, one yielding a positive culture of Gram-negative rods and the other CNS. *S. aureus* was not isolated from the MPS extracts or the samples from bottle tip. However, micro-organisms were identified from 65% of the bottle tips (Table 4.4), mostly opportunistic organisms (CNS only: 56%); the results might indicate non-compliance in handling the bottles.



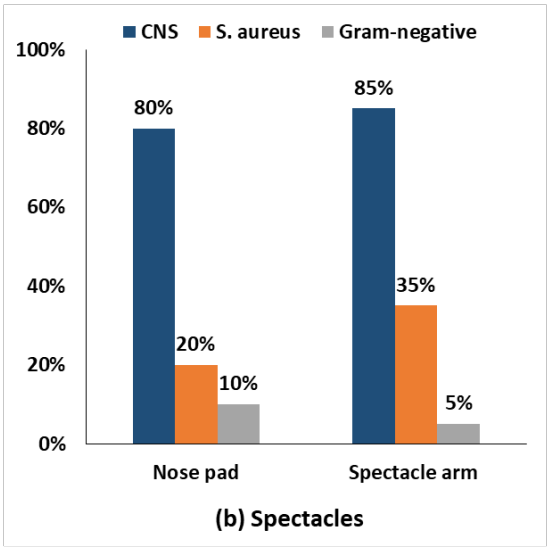


Figure 4.2 Contamination of individual organisms isolated in the accessories in subjects using (a) orthokeratology lenses, and (b) single-vision spectacles

Table 4.4 Overall contamination rates and contamination rates of pathogenic organisms and individual organisms on accessories

Sites	Overall	Positive culture of pathogenic organisms		Positive culture of individual organisms		
		Pathogens present *	CNS only	CNS	*S. aureus*	Gram-negative
Orthokeratology						
Contact lenses	78%	35%	48%	70%	22%	26%
Inner lens case	87%	30%	57%	78%	17%	13%
Outer lens case	87%	35%	52%	74%	22%	13%
Bottle tip of MPS	65%	9%	56%	61%	0	9%
MPS	9%	4%	4%	4%	0	4%
Control						
Nose pad	100%	30%	70%	80%	20%	10%
Spectacle arm	100%	40%	60%	85%	35%	5%

**S. aureus,* and other Gram-negative bacteria alone or in the presence of CNS

MPS: multipurpose solution

4.3.2 Source of contamination in the accessories

CNS was the most commonly isolated opportunistic organisms in all the four peri-orbital locations in 65–91% subjects, whereas the most commonly isolated pathogenic organisms were *S. aureus* and other Gram-negative bacteria from the skin with carriage rates varying from 0–40%. Hence, the associations between the carriage of the latter two organisms in the peri-orbital tissues and in the accessories were determined for the investigation of the source of contamination and the results shown in Table 4.5. For the ortho-k subjects, no associations between positive cultures in the eyes and positive cultures in the accessories were found ($p > 0.060$), although the probability almost reached significance. For the control subjects, no association between positive culture of Gram-negative bacteria in the eyes and in the accessories was found ($p = 0.347$) as positive culture was found only in two samples from the spectacles. On the contrary, subjects with positive culture of *S. aureus* in the eyes were likely to have positive culture in the spectacles (odds ratio: 13.5, 95% confidence interval: 1.2-152.2, $p = 0.029$).

Table 4.5 Associations between positive culture of *Staphylococcus aureus* (*S. aureus*) and Gram-negative bacteria in the peri-orbital tissues and accessories in orthokeratology and control subjects

Subjects	Positive culture in eye	Positive culture in accessories		Odds ratio	95% CI	p-value
Orthokeratology	***S. aureus***	**Yes**	**No**	3.42	0.52–22.80	3.42
	Yes	6	7			
	No	2	8			
	Gram-negative	**Yes**	**No**	6.30	0.93–42.73	0.060
	Yes	9	5			
	No	2	7			
Control	***S. aureus***	**Yes**	**No**	13.50	1.20–152.21	**0.029**
	Yes	6	4			
	No	1	9			
	Gram-negative	**Yes**	**No**	--	--	0.347
	Yes	0	8			
	No	2	10			

p-value: probability values of Fishers Exact Test; bold indicates $p < 0.05$

4.4 Discussion

4.4.1 Ocular microbiome in the peri-orbital tissues

The four peri-orbital locations were selected for sample collection because of their involvement or potential involvement during ortho-k lens wear. The upper and lower eyelids are held open by the fingers during insertion and removal whereas the lower conjunctiva touches the lenses during sleep. Contact of eyelashes with lenses should be avoided during insertion and removal; however, fingers or lenses may accidentally touch the eyelashes during these procedures. Insertion and removal of lenses may therefore affect the ocular microbiome and increase the risk of transfer of the micro-organisms, including potential pathogens.

The current results showed that the ocular microbiome in the upper and lower eyelids and eyelashes was not affected by ortho-k lens wear. The carriage rates of pathogenic organisms, the carriage rates of individual organisms, and the total number of isolates present in these two tissues in ortho-k subjects who had been on the treatment for over 12 months were comparable to those without contact lens experience (Tables 4.1–4.3). These results suggest that frequent contact of the eyelids with fingers during lens insertion and removal did not change the characteristics of the ocular microbiome on these tissues.

The total number of conjunctival micro-organisms was reduced in the ortho-k wearers compared to the control subjects (Table 4.3). It seems that the number of conjunctival micro-organisms is affected by extended wear modality but not by daily wear modality after contact lens wear. Fleiszig and

Efron (1992) observed a reduction in CNS in the conjunctiva after extended wear of rigid gas permeable lenses as in the current study, whilst Iskeleli and co-workers (2005) found an increase in CNS in conjunctiva after extended wear of silicone hydrogel lenses. There were no significant differences in the number of conjunctival micro-organisms found between subjects on daily wear contact lenses (soft and rigid) and the non-contact wearers (Larkin and Leeming, 1991; Erdoğan *et al.*, 2002). Despite the reduction in total number of isolates in the conjunctiva after ortho-k, the comparable diversity of pathogenic organisms and individual organisms between ortho-k and control subjects (see Table 4.1 and Table 4.2) suggested that the balance of ocular microbiome was not disturbed after ortho-k lens wear (*Section 1.2.5*).

4.4.2 Sources of ocular microbiome in the peri-orbital tissues

The most commonly isolated organism in the peri-orbital tissues and accessories is CNS (Singer *et al.*, 1988; Weiss *et al.*, 1993; Pernandez-Rubio *et al.*, 2009; Sthapit and Tuladhar, 2014). CNS is part of the microbiome of the skin of the body and can be easily transferred from the one part of the body to another (e.g. from eyelids to eyelashes in eye rubbing), or from the body to a device (e.g. from nose to the nose pads of the spectacles after settlement or from the eyelids to the contact lens after removal using fingers) (*Section 1.2.3.1*).

The carriage rates of *S. aureus* and other Gram-negative bacteria were lower than CNS because they are not normally found in the peri-orbital tissues. The carriage of *S. aureus* in the eyes and accessories is much lower than CNS

as nasal colonization with this organism is found in only about 24% of healthy individuals in Hong Kong (Zhang *et al.*, 2011; Ho *et al.*, 2015), of whom only 17% are persistent carriers (Ho *et al.*, 2015). Although the current study did not assess the colonization rates of *S. aureus*, the high carriage rate of *S. aureus* in the peri-orbital tissues (35%) may be attributable to the higher colonization rates in children compared to adults (Bogaert *et al.*, 2004; Kuehnert *et al.*, 2006). The presence of *S. aureus* in the skin and accessories indicates contact transmission from the nose (Wertheim eta l., 2005) to the hands, followed by transfer to other items. Gram-negative bacteria can survive on damp skin and the presence of this organism in the peri-orbital tissues and accessories indicates poor hand drying (Patric *et al.*, 1997; Merry *et al.*, 2001; Collins and Hampton, 2005; Yamamoto *et al.*, 2005; Snelling *et al.*, 2011; Huang *et al.*, 2012).

4.4.3 Sources of contamination of accessories

The modes of transmission *S. aureus* and Gram-negative bacteria are somewhat different. However, the means of transmission of *S. aureus* and Gram-negative bacteria are the same: hands and fingers. Therefore, it was hypothesized that bacterial contamination of the peri-orbital tissues would be associated with contamination of the accessories with the same organisms. In the current study, this hypothesis was only true for *S. aureus* in the control subjects such that subjects not yielding *S. aureus* from their skin tissues were less likely to have *S. aureus* in their accessories (Table 4.5). This

hypothesis was rejected for S. aureus in the ortho-k subjects and for Gram-negative bacteria in both ortho-k and control subjects.

The failure to find an association for Gram-negative bacteria is likely to be related to the small sample for different perspectives. For the control subjects, hand washing was not expected to be performed before handling spectacles. It would reduce the risk of contamination with damp skin, resulting in low positive culture found (2 out of 20 subjects), thus low statistical power to detect a difference. For the ortho-k subjects, they were expected to wash and dry hands properly before handling lenses and accessories. Although subjects with positive cultures of Gram-negative bacteria in the eyes were likely to have more positive culture in the accessories but the results did not reach statistical significance ($p = 0.060$, Table 4.5). It is likely an association would be observed if a larger sample size was included in the study.

4.4.4 Hand hygiene and non-compliance in handling procedures

Good hand hygiene is essential in contact lens wear. Hands should be adequately cleansed with soap, rinsed with tap water and dried properly with paper towels before handling lenses as recent contact lens practice recommends that contact lenses and accessories should not be in contact with tap water (Patric *et al.*, 1997; Merry *et al.*, 2001; Collins and Hampton, 2005; Yamamoto *et al.*, 2005; Snelling *et al.*, 2011; Huang *et al.*, 2012; Ustunturk *et al.*, 2012; Tilia *et al.*, 2014). Care for contact lens accessories follows the same rationale: they can be rinsed with saline after cleaning but

they have to be either wiped with paper towels or allowed to air dry after use. All lens accessories have to be rinsed with normal saline or disinfecting solution before use. *Acanthamoeba* and *Pseudomonas* are two leading causes of microbial keratitis in ortho-k lens wear. The absence of these pathogens in the current study suggested that hands are dried properly. *Acinetobacter* is another emerging cause of microbial keratitis (Fong *et al.*, 2004). This bacteria is considered as 'bathroom' bacteria and can be eliminated by proper hand hygiene (Cardoso *et al.*, 1999). It was detected in the eyelashes of one ortho-k subject and in the conjunctiva of another, but was absent in the ortho-k lenses and accessories (*Section 1.2.5*).

In addition to good hand hygiene, good handling procedures are required to reduce the risk of cross contamination of bacteria. For instance, patients should not touch other items with fingers holding lenses during lens insertion, removal or cleaning with fingers after hand washing. The lens cases should be kept clean and should only have contact with saline, MPS or cleansed lenses. The bottle tip of preserved care solutions (i.e. cleaner, saline and disinfecting solutions) should be covered to avoid contamination.

In the current study, the carriage of organisms on the ortho-k lenses and lens cases were comparable to those of the microbiome of the skin tissues (Tables 4.2 and 4.4). The results for the ortho-k lenses were expected because of the collection procedures. The ortho-k lenses were directly transferred to the transport lens case without cleaning after removal resulting. Direct transmission of the bacteria from the conjunctiva to the lens resulted in comparable diversity of ocular microbiome and contaminants

present on the lenses and similar total numbers of isolates obtained on samples from the lenses and the conjunctiva (Tables 4.2–4.4). On the other hand, the presence of micro-organisms in the lens cases suggested transfer of bacteria either by touching the cases with fingers or putting uncleansed lenses in the lens cases, or both. There was quite a high number of CNS and of total isolates found on the bottle tip of MPS solution. The presence of bacteria in the lens cases and bottle tip indicated certain amount of non-compliance.

The contamination rate of the MPS solution (9%) was much lower than that of the bottle tip (65%). The total number isolates found in the MPS extracts was negligible (< 100 CFU, Table 4.3). This trace amount of bacteria present in the MSP solutions having been used for one-month shows that the solution was still effective for disinfection.

Non-compliance is a risk factor for ocular infection in contact lens wear and deviation from good hand hygiene and good handling procedures can happen. In addition to poor hand hygiene and touching lens accessories with fingers or dirty lenses, other non-compliant behaviors include use of contaminated paper towels for drying hands and lens cases, drying lens cases facing upwards, and handling lenses and accessories in the bathroom. Practitioners should be proactive in reviewing the procedures by demonstration by patients and re-education or reminders to re-enforce good compliance should be conducted at the regular aftercare visits.

4.4.5 Ocular microbiome in young children

In Hong Kong, ortho-k is used for myopia control in children (*Section 1.1.3*). It may be offered to children as young as four years old but young children up to the age of five are likely to be carriers of virulent pathogens like *Streptococcus pneumoniae* or *Haemophilus influenza* (Mohle-Boetani *et al.*, 1993; Regev-Yochay *et al.*, 2004), and are thus more susceptible to infection caused by these pathogens. Children are also more susceptible to be carriers of *S. aureus* (Bogaert *et al.*, 2004; Kuehnert *et al.*, 2006). Ideally, screening for these pathogens may help to screen out subjects at higher risk of colonization, but increased risk of colonization is not a necessary outcome of an infection. Colonized children are at higher risk of infection only when the immune system is compromised. In ortho-k practice for myopia control, the use of lenses is usually under parental supervision and children are advised to stop lens wear when they are unwell. Therefore, screening for carriage is not currently a pre-requisite procedure for contact lens wear. Indeed, rather than screening for pathogens for very young children, it may be better to be more cautious in fitting these very young children with ortho-k.

4.4.6 Limitations of the study

The main limitation of the current study was the relatively small sample size, especially as carriage rates of the pathogenic organisms were low (e.g. Gram-negative bacteria in the eyelashes in Table 4.2) and there was considerable variability between individuals with respect to total bacterial counts (Table 4.3). Carriage can be influenced by variation in personal

hygiene, care products used, and puberty changes (Grice and Segre, 2011) in the study population of children aged seven to 14. Small sample size also affects the association study of the source of contamination. Trends for positive culture of *S. aureus* and Gram-negative bacteria in eyes and accessories were observed, but the results were statistically insignificant. The sample size may have to be increased in the future studies.

It is difficult to make direct comparison of the current findings with previous studies on ocular microbiome in contact lens wear (Larkin and Leeming, 1991; Fleiszig and Efron, 1992; Erdoğan *et al.*, 2002; Boost and Cho, 2005; Iskeleli *et al.* 2005) or contamination rates in ortho-k lenses and accessories (Patrick *et al.*, 1997; Boost and Cho, 2005; Cho *et al.*, 2009) because of the differences in lens materials investigated (rigid versus hydrogel lenses), the modality of the lens wear (extended wear versus overnight wear), the age of the subjects (children versus adults), sampling methods (e.g. ortho-k lenses were not cleansed and disinfected for cell culture; lens cases were cleansed and dried for sample), and the methodology of microbial assessment. Further investigation is warranted to confirm the current findings. In addition to the bacterial culture performed in the current study, incorporation of cell sequencing technology may provide another perspective on the microbiome of the eye.

4.5 Conclusion

The current study reported an abundant amount of opportunistic organisms especially CNS, presence of pathogenic organisms such as *S. aureus* and

Gram-negative rods on the skin tissues and in the accessories of all subjects, but no significant association between contamination of accessories and ocular microbiome in the ortho-k subjects. No *Acanthamoeba* or *Pseudomonas* were found and only two samples were identified with *Acinetobacter.*

Microbial keratitis caused by S. aureus and *Enterobacteria* are less severe and easier to treat than those caused by *Pseudomonas* or *Acanthamoeba* (Green *et al.*, 2008). Despite colonization of some subjects with *S. aureus* and Gram-negative bacteria, the absence of ocular infections in the subjects participating in this study suggests that normal skin tissues have good resistance to the presence of these less virulent organisms in children with reasonable standard of hygiene. Ocular tissues are less resistant to more virulent organisms, such as *Acanthamoeba* and *Pseudomonas*. However, these pathogens can be avoided with good hand hygiene and avoidance of contamination with tap water. Therefore, risk of microbial keratitis was not increased with ortho-k lens wear but more likely to be associated with individual susceptibility to the pathogens and level of compliance.

Publication: Cheung S. W., Boost M. V, Shi G. S. and Cho P. Microbial contamination of periorbital tissues and accessories of children. *Optom Vis Sci*. 2016;93:612-8.

CHAPTER 5

Parents' Perspective on Myopia Control Using Orthokeratology

5.1 Introduction

As the prevalence of myopia in Hong Kong is one of the world's highest, it is understandable that parents with myopic children are concerned about the progression rate of their children and are motivated to seek for myopia control treatment (*Section 1.1.3*). Various kinds of myopia control strategies are available in Hong Kong, including pharmaceutical agents, optical aids, and alternative therapies. Some of these strategies do not have strong supportive evidence for myopia control, but there is no regulation on the promotion and sale of these products in Hong Kong. There is currently abundant information about myopia control in both the traditional (e.g. television, radio, newspaper and magazine) and digital media (e.g. the internet, social networking). Parents can make their own search or be informed by friends, relatives, and health care providers. The quality of this information is not controlled and parents may receive incorrect messages regarding myopia control or myopia control strategies.

Compliance is a major concern in myopia control. Non-compliance with use may reduce the effectiveness of myopia control with soft lenses (Lam *et al.*, 2014) and can affect safety of ortho-k (*Section 1.2.5*). This study aimed to evaluate the knowledge and attitudes of parents who were considering

myopia control treatment for their children. Information collected could help bridge the gaps about needs and concerns of parents, and may help practitioners in the selection of suitable candidates with good parental support for myopia control.

5.2 Methods

A telephone interview was conducted between June and August, 2011 with parents who had concerns about myopia progression in their children and were seeking myopia control treatment. The target population was parents responding to advertisements for two clinical trials on myopia control, one on ortho-k and the other on specially designed soft contact lenses, which were to be concurrently conducted at The Hong Kong Polytechnic University. Parents were invited to participate in this telephone survey upon completion of the phone screening for the individual clinical trials. The study followed the Declaration of Helsinki and was approved by the Departmental Research Committee of the School of Optometry at The Hong Kong Polytechnic University. Oral consent was obtained from the parents before the commencement of the telephone interview. All interviews were performed by an experienced customer service officer.

Parents were required to answer questions about their opinions on myopia control treatment and experience in contact lens wear, and to provide some personal particulars, including age, gender and educational background (Appendix A). There were three main areas of interest covered in the first five questions of this telephone survey:

1. Current knowledge of myopia control treatments in the community and their sources of information
2. Preference for myopia control using ortho-k, daily wear soft contact lenses (DWSL), or spectacles (either progressive add lenses or under-correction), *assuming* all three treatments to be equally effective for slowing myopia progression and factors affecting their decisions
3. Acceptable age of children to wear contact lenses for refractive correction and factors affecting their decisions.

Parents could choose more than one option for each of these five questions. Open-ended questions were used to solicit comments from parents on the reasons behind their decisions for preference in myopia control strategy and the use of contact lenses in children.

In the rest of this report, 'soft lenses' is the general term for lens type, 'soft lens group' refers to parents seeking clinical trials using soft lenses, and 'DWSL' refers to one of the options for the preferences in myopia control strategy.

5.2.1 Statistical analysis

Since information collected in this study was categorical data, non-parametric tests were performed using the SPSS software (version 18.0, SPSS Inc., Chicago, IL, US). Chi-square tests were used to compare information collected from parents interested in the myopia clinical trial using ortho-k (ortho-k group) and those interested in the clinical trial using soft lenses (soft lens group). Mann-Whitney U tests or Kruskal-Wallis tests were used to

study the factors affecting parents' decision on age when children can wear for contact lenses for vision correction.

5.3 Results

A telephone interview was conducted with 199 respondents. The data from three respondents were excluded as they were not the legal guardians of the children. The demographic data for the remaining 196 parents, 56% seeking ortho-k (ortho-k group) and 44% seeking soft lenses (soft lens group) for myopia control, is shown in Table 5.1. Parents in the ortho-k group were slightly younger (chi-square test, $p = 0.03$), whereas parents in the soft lens group were likely to be existing contact lens wearers (chi-square test, $p = 0.02$). Otherwise, there were no significant differences in the demographic data between the two groups of parents. Overall, in the majority of cases, the parent completing the interview was the mother (83%) and most parents suffered from myopia (87%). Almost all parents had received at least secondary education and worked in a non-health care related profession (70%).

Table 5.1. Demographic data of the 196 parents seeking orthokeratology (ortho-k group) or soft lenses (soft lens group) for myopia control

	Ortho-k group	Soft lens group	All
Number of respondents	110	86	196
Relationship			
Mother	84%	83%	83%
Father	16%	17%	17%
Age*			
26–35	9%	5%	7%
36–45	77%	67%	73%
46–55	14%	28%	20%
Education			
Secondary or below	56%	63%	59%
Tertiary or above	44%	37%	41%
Occupation			
Housewives	25%	12%	19%
Health care	10%	10%	10%
Finance and business	36%	48%	41%
Others (e.g. government servant, engineering etc.)	28%	30%	29%
Refractive status			
Emmetropia or hyperopia (> -0.50 D)	17%	9%	13%
Low to moderate myopia (between -6.00 to -0.50 D)	55%	61%	58%
High myopia (<-6.00D)	29%	29%	29%
Contact lens experience*			
No	38%	23%	32%
Ceased	38%	36%	37%
Current	24%	41%	31%

* Significant differences in age and contact lens experience in parents seeking orthokeratology and those seeking soft lenses for myopia control (Pearson chi-square > 6.91, $p < 0.05$); otherwise, no significant differences in the demographic data were found between the two groups of parents

5.3.1 Known myopia control treatments and the sources of information

The most commonly known myopia control treatment was ortho-k (86%), followed by progressive spectacles (35%) and soft lenses (29%) (Figure 1a). About 21% of parents had heard of the use of under-correction for slowing myopia progression. Most parents obtained information about myopia control from friends and relatives (52%), or from newspapers and magazines (50%). Only 6% were from other sources, such as the internet (Figure 5.1b). There were no significant differences in the known treatment for myopia control (chi-square tests, $0.34 > p > 0.11$) and the source of information (chi-square tests, $0.97 > p > 0.28$) between the two groups of parents.

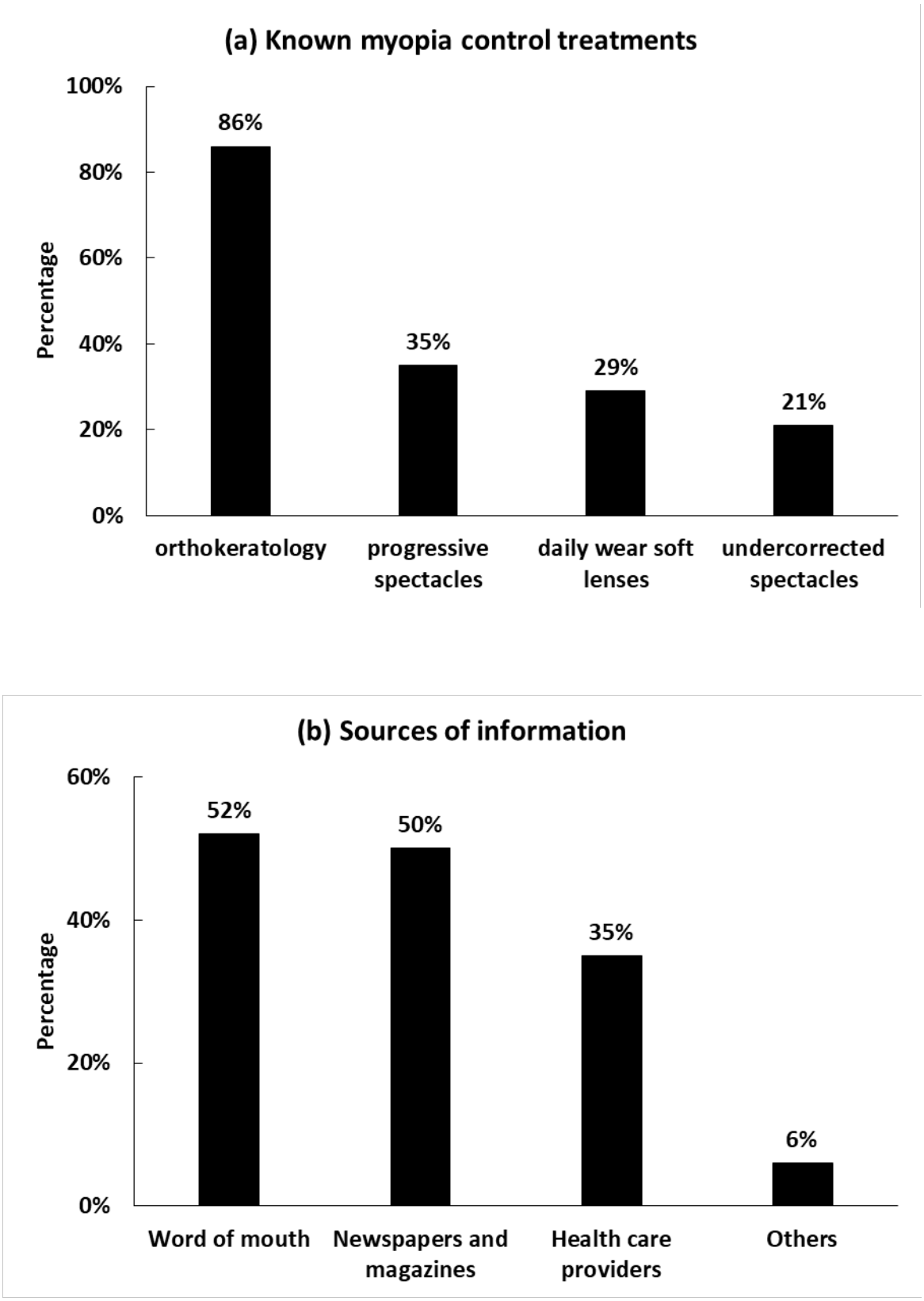


Figure 5.1 Myopia control treatments (a) known to the parents and (b) the sources of information

5.3.2 Preference in myopia control using orthokeratology, daily wear soft contact lenses and spectacles

Parents were asked to prioritize their options for myopia control treatments with the assumption that all the three options were equally effective for myopia control. The responses to the most and the least preferred options were affected by the study groups (chi-square tests, $p < 0.001$). Parents in the soft lens group had no overall preference for myopia control strategy (ortho-k 38%; spectacles: 29%; DWSL: 33%) (chi-square test, $p = 0.57$) (Figure 5.2). In contrast, parents in the ortho-k group strongly preferred ortho-k (49%) and spectacles (45%) to DWSL (6%) for myopia control (chi-square test, $p < 0.001$) (Figure 5.2).

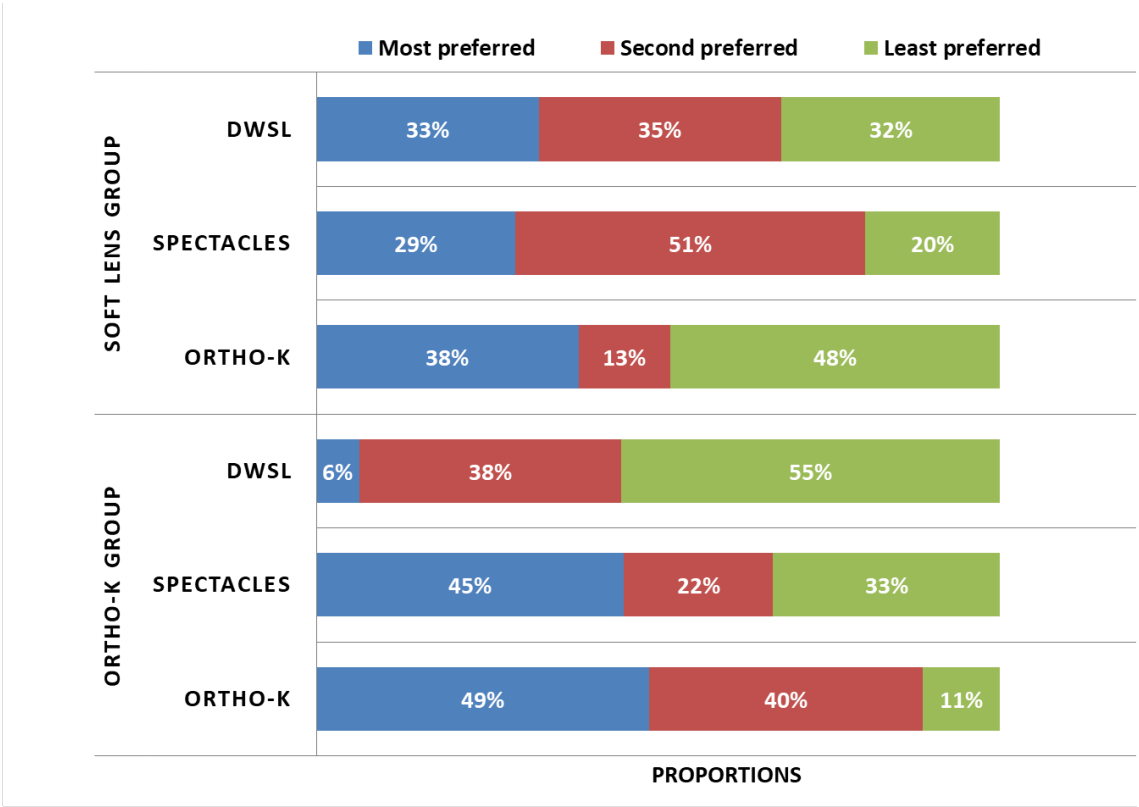


Figure 5.2 Preference in the three myopia control strategies (assuming all three strategies were equally effective for slowing myopia progression), in parents seeking orthokeratology (ortho-k; ortho-k group) or soft lenses (soft lens group) for myopia control (DWSL: daily wear soft lenses)

5.3.3 Preference in myopia control using orthokeratology, daily wear soft contact lenses and spectacles

The decisions on the most and the least preferred options were not influenced by the demographic data shown in Table 5.1 (chi-square tests, $0.98 > p > 0.36$). It was not affected by prior knowledge of myopia control using spectacles or DWSL (chi-square tests, $0.73 > p > 0.05$), but was affected by the prior knowledge of myopia control using ortho-k (chi-square tests, $0.012 > p > 0.007$). Figure 5.3 shows the influence of prior knowledge of myopia control using ortho-k on the most and the least preferred myopia control strategies. For the 84% of parents with prior knowledge of ortho-k for myopia control, 82% were willing to use contact lenses (ortho-k: 47%; DWSL: 35%) for the treatment (chi-square test, $p < 0.001$) (Figure 5.3a) and had no effect on the least preferred options (chi-square test, $p = 0.09$). For the 14% parents without prior knowledge in ortho-k, they preferred spectacles to contact lenses with only 33% were willing to use contact lenses (ortho-k: 26%; DWSL: 7%) for myopia control (chi-square test, $p = 0.001$) and 93% considered contact lenses (ortho-k: 37%; DWSL: 56%) as the least preferred strategy for myopia control (chi-square test, $p = 0.008$) (Figure 5.3b).

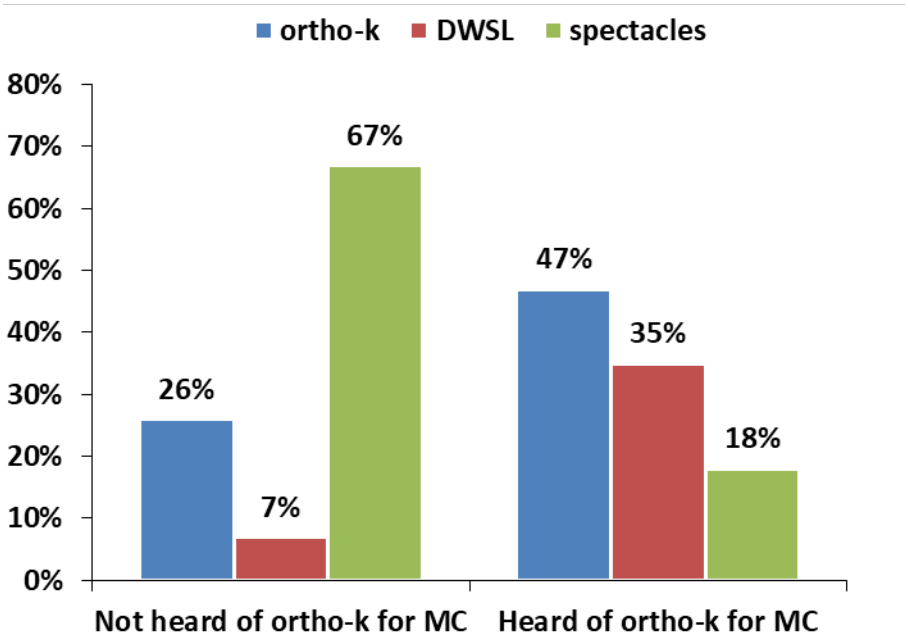


Figure 5.3a Prior knowledge of orthokeratology (ortho-k) for myopia control on the decision on <u>the most preferred</u> myopia control strategies

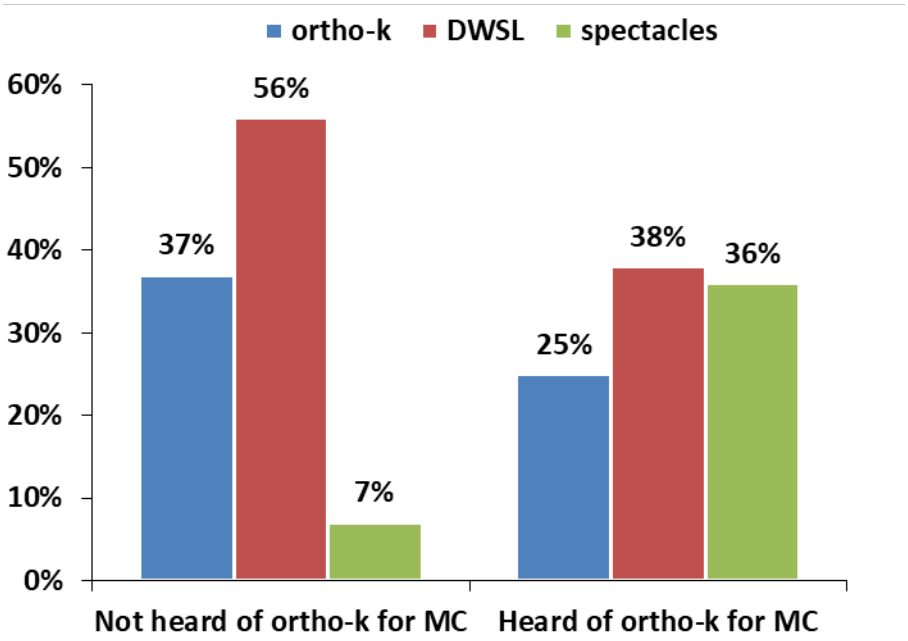


Figure 5.3b Prior knowledge of orthokeratology (ortho-k) for myopia control on the decision on <u>the least preferred</u> myopia control strategies

5.3.4 Parental concerns in myopia control strategy

Parents were required to give comments on their prioritization of the three myopia control strategies. These comments were categorized into five areas: safety concerns, convenience for daily life, confidence in efficacy for myopia control, comfort of treatment, and effect on appearance. Parental concerns in choosing the most and the least preferred myopia control strategy were not significantly different between the two groups of parents (chi-square tests, $0.53 > p > 0.25$) but varied with their preference for control treatment (chi-square tests, $p < 0.001$) (Table 5.2).

For the most preferred myopia control strategy, the two major factors affecting the decision were safety concerns versus convenience for daily life provided by the treatment. Convenience outweighed safety for those choosing ortho-k as their preferred option (convenience: 61%; safety: 33%) and vice versa for those choosing spectacles as their preferred option (convenience: 27%; safety: 69%) (Table 5.2a). For the least preferred myopia control strategy, the major concern for not favoring spectacles was inconvenience in daily activities (61%), compared to safety among those not favoring contact lenses (ortho-k: 78%; DWSL: 89%) (Table 5.2b). The reasons of concern differed between parents disapproving of ortho-k and DWSL. Parents with low motivation for ortho-k were worried about damage to the eye caused by eye movement (28%) and eye rubbing (20%) during sleep, and compliance with lens handling (13%). In contrast those with low motivation for DWSL were worried about the lack of parental supervision

when the child was using the lenses (36%), compliance with lens cleaning and handling (28%), and the risk associated with eye rubbing (21%).

Table 5.2 Parental concerns in choosing (i) the most and (ii) the least preferred myopia control strategies

(i) The most preferred myopia control strategy	Ortho-k	DWSL	Spectacles	All
1. Confidence in strategy (a+b)	**33%**	**44%**	**69%**	**48%**
(a) Safety	*(22%)*	*(25%)*	*(69%)*	*(40%)*
(b) Confidence in strategy	*(11%)*	*(19%)*	*(0%)*	*(8%)*
2. Additional benefits of strategy (c+d+e)	**66%**	**56%**	**30%**	**51%**
(c) Convenience	*(61%)*	*(31%)*	*(27%)*	*(43%)*
(d) Comfort	*(4%)*	*(19%)*	*(3%)*	*(6%)*
(e) Appearance	*(1%)*	*(6%)*	*(0%)*	*(2%)*

(ii) The least preferred myopia control strategy	Ortho-k	DWSL	Spectacles	All
1. Lack of confidence in the strategy (a+b)	**84%**	**91%**	**12%**	**60%**
(a) Not safe	*(78%)*	*(89%)*	*(3%)*	*(55%)*
(b) No confidence in strategy	*(6%)*	*(2%)*	*(9%)*	*(5%)*
2. Affect daily activities (c+d+e)	**16%**	**9%**	**88%**	**40%**
(c) Causing inconvenience	*(6%)*	*(6%)*	*(61%)*	*(26%)*
(c) Poor comfort	*(10%)*	*(3%)*	*(24%)*	*(13%)*
(e) Poor appearance	*(0%)*	*(0%)*	*(3%)*	*(1%)*

Ortho-k: orthokeratology; DWSL: daily wear soft lenses

5.3.5 Acceptable age when children can use contact lenses for refractive correction

One hundred and eighty-one out of the 196 parents responded to this question; 15 parents were reluctant to give a response. The youngest age considered for contact lens wear was eight and only 15% of parents would consider contact lenses for vision correction for children age eight to 12. The percentages of parents who approved contact lens wear for older children age 13 to < 15 and early adolescents age 15 to < 18 were 32% and 37%, respectively. There was 16% of parents who would consider contact lens wear only after) age 18 or older.

The decision on age for contact lens wear for vision correction was not affected by age, gender, occupation, refractive status, study groups, and preference in myopia control strategy ($p > 0.05$). However, the decision on age was affected by the parental education level ($p = 0.004$) and prior contact lens experience ($p = 0.008$), such that parents currently using contact lenses or those with a higher educational background were more willing to allow commencement of contact lens wear in younger children (Figure 5.4).

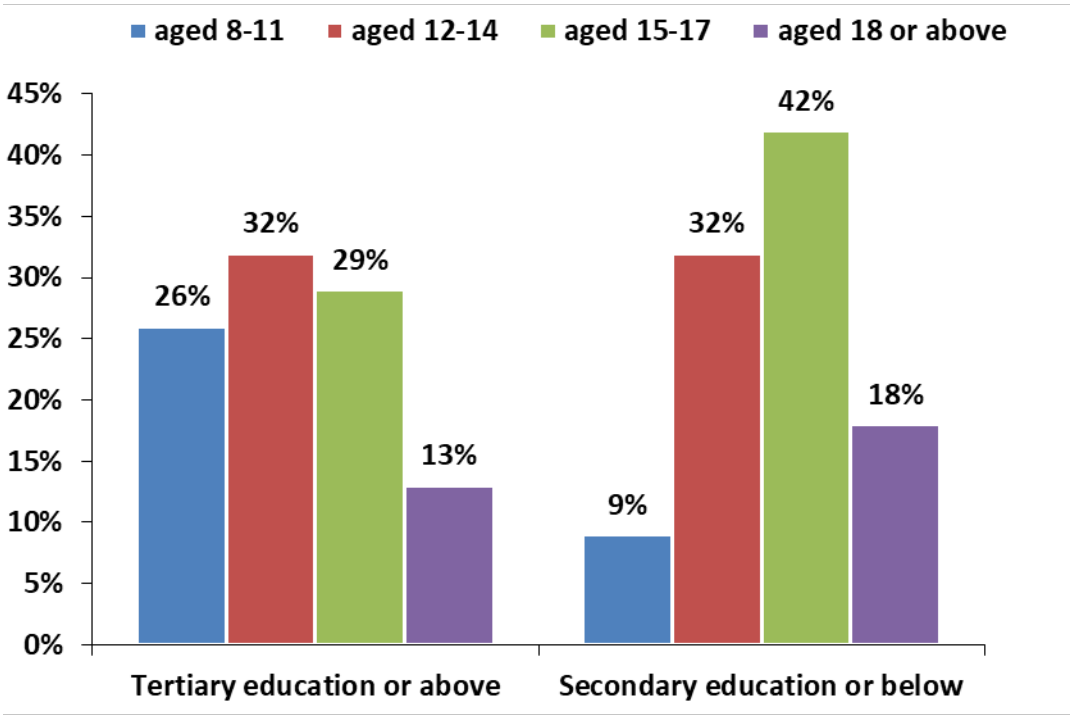


Figure 5.4a Parental education level on the decision on age at which children can commence contact lens wear for vision correction

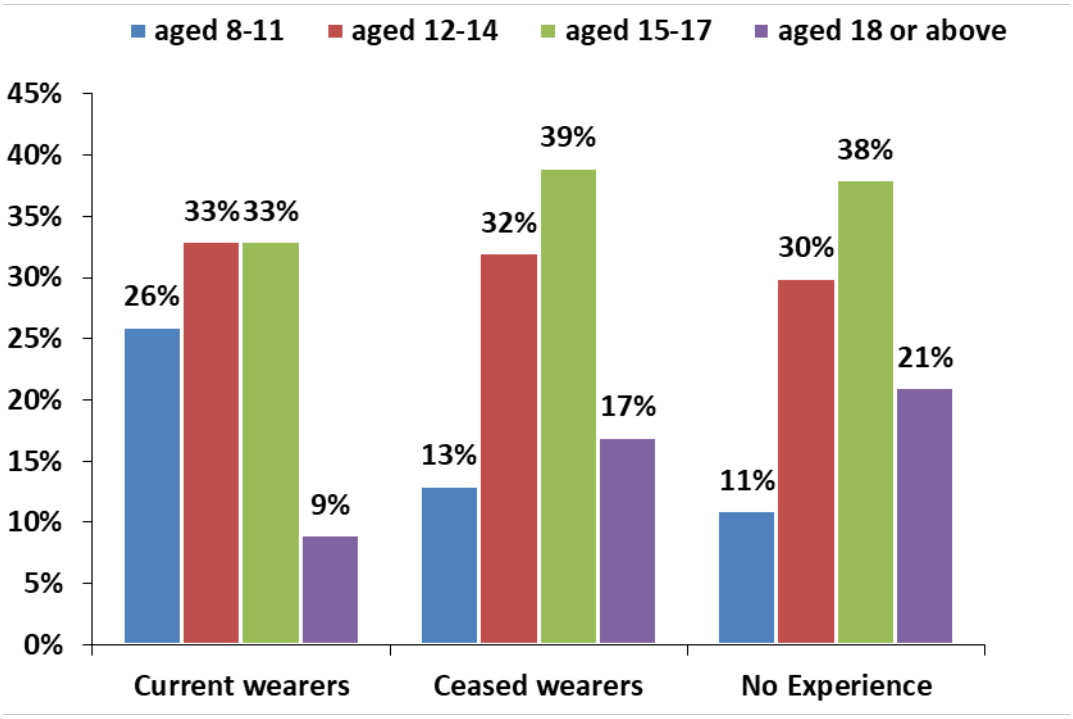


Figure 5.4b. Parental contact lens experience on the decision on age at which children can commence contact lens wear for vision correction

5.3.6 Parental concerns in prescribing contact lenses for children

The reasons for parents' opinion on age when children can commence contact lens wear for vision correct were categorized into four areas: safety concerns, influence on eyeball development, benefits of contact lens wear (including convenience, comfort and appearance), and willingness of the children to wear contact lenses. Only two parents were concerned about the cost of contact lenses.

Safety was the main concern about pediatric contact lens wear in 75% of parents. Concerns were significantly different between the two groups of parents (chi-square test, $p = 0.007$). More parents were concerned about safety in the ortho-k group (ortho-k group: 80%; soft lens group: 70%) (Figure 5.5a). On the other hand, more parents would appreciate the additional benefits of contact lens wear in the soft lens group (ortho-k group: 7%; soft lens group: 15%) (Figure 5.5a).

Parental concerns were also dependent on the age when parents considered it to be suitable to commence contact lens wear (chi-square test, $p = 0.007$). Parents who approved of contact lens wear in children age eight to 12 were less worried about the influence of contact lens wear on eyeball development (Figure 5.5b). However, they were more concerned about benefits of the lens wear, and the willingness of their children to wear the lenses (Figure 5.5b)

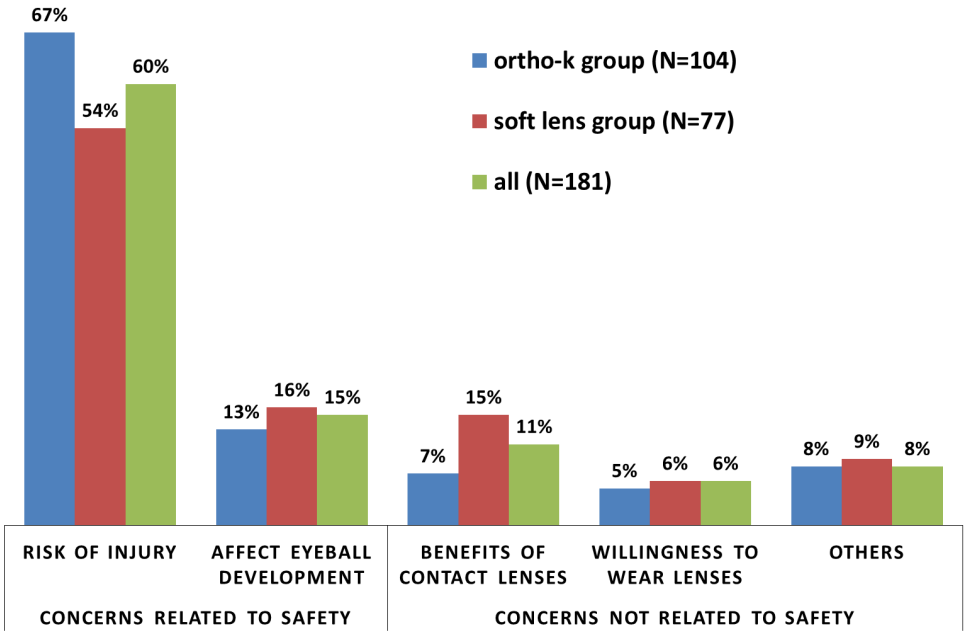


Figure 5.5a Effect of study group on parental concerns

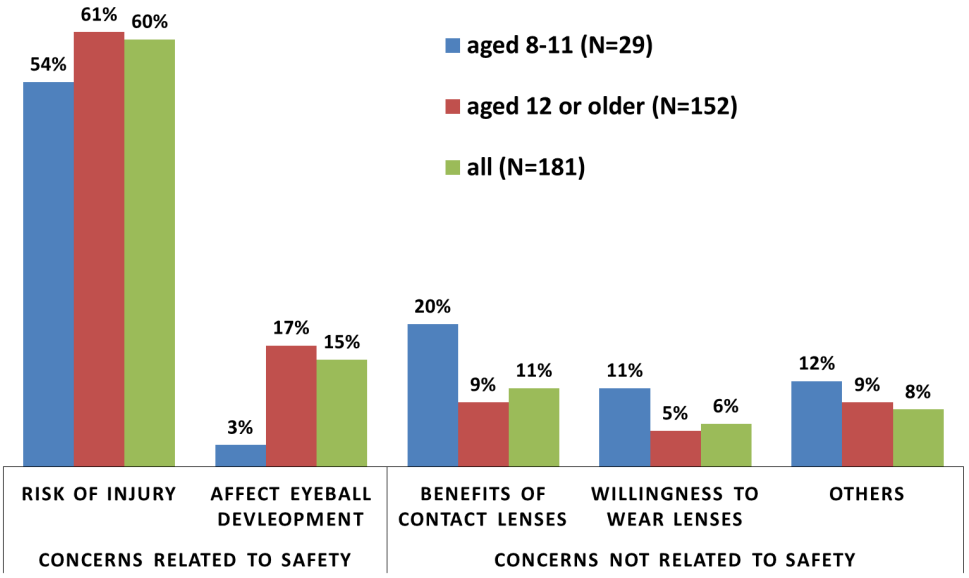


Figure 5.5b Parental concerns in prescribing contact lenses for children for vision correction with respect to the he age they consider appropriate for contact lens wear

5.4 Discussion

The current study reported on the knowledge of and attitudes to myopia control and contact lens wear of a group of parents, mostly working mothers of age 36 to 45. These parents were mostly myopic, but only one-third used contact lenses. They were motivated for their myopic children to attempt myopia control using contact lenses. The majority (86%) had learnt about the use of ortho-k for myopia control from interaction with friends and relatives, or from reading newspapers and magazines. Overall, the most and least preferred myopia control strategies were ortho-k (44%) and DWSL (45%), respectively.

5.4.1 Factors affecting decision on contact lens wear in children

In general, parents in the current study were conservative about the use of contact lenses wear in children for refractive correction, as over 50% would consider contact lenses only for older teenagers (age 15 or above). The primary factors driving for contact lens wear in adults are convenience, comfort, appearance, and cost (Hickson-Curran *et al.*, 2011), but these factors are as not crucial as safety to parents in Hong Kong, when considering contact lens wear for their young or teenage children.

The primary concerns in safety are the risk of direct injury to the eye (e.g. eye rubbing) (60%) and the risk of influence on eyeball development during puberty (15%). The expressions commonly used by the parents were 'children cannot handle the lenses by themselves' and 'growth of the eye(ball) is not stable yet'. Other considerations in the use of contact lenses before

adulthood included concerns of eye rubbing and the use of the lenses without parental supervision (e.g. in case of lens falling out or ocular discomfort at school). These results were in agreement with the results from a survey of parents in the United States, who were also concerned about non-compliance and risk of injury due to wearing 'mis-fit' lenses designed for adult eyes (Janes, *et al.*, 2009; Li *et al.*, 2009; Kwan, 2010).

The concern of safety is understandable as contact lens wear involves various degree of risk. The risks can be minimized by education and regular reminders and re-education. However, the concerns of eyeball development as reported in the current study or the concern about poor lens fit reported by parents in US are misconceptions due to inadequate knowledge or understanding.

In the current study, the parental decision on contact lens wear in children was affected by personal experience (i.e. through better education and/or self-experience in contact lens wear), but not by experience shared by the others (e.g. information provided by friends, relatives or eye care practitioners). Eye care practitioners can influence parental knowledge and attitudes by providing updated and accurate information regarding the use of contact lenses for refractive correction as well as myopia control, allowing parents to make an informed decision for their children.

5.4.2 Factors affecting decision on myopia control

5.4.2.1 Needs for myopia control and evidence of effective myopia control strategy

Needs and evidence of effectiveness for the treatment are the two fundamental conditions for myopia control. In the current study, despite the low motivation for contact lens for refractive correction, parents were more receptive to the use of contact lenses for myopia control as they were seeking myopia control treatment for their children (needs). They considered contact lenses for myopia control as 'treatment' and not simply as a 'correction device'. Ortho-k was the only available optical method with promising effects for myopia control at the time of study (evidence on effectiveness) and was known by 86% of parents in the current study. The percentage of parents willing to attempt myopia control strategies using contact lenses (either ortho-k or DWSL)increased from 33% (ortho-k: 26%; DWSL: 7%) to 82% (ortho-k: 47%; DWSL: 35%) if they had prior knowledge of the treatment for myopia control. Otherwise, they would opt for conventional treatment (i.e. spectacles) (Figure 5.3).

5.4.2.2 Concerns of safety, additional benefits and confidence in treatment

The most important factors affecting the parental decision for myopia control strategy are (in order): evidence on safety of the treatment, additional benefits offered by the treatment, and confidence in the treatment (Table 5.2). Safety was the main concern of parents when considering myopia control if all three treatments were equally effective for myopia control. Spectacles are

considered to be safer than contact lenses, either ortho-k or soft lenses. Parents who were more concerned with the risk involved would choose spectacles as their most preferred treatment option (safety concerns: 69%), whereas those who had less safety concerns (ortho-k: 22; DWSL: 25%) would choose one of the contact lenses as their most preferred option (Table 5.2). A similar conclusion was derived from the results of the least preferred treatment option.

The final decision on parental preference on myopia control treatment may be modified by additional benefits and confidence in the treatment. Additional benefits include daytime spectacle-free convenience and comfort, and better appearance, whilst confidence in the strategy is the subjective perception of the evidence (effectiveness and safety) of the myopia control treatment. These two factors differentiate the decision on the use of ortho-k versus the use of DWSL. In this study, of those parents choosing either contact lens format as their most preferred option, parents chose ortho-k over DWSL if they appreciated the convenience provided in the daytime (ortho-k: 61%; DWSL: 31%), and chose DWSL over ortho-k if they had more confidence in the treatment (ortho-k: 11%; DWSL: 19%).

Safety concerns, confidence in a treatment and appreciation of additional benefits are processed perceptionally by individuals, i.e. different people can make different decisions based on the same information. For instance, two parents attended a public seminar on myopia control. One decided to use ortho-k for myopia control and the other decided on soft lenses. In this study, 86% parents knew about ortho-k, of whom 56% opted for ortho-k, whilst 44%

opted for soft lenses for myopia control. The perception of the benefits, risks and effectiveness of myopia control strategies may be influenced by how the information was received, processed, and interpreted by the individuals. Intrinsic factors that influence individual perception of information were beyond the capacity of the current study.

5.4.3 Roles of eye care practitioners

There is a need for myopia control strategies in the community in Hong Kong and parents are looking for options for their myopic children. The main sources of information are personal communication and mass media. Information from such sources may not be accurate, true, or updated. For instance, parents were misled to believe that contact lens wear would affect the growth of the eyeball. Eye care practitioners should be the best source of information, but unfortunately, only one-third of the parents received myopia related information from their practitioners.

As the current study shows that prior knowledge influenced the parental decision regarding myopia control treatment, practitioners should take the initiative to educate parents on myopia and eye growth and options for myopia control. It is therefore important for practitioners to keep abreast with advances and development in this area. Practitioners may have different attitudes towards different treatment strategies, but it does not necessarily imply making decision for the parents. A fair and balanced overview of information on available treatments should be provided to parents considering myopia control and practitioners should discuss the options

according to the individual needs of the child (e.g. refractive status and need for myopia control, cooperation with eye examination, willingness to wear contact lenses) to help parents made an informed decision. Parents should be educated and reassured about the risks involved and preventive measures for reducing risks. It is of paramount importance that practitioners provide good quality of professional services re-enforced by monitoring of patients to ensure good compliance and safe treatment.

5.4.4 Limitations and further investigation

The major limitation of this study was that only parents who had enrolled their children in two myopia control studies, one using ortho-k and the other using specially designed soft lenses, were recruited. They were asked to assume that all three optical strategies, ortho-k, DWSL, and spectacles for myopia control, were equally effective in their responses to the questionnaire, although they may have heard about the effectiveness (or ineffectiveness) of one or more of the strategies. This study was conducted in 2011 when ortho-k has been shown to slow progression by case reports (Cheung et al., 2004) and a pilot study (Cho et al., 2005), which were fairly widely reported in the local media. The pilot study on myopia control using defocused soft lenses (Anstic and Philips, 2011) was published at about the same the time, but the result was not as well disseminated in Hong Kong as those for ortho-k. Spectacles specially designed for myopia control were not available at that time. As such, even though parents were asked to make their decisions assuming all three strategies were equally effective, their decisions may be

affected by what they actually knew and/or by their (already made) decision to enroll their children in the particular myopia control study when the interview was conducted. However, attempting to conduct such a survey in an entirely random group would likely also lead to bias as refusals to participate would be likely to be much more frequent in parents with little interest in treating myopia rather than just correcting refraction error.

Level of confidence in a treatment can be affected by the quality of evidence (i.e. availability, publicity and circulation of the information), quality of the media of providers (i.e. personal contact or from mass media) and the source of providers (e.g. friends, relatives and health care providers). However, these were not assessed in this study. Parental need or urge for myopia control for their myopic children may be affected by the onset, duration, progression and current level of myopia control of the myopic children. It may also be affected by the number of siblings, number of siblings with myopia, and prior experience with myopia control treatment. Studies on these factors could improve knowledge of motivation for myopia control, not only for optical treatment, but also for the non-optical treatments, such as the pharmaceutical agents.

5.5 Conclusion

Parents were conservative in the use of contact lenses in children, and less than 50% parents would consider contact lenses for visual correction before the age of 15 years. However, they were more receptive to their use for myopia control treatment in children. The two fundamental elements in

myopia control were needs and the presence of an effective treatment. The primary factor affecting parental decision on myopia control treatment was the evidence of safety of the treatment. The final decision was modified by additional benefits provided by the treatment and confidence on the strategy. Ortho-k was the most preferred strategy among the parents interviewed in this study, but this may be because ortho-k was the most well-known myopia control treatment among the parents included in the study.

Publication: Cheung S. W., Lam C. and Cho P. Parents' knowledge and perspective of optical methods for myopia control in children. *Optom Vis Sci.* 2014;91:634-41.

CHAPTER 6

Evidence-based Approach for the Selection of Children for Orthokeratology for Myopia Control

6.1 Introduction

Ortho-k has been demonstrated to slow axial elongation by 32% to 56% in children (*Section 1.1.3*). One of the concerns in prescribing ortho-k for myopia control is the risk of microbial keratitis. Although this risk is similar to other contact lenses on overnight or extended wear modality (*Section 1.2.3*), and can be minimized by good patients' and parents' compliance (*Section 1.2.5*), as ortho-k for myopia control is targeted at children, avoiding unnecessary exposure to this risk is important. Adherence to guidelines on myopia control and refractive correction can increase the safety of the treatment. Currently, ortho-k is offered empirically to children presenting with myopia as there are no guidelines to assist in the identification of appropriate patients for this treatment. In this chapter, we propose a structural management plan for myopia control with ortho-k, intended to select 'suitable' candidates for this treatment to optimize the myopia control effect, as well as minimizing the risks involved.

The effectiveness of myopia control in ortho-k has been proposed to be affected by age and refractive error (*Section 1.1.3.2*). Children with a history of rapid myopia progression are believed to gain benefit more from myopia control interventions, however, little has been done to measure and

investigate this factor in clinical trials on myopia control. In this 14-month study, a group of children age six to less than 16 years were prescribed with single vision spectacles before switching to ortho-k. Axial elongation was compared after each treatment period to determine the effect on myopia progression and response to the treatment for different age groups of children. This allowed the development of a simple decision tree for the identification of children who would most benefit from ortho-k.

6.2 Methods

In this 14-month prospective cohort study, low myopic children aged six to <16 years were recruited. They were prescribed a pair of single-vision spectacles for seven months and prescribed ortho-k for another seven months. Effect of age, gender, initial refractive error, and initial axial length on axial elongation in the two periods was investigated. The study followed the tenets of the Declaration of Helsinki. It was approved by the Ethics Committee of the School of Optometry of The Hong Kong Polytechnic University and registered at ClinicalTrials.gov, number NCT01236755.

6.2.1 Subjects

The inclusion and exclusion criteria for the current study are shown in Table 6.1. Subjects were classified as Younger Children (YC) (6 to < 9 years), Older Children (OC) (9 to < 13 years), and Early Adolescent (EA) (13 to < 16 years). The stratification for the younger and older children was in

accordance with the stratification adopted in the study by Cho and Cheung (2017) and the remaining older subjects were classified as early adolescents. All subjects recruited for this study had no previous experience in myopia control or contact lens wear, and had no contra-indication for ortho-k lens wear. Informed consent was obtained from the subjects and their parents prior to the commencement of the study.

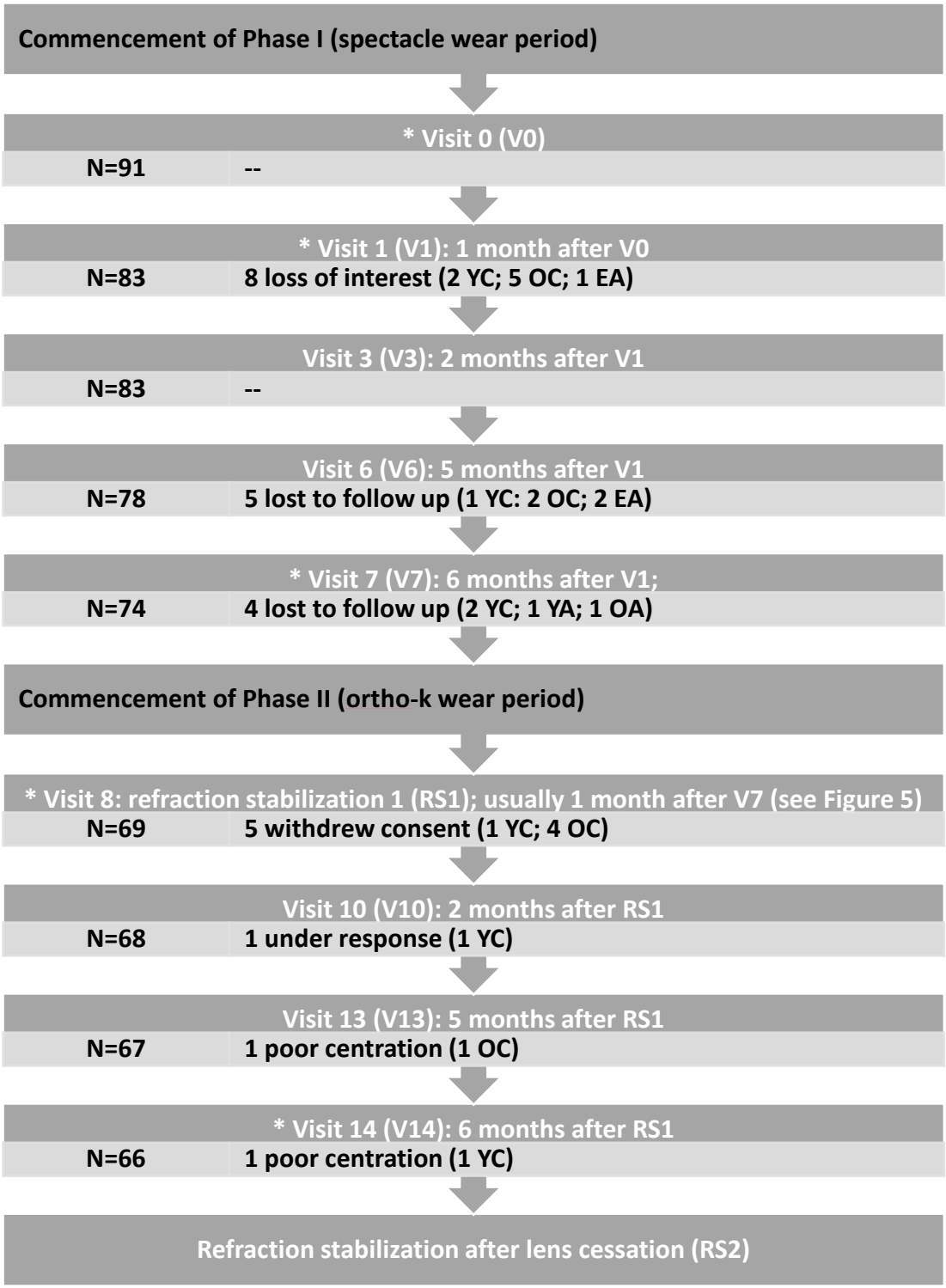
Table 6.1 Inclusion and exclusion criteria for subject recruitment

Inclusion criteria	**Exclusion criteria**
Age 6 to <16 years	Strabismus at distance or near
Cycloplegic refractive sphere: -0.75 to -4.50 D	Contraindication for contact lens wear and ortho-k (e.g. limbus to limbus corneal cylinder and dislocated corneal apex)
Cycloplegic refractive cylinder: ≤ -3.00 D	Systemic or ocular conditions which may affect contact lens wear (e.g. allergy and medication)
Anisometropia: ≤ -1.50 D	Systemic or ocular conditions which may affect refractive development (e.g. Down syndrome, ptosis)
Best corrected monocular visual acuity: equal to or better than 0.10 in logMAR scale in the worse eye	Prior experience with the use of rigid lenses (including ortho-k)
Willingness to wear contact lenses and spectacles on a daily basis	Prior experience with myopia control treatment (e.g. refractive therapy or progressive spectacles)
Willingness to comply to the follow up schedules of this 14-month study	Non-compliance to the study procedures (e.g. follow up schedule, and use of prescribed optical correction)
	Poor response to ortho-k lens wear (e.g. poor ocular health or significant residual refractive error after ortho-k treatment resulting in poor unaided vision (worse than 0.18 in logMAR scale))

6.2.2 Study visits and examination procedures

Figure 6.1 shows the flow chart of the study. There were five cycloplegic data collection visits, V0, V1, V7, V8, and V14 (number referring to months after commencement of study), and four non-cycloplegic scheduled visits, V3, V6, V10, and V13. Primary outcome measurement (axial length) was performed by an examiner masked to the current treatment of each child, at the data collection visits after cycloplegia. Vision and refraction, corneal topography, corneal thickness, corneal biomechanical properties, and slit-lamp biomicroscopy (TOPCON slit-lamp SL7 and TOPCON IMAGEnet system, Topcon Corp., Tokyo, Japan) were performed before cycloplegia with one drop of 0.5% a proparacaine followed by 1% tropicamide and 1% cyclopentolate, five minutes apart; whereas, axial length (IOLMaster; Carl Zeiss Meditec, Inc., CA, US), ocular aberration, and cycloplegic subjective and objective refraction (Shin-Nippon NVision K5001, Shin-Nippon Commerce Inc., Tokyo, Japan) were performed after cycloplegia.

Eligible subjects were prescribed with a pair of spectacles which were delivered within two weeks after V0. Due to the short duration of the current study, non-cycloplegia scheduled visits, every 2- to 3-monthly, were arranged to review refraction and vision (Figure 6.1). V6 also served to determine the target refraction of the ortho-k lenses to be ordered. Additional visits on lens handling were arranged between V6 and V7, and ortho-k lenses were delivered at V7 to those who could successfully handle the lenses.



* Data collection visit with cycloplegic examination

YC: Young Children aged 6 to < 9 years

YA: Older Children aged 9 to < 13 years

EA: Early Adolescent aged 13 to < 16 years

Figure 6.1 Study flowchart and reason for dropout

All subjects had to return for regular ortho-k aftercare visits: one night, one night, one week, two weeks, three weeks, and one month after commencement of lens wear. Visit 8 would be performed once optimal refractive correction was achieved. Subjects would be excluded if optimal refractive correction could be not achieved after three lens modification (Figure 6.2) or if they could not comply with the use of lenses (Table 6.1).

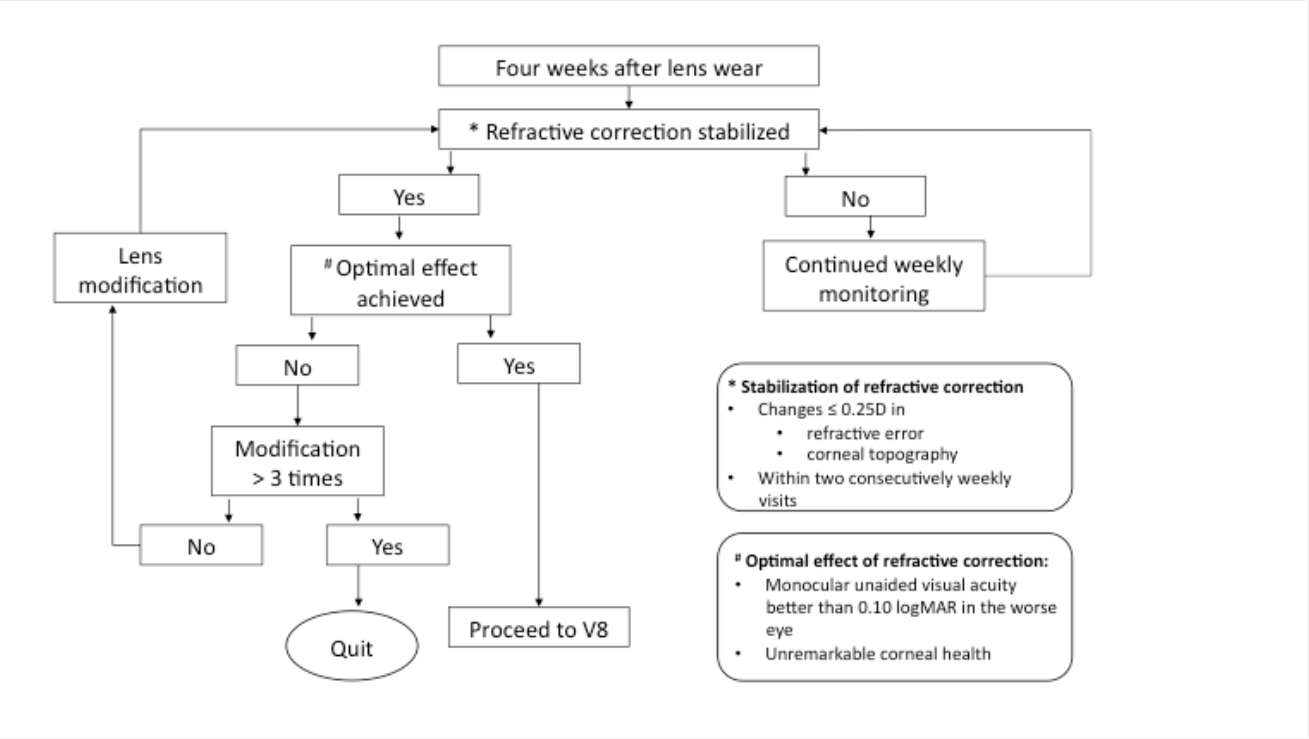


Figure 6.2 Management plan for refractive correction

6.2.3 Spectacle glasses, orthokeratology lenses and lens care products

All eligible subjects were prescribed with a pair of single-vision glasses with refractive index 1.56 of spherical design (Founder Optical Company, Hong Kong) at the beginning of the study. The ortho-k lenses used in the current study were the Menicon Z Night or Menicon Z Night Toric ortho-k lenses (NKL Contactlenzen BV, Emmen, The Netherlands) and the lens parameters were determined by the Easyfit software (NKL Contactlenzen BV, Emmen, The Netherlands). The center thickness of the lens was about 0.24mm and Dk/t was about 68 ISO / 79 Fatt. Subjects were required to use the ortho-k lenses every night during the ortho-k wear period, and to use the recommended solutions to care for their lenses (Table 6.2). Artificial tears were used for lubrication prior to lens insertion and for prior to lens removal in case of lens binding. Non-preserved unidose artificial tears and preservative free saline were selected to reduce the risk of solution allergy. All ortho-k lenses and solutions prescribed to the subjects had to be returned at the completion or early termination of study.

Table 6.2 Lens care products for orthokeratology used in the current study

Product	Purpose	Replacement frequency
Menicon Spray & Clean	Daily cleaning	Monthly
MeniCare Plus	Daily disinfection	Monthly
Menicon Progent	Weekly protein removal	NA
Alcon Tears Naturale Free	Lens insertion	NA
AMO Lens Plus Saline	Lens rinsing	Monthly

6.2.4 Statistical analysis

The rate of myopia progression may differ between the two eyes. In general practice, myopia control will be recommended, even if only one eye is progressing. In this study, the data from the eye with the greater axial elongation during spectacle wear period (the worse eye) were used in the analysis. In case of similar progression in axial elongation, the eye with the greater increase in myopia was selected. The right eye was selected if the axial elongation and myopia progression were the same in both eyes.

Parametric tests were used for data following Gaussian distributions, whilst non-parametric tests were used for data following non-Gaussian distribution. Between-group differences in baseline characteristics were determined using one-way ANOVA (refractive sphere, spherical equivalent and axial length), Kruskal-Wallis test (refractive cylinder) and Chi-square test (gender). Changes in axial length before (V0) and after the two interventions (i.e. V7 and V14) in the three age groups were investigated using repeated measures ANOVA. Between-group effects on axial elongation in the two study phases were determined using one-way ANOVA, if there were significant effects found in the repeated measures ANOVA. Post-hoc tests with Bonferroni corrections were performed to compare the changes among the three age groups. Linear regressions were used to explore factors affecting axial elongation after ortho-k wear. Univariate linear regression was performed to investigate the effect of initial age, gender, refractive error, axial length and axial elongation during spectacle wear. An alpha value of 0.01 was adopted to adjust for the multiple factors being tested and to increase

the threshold for statistical significance. Multiple linear regression was then followed to investigate the association between axial elongation after ortho-k with factors identified in the univariate analysis.

6.3 Results

A total of 91 eligible subjects were recruited, but of these, only 66 completed the 14-month study. Of the 25 subjects dropping out, 17 left during the spectacle wear period and the remaining eight during the ortho-k wear period (Figure 6.1). The dropout rate was slightly higher in the younger subjects (YC: 36%; OC: 27%; EA: 20%). No subject was excluded due to ocular complications and there were no significant adverse events reported for any subject fitted with ortho-k lenses. Of the 66 subjects who completed the study, 85%, 97%, and 100% subjects were able to achieve optimal correction in four, five, and eight weeks, respectively, after commencement of lens wear, i.e. all V8 were performed four to eight weeks after V7.

At the end of the spectacle wear period, 28 right eyes and 38 left eyes were identified to have faster axial elongation than their fellow eyes. The baseline demographic data of the 66 subjects and ocular parameters from the worse eyes are shown in Table 6.3.

The study intended to recruit equal number of subjects for each age group. However, the response rates for the YC and EA groups were not as good as for the OC group, resulting in more subjects in the OC group than the other two groups (YC: 21%; OC: 55%; EA: 24%). There were also more boys in the OC groups than the other groups (percentage of boys; YC: 21%; OC:

58%; EA: 19%) (Chi-square, p = 0.007). It is noted that subjects in the YC group had shorter initial axial length than those in the other two groups (one-way ANOVA, p < 0.015) even though their refractive statuses were comparable (one-way ANOVA; refractive sphere, p = 0.43; spherical equivalent, p = 0.30; Kruskal-Wallis test; refractive cylinder, p = 0.39).

Table 6.3 Demographic data and baseline ocular parameters of the worse eyes for the three groups of subjects

Demographic data	Younger Children	Older Children	Early Adolescents
Number of subjects	14	36	16
Median age (years)	8.10	11.48	13.90
Age range (years)	6 to < 9	9 to < 13	13 to < 16
Male / Female	3 /11	21 / 15	3 / 13
Sphere (D)	-2.34 ± 0.74	-2.43 ± 0.85	-2.70 ± 0.83
Cylinder (D)	-0.32 ± 0.27	-0.34 ± 0.39	-0.50 ± 0.42
Spherical equivalent (D)	-2.50 ± 0.74	-2.60 ± 0.89	-2.95 ± 0.91
Axial length (mm)	23.94 ± 0.51	24.65 ± 0.80	24.77 ±0.90

6.3.1 Axial elongation and myopia control effect

Figure 6.3 shows the axial length for the three age groups of subjects. There was significant axial elongation over the 14-month study period for all subjects (repeated measures ANOVA, p < 0.001) and significant interaction between time and age group (repeated measures ANOVA, p < 0.001).

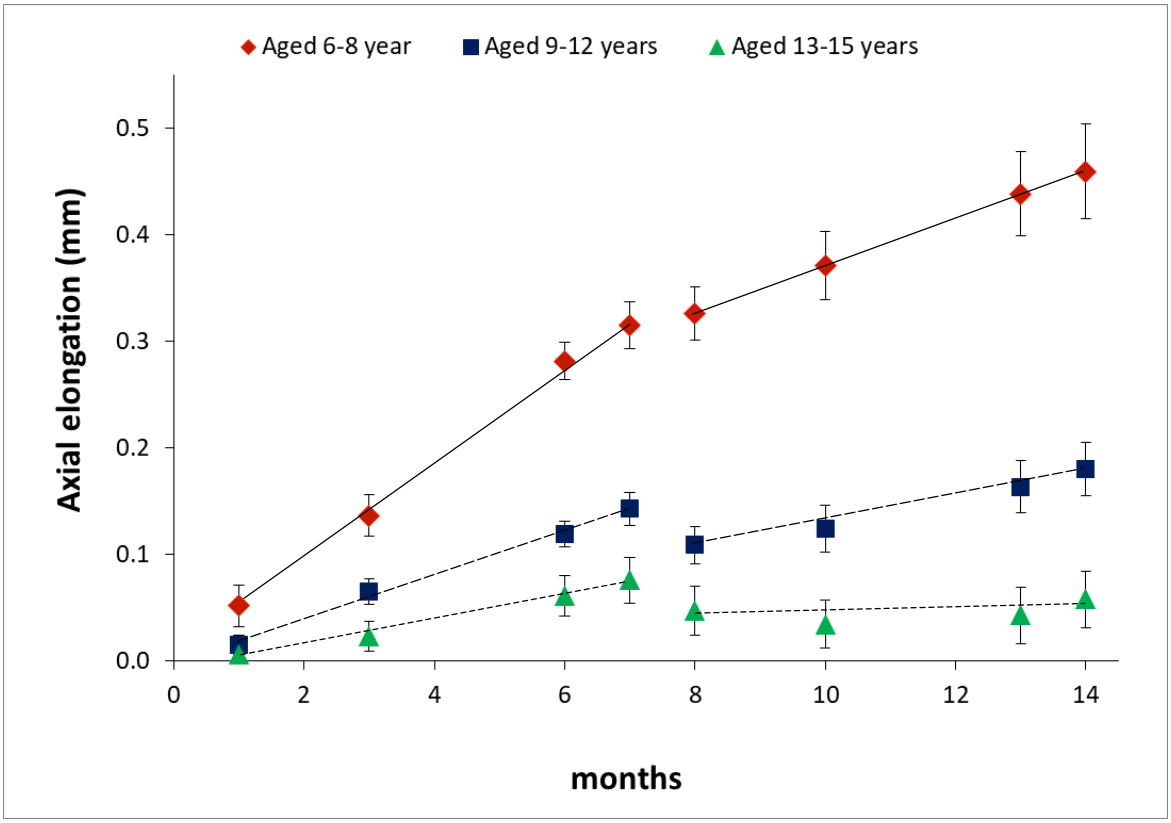


Figure 6.3 Increase in axial length during the 14-month study (error bars show the standard error of means)

Table 6.4 shows the mean ± SD axial elongation over the study period. Subjects in the YC group demonstrated faster progression than those in the other two groups in the both study phases (one-way ANOVA; post-hoc; $p < 0.001$). Subjects in the OC group demonstrated significantly faster axial elongation than those in the EA group in the spectacle wear period (one-way ANOVA; post-hoc; $p = 0.044$) but not in the ortho-k-wearing phase (one-way ANOVA; post-hoc; $p = 0.192$). Axial elongation was significantly reduced after ortho-k in all three groups of subjects (repeated measures ANOVA, $p <$

0.001) (Table 6.4, Figure 6.3). Although the reduction in axial elongation was greatest in the YC group (-0.171 ± 0.134 mm) than the other two groups (OC: -0.105 ± 0.110 mm; EA: -0.094 ± 0.130 mm), the percentage change was the smallest in the YC group (54%) compared to the other two groups (OC: 73%; EA: 124%). As the percentage changes were derived from dividing changes in axial elongation by the axial elongation during spectacle wear, the discrepancy between changes in axial elongation and percentage changes was due to small axial elongation during spectacle wearing period in the OC and EA groups, especially the latter group (Table 6.4).

Table 6.4 Increase in axial length during the study period for all subjects, and subjects in the three age groups

	N	Axial elongation		
		Spectacle wearing period	**Orthokeratology wearing period**	**Difference**
All subjects 6 to < 16 years	66	0.163 ± 0.121	0.047 ± 0.112	-0.116 ± 0.122
Younger Children 6 to < 9 years	14	0.315 ± 0.081	0.144 ± 0.128	-0.171 ± 0.134
Older Children 9 to < 13 years	36	0.143 ± 0.092	0.038 ±0.092	-0.105 ± 0.110
Early Adolescents 13 to < 16 years	16	0.076 ± 0.086	-0.018 ± 0.081	-0.094 ± 0.130

Univariate linear regression showed that axial elongation during ortho-k lens wear was associated with initial age (Pearson: $r = -0.485$, $p < 0.001$) and axial elongation during spectacle wear (Pearson $r = 0.455$, $p < 0.001$), but not with gender, initial refractive error, or initial axial length (Pearson: $-0.223 < r < 0.228$, $p > 0.036$). The results for the multiple linear regression are shown in Table 6.5 and show that axial elongation after ortho-k was faster in younger subjects and those with a faster history of progression.

Table 6.5 Multiple linear regression for the association of age and history of progression on axial elongation after orthokeratology

	Adjusted R^2	F	p-value
Model summary	0.233	10.884	**0.000**
		Unstandardized beta	**Standardized beta**
	Constant	0.197	--
	Age	-0.016	-0.326
	Axial elongation (spectacles)	0.200	0.217

p-value: probability value for regress model; bold value indicates $p < 0.05$

The axial elongations for the individual subjects in the three age groups are shown in Figure 6.4. The two dashed lines in the figure divides subjects into three categories (rapid, moderate and slow) according to their rate of elongation. Rapid, moderate and slow elongations were defined as elongation ≥ 0.20 mm in seven months (equivalent annual progression of ≥ 1.00 D), between 0.10 to < 0.20 mm in seven months (equivalent to annual progression of 0.50 D to < 1.00 D) and < 0.10 mm in seven months (equivalent to annual progression of < 0.50 D), respectively. The data show that most of the subjects who demonstrated rapid progression were in the YC group, whereas most of the subjects with slow progression were in the OC and EA groups. The results agreed with previous findings that axial elongation after ortho-k was affected by age and history of progression as younger subjects were likely to progress faster than older subjects. However, some exceptions to these general conclusions were observed.

Table 6.6 shows the number of subjects with rapid, moderate, and slow progression in the two study phases. After ortho-k, the percentage of subjects with rapid progression significantly reduced from 93% to 29% in the YC group and from 25% to 0% in the OC group. In the EA group only two subjects demonstrated rapid progression in the spectacle wearing period and none after ortho-k. However, the percentage of subjects with slow progression significantly increased from 63% to 94% in the EA group after ortho-k.

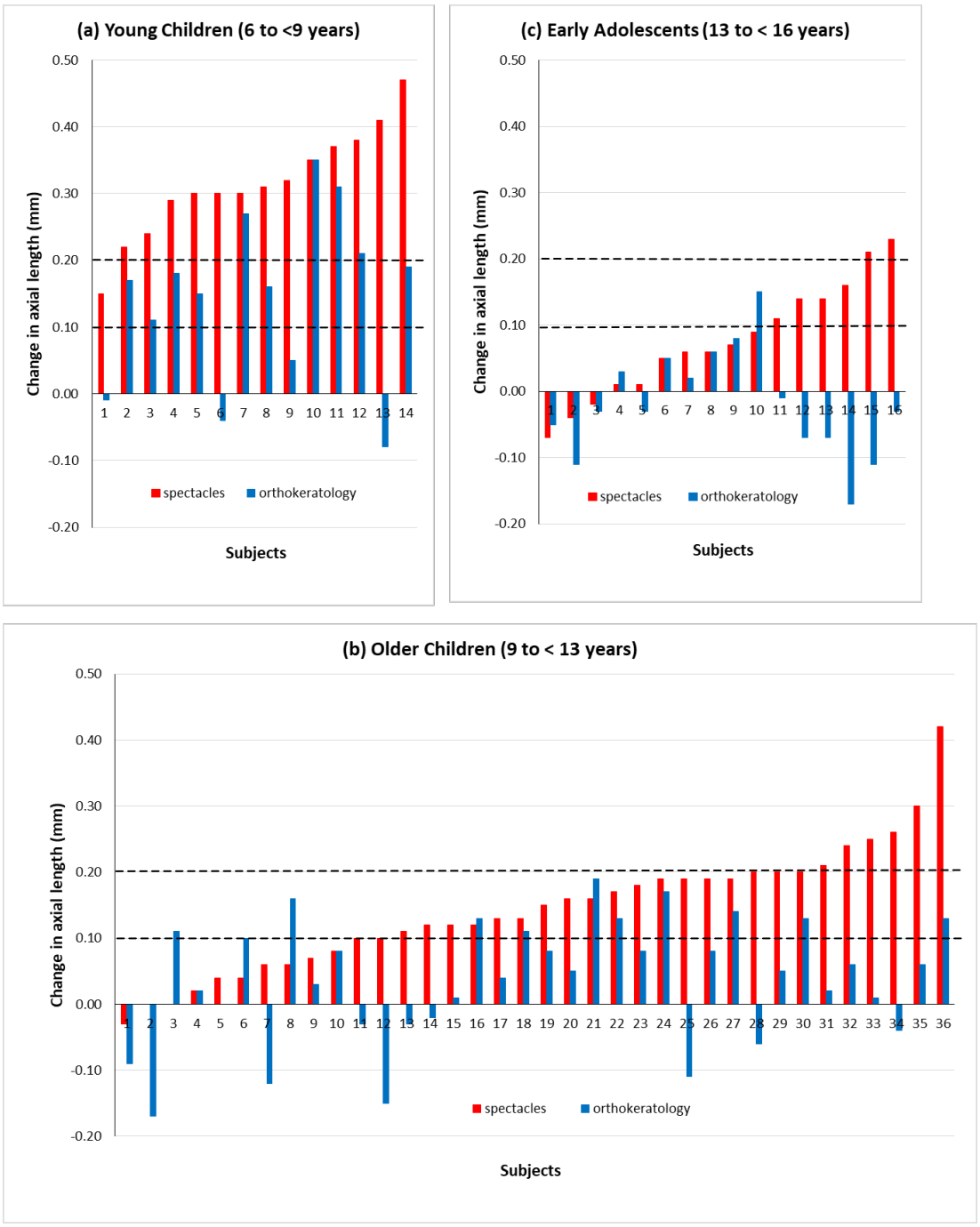


Figure 6.4 The 7-monthly changes in axial length in the individual subjects during the two study phases: (a) Younger Children, (d) Older Children and (c) Early Adolescents. (Data were sorted according to the elongation during spectacle wearing period for the three age groups: *data above the upper dashed line*: fast progression; *data between the two dashed lines*: moderate progression; *data below the bottom dashed line*: slow progression)

Table 6.6 Subjects demonstrating rapid (elongation ≥ 0.20 mm), moderate (elongation ≥ 0.10 and < 0.20 mm) and slow (elongation < 0.10 mm) progression before and after the use of orthokeratology

	Spectacle wearing period	Orthokeratology wearing period	RR (95%CI)	p-value
Younger Children (6 to < 9 years)				
Rapid	13 (93%)	4 (29%)	0.31 (0.13–0.71)	**0.006**
Moderate	1 (7%)	6 (42%)	--	**--**
Slow	0 (0%)	4 (29%)	9.00 (0.53–152.93)	0.128
Older Children (9 to < 13 years)				
Rapid	9 (25%)	0 (0%)	0.05 (0.00–0.87)	**0.040**
Moderate	17 (47%)	11 (31%)	--	--
Slow	10 (28%)	25 (69%)	0.96 (0.71–1.29)	0.800
Early Adolescents (13 to < 16 years)				
Rapid	2 (12.5%)	0	0.20 (0.01–3.86)	0.287
Moderate	4 (25%)	1 (6%)	--	--
Slow	10 (62.5%)	15 (94%)	1.50 (1.01–2.24)	**0.047**

RR: relative risk of rapid or slow progression after ortho-k treatment

CI: confidence interval

p-value: probability values for significance of relative risk; bold values indicate $p < 0.05$

6.3.2 History of progression and efficacy of myopia control

Axial elongation after ortho-k was affected by history of progression as shown Figures 6.4 a-c. All 24 subjects with rapid progression (YC: 13; OC: 9; EA: 2) and 20 of the 22 subjects with moderate progression (YC: 1; OC: 17; EA: 4) demonstrated reduction in elongation after ortho-k. The remaining two subjects with moderate progression in the OC group showed an increase in elongation after ortho-k. For the 20 subjects with slow progression (YC: 0; OC: 10; EA: 10), the response to ortho-k varied from faster elongation or no change to reduction in elongation.

The mean changes in axial elongation in the two study phases for subjects demonstrating rapid, moderate and slow progression in spectacle wearing period are shown in Table 6.7. The reduction in elongation was significantly fastest in those with rapid progression and slowest in those with slow progression (one-way ANOVA, post-hoc: $p < 0.001$). However, axial elongation was significant reduced after ortho-k only in subjects with rapid and moderate progression (paired t test, $p < 0.001$) but was not changed in subjects with slow progression (paired t test, $p = 0.422$). On average, axial elongation was slowed by 71% and 88% in those with rapid and moderate progression, respectively.

In 46 subjects with moderate to rapid axial elongation, the rate was reduced in 43 subjects (YC group: 13; OC group: 24; EA group: 6), remaining unchanged in one subject in the YC group and increased in two subjects in the OC group. Of the 43 subjects, 32 had reduction of ≥ 0.10 mm in seven months (Table 6.7). Of the 20 subjects with slow axial elongation during

spectacle wear, only two subjects from the OC group showed reduction in elongation ≥ 0.10 mm. All other subjects showed no significant clinical benefit in reducing axial growth rate (seven had small reduction in elongation, four had no change in rate, and seven had increased in elongation rate).

Table 6.7 Mean and standard deviation (SD) of the axial elongation (AE, mm) in subjects with rapid, moderate and slow progression in the spectacle wearing period (PI) and the ortho-k wearing period (PII). Effectiveness of myopia control in terms of change in elongation (AE Change), percentage of reduction (% change), percentage of subjects with reduction more than 50% (MC ≥ 50%) and percentage of subjects with reduction for more than 0.10 mm in seven months (AEC ≥ 0.10) were presented

		AE in PI	AE in PII	AE change	% change	MC ≥ 50%	AEC ≥ 0.10
Rapid	mean±SD	0.291±0.077	0.094±0.124	0.197±0.116	71±41%	67%	79%
(N=24)	range	(0.20, 0.47)	(-0.11, 0.35)	(0.00, 0.49)	(0%, 152%)		
Moderate	mean±SD	0.156±0.030	0.025±0.101	0.121±0.094	88±70%	68%	59%
(N=22)	range	(0.10, 0.19)	(-0.17, 0.19)	(-0.03, 0.33)	(-18%, 250%)		
Slow	mean±SD	0.028±0.044	0.015±0.090	0.014±0.074	36±151%	45%	10%
(N=20)	range	(-0.07, 0.09)	(-0.17, 0.16)	(-0.11, 0.18)	(-200%, 400%)		

6.4 Discussion

The current study confirmed that ortho-k could slow axial elongation in children aged six to <16. Success of myopia control is often reported as a percentage reduction in the amount of axial elongation compared to a control group and a 50% reduction in axial growth has been considered as a meaningful endpoint in myopia control (Smith and Walline, 2015). However, it is important to apply caution when considering percentage reduction. As demonstrated in this study, in 31 of 46 subjects (67%) with moderate to rapid progression, axial elongation was reduced by at least 50%, whilst 45% of subjects with slow progression also showed 50% reduction in axial elongation (Table 6.7). However, this use of percentage reduction in axial elongation is misleading. If a reasonable myopia control effect is deemed a reduction in elongation by at least 0.10 mm in seven months (or about 0.50 D in one year), then the 50% effect of ortho-k on axial elongation in the slow progressors (i.e. those with progression less than 0.10 mm in seven months) is not clinically significant.

6.4.1 Development of management plan for myopia control using orthokeratology

A greater reduction in axial elongation was observed in the YC group, which was likely to be due to their history of faster progression. Older subjects benefited from myopia control if their axial elongation was still progressing, either moderately or rapidly. Subjects with slow progression did not benefit from ortho-k treatment. Based on these findings, a 12-month management

plan for myopia control for children is proposed with respect to (a) history of progression in spectacle wear and (b) efficacy of myopia control (Figure 6.5).

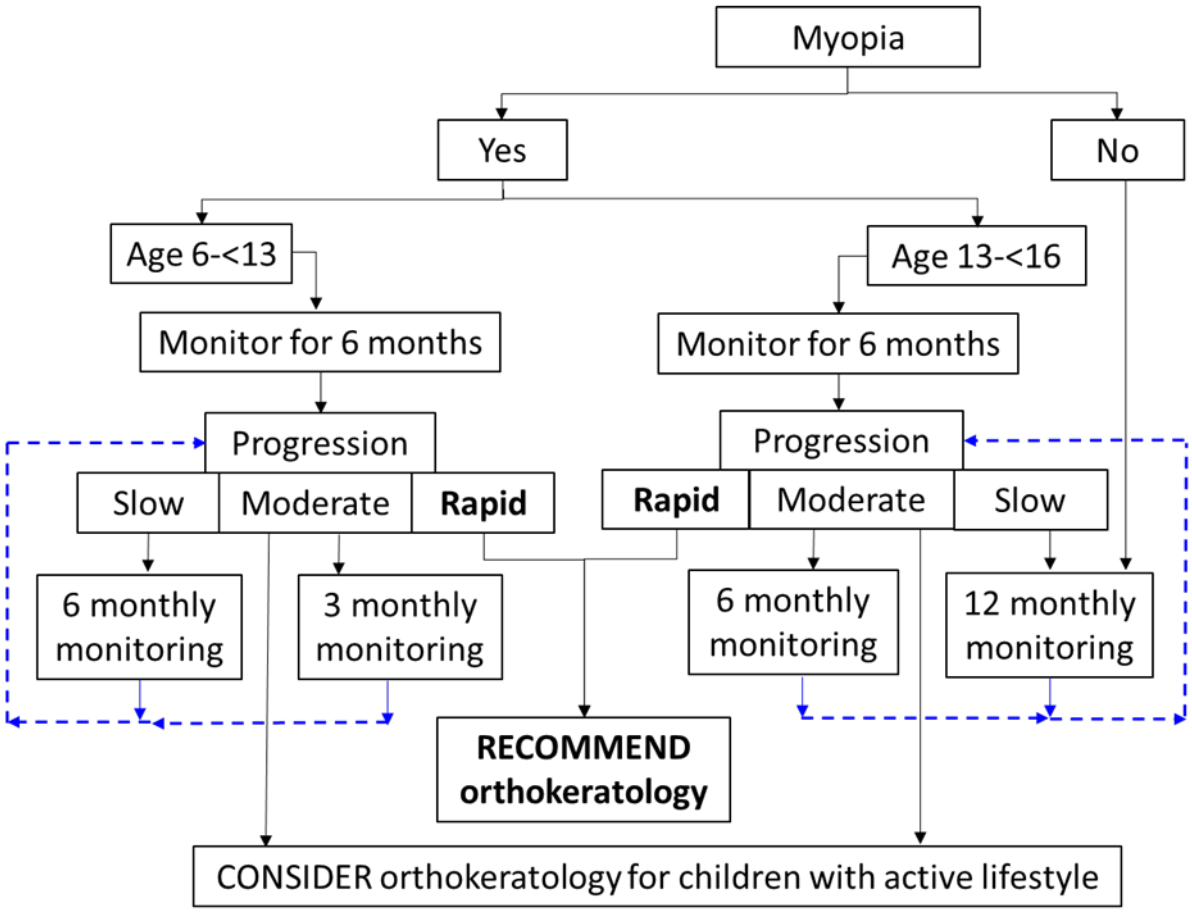


Figure 6.5 Decision tree for myopia control management for children age 6 and below 16 (< 16)

The key element in the proposed decision tree is to understand whether there is a need for myopia control. A run-in period of six months is proposed to monitor the progression rate of myopia. The current study used axial length as a measure of progression but change in refractive error also does well as a parameter for monitoring myopia progression. Ortho-k (or other myopia control treatment) is recommended for those with rapid progression of more than 0.50 D in six months. The myopia control effect should be reviewed every six months. Other myopia control treatment could be recommended if subjects do not respond well to the treatment (i.e. no change or faster axial elongation). This could be continuation of ortho-k in combination with other therapy or switching to a different therapy. For those with moderate myopia progression of 0.25–0.50 D in six months, parents may consider ortho-k as a prophylactic treatment for myopia control. The management plan will be similar as those with rapid progression. However, as they have no urgent need for myopia control, those with slight but not optimal improvement in axial elongation, may continue with ortho-k treatment as a refractive correction, rather than myopia control. For children with slow progression, not more than 0.25 D in six months, a 3- to 6-monthly monitoring period is recommended. Because younger children are likely to have faster progression, children aged less than nine should be monitored every three months, whereas for the older children, this could be extended to every six months. Myopia control should be recommended if progression becomes faster.

6.4.2 Benefits of evidence-based myopia control protocol

Myopia control with ortho-k improves unaided vision in the daytime but it also involves a degree of risk. The dropout rate in the current study shows that ortho-k may not be suitable for all children. The cost may be higher than other optical and pharmaceutical treatments because of the regular replacement of lenses and care solutions. A cost effective management plan to screen for the best candidates for myopia control treatment and such a structural plan for decision-making can be beneficial to both parents and practitioners. The decision tree (Figure 6.5) provides guidance to practitioners and patients to make clinically informed choice on initiation and termination of treatment. Good patient selection can improve the efficacy of the myopia control effect whilst early termination of the treatment for children with poor response to the treatment can reduce unnecessary risk to the children, thus, improving the safety of the treatment. For example, in the current study, 79% of subjects with history of rapid progression were likely to benefit from ortho-k (Table 6.7). Subjects with slow progression would be screened out after the spectacle wear period. They would be recommended to have six monthly monitoring while wearing spectacles, rather than commencing treatment for myopia control. However, if parents insist to try ortho-k for children with moderate axial elongation, a six monthly follow up could monitor the myopia control effect. The decision tree could be useful for practitioners to explain the treatment plan for myopic children to their parents as it could enhance understanding of the necessity for myopia control.

The current management plan for myopia control is intended for children of age < 16 years. Although the sample size for the YC and EA groups were not large, the development of the current protocol is derived based on the individual history of progression and response to ortho-k treatment. The six months run-in period helps the practitioners to identify children at risk and initiate treatment in the timely manner for those with higher risk.

Seasonal change in myopia progression has been reported such that the rate of change is slower in summer and faster in winter (Tan *et al.,* 2000; Fulk *et al.,* 2002; Donova *et al.,* 2012; Gwiazda *et al.,* 2014). The change may be related to the amount of sunlight and time spent indoors. The difference in progression between summer and winter time was about 0.18-0.22D (Fulk *et al.,* 2002; Donova *et al.,* 2012; Gwiazda *et al.,* 2014) which was statistically but not clinically significant. It has been suggested that the duration for any myopia control treatment should be at least 12 months to take into account the seasonal variations (Donova *et al.,* 2012), however, it has little effect on the proposed 6 monthly run-in period in the current study. The key element of the decision tree is to identify fast progressors. Those who progress faster within 6 months will be indicated for myopia control. If they are missed during the slow progression period, they will be captured again in the second 6-monthly period. The aim of the decision tree is to offer myopia control at the right time, not just any time for anybody.

It has been reported that the myopia control effect is most significant in the first six months of treatment (Cho and Cheung, 2012). The 6-monthly follow up period on myopia control allows the practitioners to review the myopia

control effect, and decide whether to continue with the current treatment or to modify the treatment plan by switching to other treatment, using combination therapy (e.g. ortho-k with low concentration atropine) or even cessation of treatment if the initial response was poor. The proposed guidelines not only apply to ortho-k, but also can also extend to other myopia control treatments.

The current protocol is a tailor-made decision process in myopia control planning. Subjects with good response to the ortho-k for myopia control after the initial six months are recommended to continue with the treatment. It is expected that the progression in the second year will be slower than the first year. Children would be recommended to continue with the treatment as long as the axial elongation in the following years is not greater than the previous year. It has been suggested that myopia control treatment can be stopped upon the end of the puberty when the growth of the eyeball stops (Cho and Cheung, 2017). However, as there is individual variation in myopia control effect, the treatment effect should be considered case by case.

6.4.3 Myopic control in Hong Kong

In Hong Kong, all school aged children, from primary one to secondary three, receive complimentary health assessment on physical health, which is usually conducted in the second semester of the school year. The assessment includes vision screening, and those who fail are recommended to have a comprehensive eye examination, either at the eye clinic of the Department of Health, or in the private sector. For a progressing myope, the refraction may increase by over 0.50 D in six months if not detected and

treated or given proper advice on vision hygiene. Timely intervention can reduce the risk of development of high myopia and its associated complications. Recent study shows that the prevalence of myopia may be increased in lower income families living in small homes in highly populated area (Choi *et al.*, 2017). Children of these families may be likely to be in need but unable to afford regular eye examination and myopia control treatment. Government or other assistance with the cost of examination and treatment could help these children avoid high myopia in later life.

6.5 Conclusion

The proposed decision tree for myopia control planning can help to improve the identification of suitable subjects for intervention and monitoring of treatment effects, while providing management plan for those who may not currently be deemed appropriate to receive myopia control treatment.

Publication: Cheung, S. W., Boost, M. V., Cho, P. (2018) Pre-treatment observation of axial elongation for evidence-based selection of children in Hong Kong for myopia control. *Cont Lens Anterior Eye*. (in press).

CHAPTER 7

Summary and Recommendations

7.1 Summary

The effectiveness of ortho-k for myopia control has been demonstrated by numerous studies (*Section 1.1.3.1*). However, there is still the concern of the long term safety of the use of ortho-k in children. In principle, a properly prescribed ortho-k lens should not do any harm to the cornea during refractive correction for low to moderate myopes (*Section 1.1.2.1*). However, safety is also dependent on good compliance with use and care, as the treatment can be associated with severe adverse events such as microbial keratitis (*Section 1.2.3*) and users are rarely completely compliant (*Section 1.2.3.1*). This thesis reports four studies which provide additional information about the safety of ortho-k treatment. The studies include long term effects on the corneal endothelium and the ocular microbiome, association between carriage of peri-orbital tissues and contamination of the lenses and accessories, attitudes of the users (i.e. the children and their parents) on the treatment, and finally a guideline for practitioners for improving the practice of ortho-k for myopia control in children.

The long term use of ortho-k lenses was found to be associated with reduction in cell density, polymegathism, and pleomorphism in the central corneal endothelium and pleomorphism in the superior cornea of children; however, these changes were mainly due to the normal ageing (*Chapter 3*).

Although the modification of reduction in cell density after ortho-k requires further investigation, the results of the current study showed that the effects of the chronic stress exerted on the corneal physiology by this treatment were minimal.

The use of ortho-k had little influence on the colonization rates of the peri-orbital tissues (*Chapter 4*). Although the total bacterial count in the conjunctiva after ortho-k was reduced, the balance of the environment was not affected because of the comparable diversity of bacteria in this area. Spectacles were more likely to be contaminated with *S. aureus* in control subjects colonized with this bacteria, but the association did not reach statistical significance in ortho-k subjects, i.e. source of contaminants in ortho-k lenses and accessories could not be identified in the current study. Better compliance with handwashing before lens insertion and removal could reduce the risk of transfer of nasal organisms to the eye.

Interviews with parents and children showed that their attitudes towards the use of ortho-k for myopia control was affected by evidence on safety and effectiveness for myopia control and prior knowledge of this strategy (*Chapter 5*). The parents were more in favor of the treatment in the presence of prior positive experience, either personal or from friends (word of mouth).

In the last study, the efficacy of myopia control was shown to be affected by the initial age of the children and their history of myopia progression (*Chapter 6*). No beneficial effects were observed for myopic children with no or slow progression. A decision tree incorporating a run-in period for the

determination of individual progression rate was developed to aid practitioners in patient selection.

7.2 Recommendation for practitioners

Patient selection and compliance are the two key issues in safe ortho-k practice. Ortho-k for myopia control should be not prescribed for all myopic children, but should be customized for those with needs (i.e. with history of progression and with motivation for commitment to comply with the treatment schedules and correct handling (*Chapter 5*). Safe ortho-k practice can be improved with proactive measures taken by practitioners providing myopia control treatment:

1. Stay abreast of latest development and research, and update practice accordingly
2. Determine the needs for myopia control based on individual characteristics, discuss the choices available and suitable for the children in a fair and balanced manner, and make recommendations of the management of their refractive status accordingly (*Chapters 5 & 6*), e.g. regular monitoring for children with stable (non-progressing) refractive errors and myopia control for the progressing myopes
3. Recommend ortho-k to parents and children who are motivated and willing to take responsibility and commit to the requirements of the treatment (*Chapters 4 & 5*)
4. Prescribe ortho-k according to the guidelines for safe ortho-k practice for refractive correction

5. Review the hand hygiene and handling procedures at 3- or 6-monthly regular aftercare visits by asking the children and/or their parents to demonstrate the procedures to ensure good compliance (*Chapter 4*).

Note that the last point can be easily overlooked by both the patients/parents and practitioners, as microbial keratitis has been reported in ortho-k practice and contamination of care accessories is common.

7.3 Limitations

The major limitation of this thesis is related to the sample size. To date, there have been no similar studies comparing the changes in the corneal endothelium or examining the association between colonization of per-orbital tissues and contamination in the accessories in children wearing ortho-k and spectacles. The lack of significant between-group differences in changes in corneal endothelial morphology (*Chapter 3*), total bacteria cell counts and association between colonization of per-orbital tissues and the accessories (*Chapter 4*) could be true findings (i.e. ortho-k did not affect these factors) or false findings due to relatively low statistical power. Study on the colonization of *S. aureus* is more feasible as colonization on skin and contamination in accessories could be found in about one in four subjects. However, study on the colonization of Gram-negative bacteria would be difficult because of the low contamination rate in the accessories in ortho-k subjects with good compliance.

In the early stage of planning for this thesis study, confocal microscopy using the Nidek ConfoScan 4 was considered to be incorporated in the longitudinal

study in *Chapter 6*. However, this examination was excluded from the final study because the pilot study showed that valid measurement could not be obtained from children due to the long acquisition time and clinically significant corneal staining created by the Z-ring attachment (Chan *et al.*, 2011, 2012).

7.4 Future directions

Safety in ortho-k covers a wide range of topics. In this thesis, the macroscopic effect in terms of endothelial morphology and ocular microbiome were investigated. Future examination of the effects of the treatment, especially examination of tear components, could provide different perspectives to help in the understanding of other in-depth changes induced in the eyes. Corneal health is affected by the host defense of the eyes and was not investigated in this thesis. Recent technology allows the evaluation of the immunological response of the eyes, e.g. microscopic examination of Langerhan's cell density in the stroma, examination of inflammatory mediators and other compounds in tears using advanced mass spectrometry techniques to characterize proteins present or changes of ocular microbiome using gene sequencing technology. The duration of ortho-k lens wear for the two studies on corneal endothelium and ocular microbiome ranged from one to three years (*Chapters 3 and 4*). As the myopia control treatment can last for up to 12 years if it is commenced at age six and terminated at age 18, investigation of the ocular response for longer term of lens wear in terms of histology, proteomics and the microbiome is warranted. Finally, assessment

of the value of the decision tree after use by practitioners should be performed to determine its usefulness and acceptability.

7.5 Conclusions

Changes in corneal endothelium and the ocular microbiome indicate influences of long term ortho-k lens wear on the cornea. These changes were not associated with adverse effects, providing further support for the safety of this treatment. As safety in ortho-k lens wear is highly dependent on the compliance of children, parents, and practitioners and the treatment period for myopia control in children is usually several years, deviation from recommended procedures by any of those involved, especially from the children and the parents, can lead to increased risks of infection. Thus, the role of practitioners is even more important to ensure good compliance and re-enforcement of correct handling techniques should be repeated frequently throughout the whole treatment period, especially as responsibility for lens care passes from the parent to a young adolescent. In general, ortho-k treatment was found to be safe and had little effect on cornea, and the practice of myopia control in children using ortho-k can be performed without adverse effects, provided there is good compliance from all parties involved.

APPENDIX A

Questionnaire for the Telephone Survey in Chapter 5

Myopic Control Treatments - Parents' perspective

Caller Number : _____
Date of interview : _____ **Time of interview** : _____--_____

We would like to know the attitude of parents towards children using contact lenses. Do you mind to spend about 5-10 minutes to answer 13 questions in a short survey? You will need to provide some personal information including the education level and age range. Please be assured that no personal information will be disclosed. Do you agree to continue? □ **Yes** □ **No**

1. Have you heard of the following treatment for slowing myopic progression?

a) Single-vision (under minus) spectacles	□ Yes	□ No
b) Progressive spectacles (PAL)	□ Yes	□ No
c) Ortho-K (night wear)	□ Yes	□ No
d) Soft contact lenses (day wear)	□ Yes	□ No

2. Where did you hear about the above treatment(s) for slowing myopic progression?

□ Friends/Relatives □ Parents from school
□ Eye care practitioners □ Doctors □ Other health care provider (specify)
□ Newspaper/Magazine Advertisement □ Newspaper/Magazine articles
□ NA □ others (specify)_____

3. Have you sought any of the following treatments for slowing myopic progression for your child?

* Single-vision spectacles	□ Yes	□ No
* Progressive spectacles	□ Yes	□ No
* Ortho-K (night wear)	□ Yes	□ No
* Soft contact lenses (day wear)	□ Yes	□ No

4. *If* three methods (spectacles (under minus/PAL), specially-designed daily wear soft lenses and overnight wear ortho-k lenses) are proven to be equally effective in slowing myopic progression, please rank the choice of treatment for your child. '1' first choice, and '3' last choice.

	REASONS
□ Spectacles (under minus/PAL)	
□ Ortho-K (night wear)	
□ Soft contact lenses (day wear)	

5. *If* contact lens is solely used for vision correction, not myopic control, what age do you think children can start wearing contact lenses? Why

Age _____ Reasons

(About the contact person)
Now we will need to ask some personal particular about yourself.

6. Your relationship with the child
□ Mother □ Father □ Guardian (specify)

7. Have you or any family members received refractive surgery before?
(You) □ No □ Yes
(Family member) □ No □ Yes (specify)

8. What is your current (before treatment if any) prescription for distance (excluding presbyopia)?
□ Hyperopia +0.50 or above
□ Emmetropia -0.50 to +0.50
□ Low myopia -0.50 to -3.00
□ Moderate myopia -3.00 to -6.00 (exclusive)
□ Higher myopia -6.00 or above
□ Unknown

□ No astigmatism
□ Low astigmatism -0.75 to -3.00
□ High astigmatism above -3.00)
□ Unknown

9. Have you worn contact lenses before?
□ No. Go to Question 9
□ Yes. **Are you still wearing contact lenses?**
□ No. Go to next Question 9
□ Yes.
(a) How long have you been using contact lenses? ___years ____months
(b) What kind of contact lenses are you currently using / have you used?
□ Rigid lenses □ Conventional soft lenses □ Disposable soft lenses
(□ Hydrogel lenses □ Silicone hydrogel lenses)
(c) Are/Were you a constant wearer or an occasional wearer?
□ Constant (at least 8 hours per day and 5 days per week (ie. >39 hrs per week))
□ Occasional (less than 40 hours per week)

10. Your educational background
□ Primary □ Secondary □ Tertiary □ Graduate school

11. Your age falls in which age groups
□ 21- □ 21-25 □ 26-30 □ 31-35
□ 36-40 □ 41-45 □ 46-50 □ 50-55 □ 55+

12. Your occupation
(A) Status: □ Housewife □ Self employed □ Employed
(B) Field: □ Health related □ Finance/Business related □ Teaching/Academic
□ Others (please check or specify by parents)

13. Where did you hear of this research?
□ Newspaper Adv. □ PolyU/SO website □ Clinic flyers
□ Friends/Relatives □ Parents from school
□ Others (specify) _____

Thank you for answering the questions.

- END -

REFERENCES

Abib, F. C., Barreto, J. Jr. (2001). Behavior of corneal endothelial density over a lifetime. *J Cataract Refract Surg.* 27: 1574–1578.

Adler, D., Millodot, M. (2006). The possible effect of undercorrection on myopic progression in children. *Clin Exp Optom.* 89: 315–321.

Alharbi, A., La Hood, D., Swarbrick, H. A. (2005). Overnight orthokeratology lens wear can inhibit the central stromal edema response. *Invest Ophthalmol Vis Sci.* 46: 2334–2340.

Alharbi, A., Swarbrick, H. A. (2003). The effects of overnight orthokeratology lens wear on corneal thickness. *Invest Ophthalmol Vis Sci.* 44: 2518–2523.

Al-Otaibi, A. G. (2012) Non-viral microbial keratitis in children. *Saudi J Ophthalmol.* 26: 191–197.

Alter, S. J., Vidwan, N. K., Sobande, P. O., Omoloja, A., Bennett, J. S. (2011) Common childhood bacterial infections. *Curr Probl Pediatr Adolesc Health Care.* 41: 256–283.

Amann, J., Holley, G. P., Lee, S. B., Edelhauser, H. F. (2003). Increased endothelial cell density in the paracentral and peripheral regions of the human cornea. *Am J Ophthalmol.* 135: 584–590.

Atchison, D. A., Li, S. M., Li, H., Li, S. Y., Liu, L. R., Kang, M. T., Meng, B., Sun, Y. Y., Zhan, S. Y., Mitchell, P., Wang, N. (2015). Relative peripheral hyperopia does not predict development and progression of myopia in children. *Invest Ophthalmol Vis Sci.* 56: 6162–6170.

Barr, J. T., Rah, M., J., Jackon, J. M., Jones, L. A., (2003). Orthokeratology and corneal refractive therapy: a review and recent findings. *Eye Contact lens.* 29(1 Suppl): S49–53.

Bausch & Lomb. (2004). Premarket approval application P010062/S001/S002; June 7, 2004. Available at: https://www.accessdata.fda.gov/cdrh_docs/pdf/P010062B.pdf. Accessed on October 18, 2017.

Benavente-Pérez, A., Nour, A., Troilo, D. (2014). Axial eye growth and refractive error development can be modified by exposing the peripheral retina to relative myopic or hyperopic defocus. *Invest Ophthalmol Vis Sci.* 55: 6765–6773.

Berntsen, D. A., Barr, C. D., Mutti, D. O., Zadnik, K. (2013). Peripheral defocus and myopia progression in myopic children randomly assigned to wear single vision and progressive addition lenses. *Invest Ophthalmol Vis Sci.* 54: 5761–5770.

Bogaert, D., van Belkum, A., Sluijter, M., Luijendijk, A., de Groot, R., Rümke, H. C., Verbrugh, H. A., Hermans, P. W. (2004). Colonisation by Streptococcus pneumoniae and Staphylococcus aureus in healthy children. *Lancet.* 363(9424): 1871–1872.

Boost MV1, Shi G, Cho P. (2012). Comparison of contamination rates of designs of rigid contact lens cases. *Optom Vis Sci.* 89: E1030–1034.

Boost, M. V, Cho, P. (2005). Microbial flora of tears of orthokeratology patients, and microbial contamination of contact lenses and contact lens accessories. *Optom Vis Sci.* 82: 451–458.

Bourne, W. M. (2001). The effect of long-term contact lens wear on the cells of the cornea. *CLAO J.* 27: 225–230.

Bourne, W. M., McLaren, J. W. (2004). Clinical responses of the corneal endothelium. *Exp Eye Research.* 78: 561–572.

Breslin, K. M., O'Donoghue, L., Saunders, K. J. (2013). A prospective study of spherical refractive error and ocular components among Northern Irish schoolchildren (the NICER study). *Invest Ophthalmol Vis Sci.* 54: 4843–4850.

Bullimore, M. A., Sinnott, L. T., Jones-Jordan, L. A. (2013). The risk of microbial keratitis with overnight corneal reshaping lenses. *Optom Vis Sci.* 90: 937–944.

Cardoso, C. L., Pereiraa, H. H., Zequimb, J. C., Maringá, M. G., Brazil, P. (1999). Effectiveness of hand-cleansing agents for removing Acinetobacter baumannii strain from contaminated hands. *Am J Infect Control.* 27: 327–331.

Chan, B., Cho, P., Cheung, S. W. (2008). Orthokeratology practice in a university clinic. *Clin Exp Optom.* 91: 453–460.

Chan, K. Y, Cheung, S. W, Chan, Y. Y., Cheung, S. W., Swann, P. G. (2013). Prevalence of papillary changes and folliculosis of the palpebral conjunctiva in asymptomatic Chinese children. *Cont Lens Anterior Eye.* 36: 62–65.

Chan, K. Y, Cheung, S. W, Cho, P. (2012a). Clinical performance of an orthokeratology lens fitted with the aid of a computer software in Chinese children. *Cont Lens Anterior Eye.* 35: 180–184.

Chan, K. Y, Cheung, S. W, Cho, P. (2012b). Nidek ConfoScan 4 (z-ring) measurements over soft contact lenses. *Eye Contact Lens.* 38: 80–85.

Chan, K. Y, Cheung, S. W, Cho, P. (2012c). Corneal parameters of six- to 12-year-old Chinese children. *Clin Exp Optom.* 95: 160–165.

Chan, K. Y, Cheung, S. W, Cho, P. (2014). Orthokeratology for slowing myopic progression in a pair of identical twins. *Cont Lens Anterior Eye.* 37: 116–119.

Chan, K. Y, Cheung, S. W, Lam, A. K., Cho, P. (2011). Corneal sublayer thickness measurements with the Nidek ConfoScan 4 (Z Ring). *Optom Vis Sci.* 88: E1240–1244.

Chan, T. C., Li, E. Y., Wong, V. W, Jhanji, V. (2014). Orthokeratology-associated infectious keratitis in a tertiary care eye hospital in Hong Kong. *Am J Ophthalmol.* 158: 1130–1135.

Chang, S., Tsai, I., Hu, F., Lin, L. L., Shih, Y. (2001). The cornea in young myopic adults. *Br J Ophthalmol.* 85: 916–920.

Charm, J., Cheung, S. W., Cho, P. (2010). Practitioners' analysis of contact lens practice in Hong Kong. *Cont Lens Anterior Eye.* 33: 104–111.

Charm, J., Cho, P. (2013). High myopia-partial reduction ortho-k: a 2-year randomized study. *Optom Vis Sci.* 90: 530–539.

Charman, W. N., Mountford, J., Atchison, D. A., Markwell, E. L. (2006). Peripheral refraction in orthokeratology patients. *Optom Vis Sci.* 83: 641–648.

Cheah, P. S., Norhani, M., Bariah, M. A,, Myint, M., Lye, M. S., Azian, A. L. (2008). Histomorphometric profile of the corneal response to short-term reverse-geometry orthokeratology lens wear in primate corneas: a pilot study. *Cornea.* 27: 461–470.

Chen, C., Cheung, S. W., Cho, P. (2012). Toric orthokeratology for highly astigmatic children. *Optom Vis Sci.* 89: 849–855.

Chen, C., Cheung, S. W., Cho, P. (2013). Myopia control using toric orthokeratology (TO-SEE Study). *Invest Ophthalmol Vis Sci.* 54: 6510–6517.

Chen, D., Lam, A. K., Cho, P. (2010). Posterior corneal curvature change and recovery after 6 months of overnight orthokeratology treatment. *Ophthalmic Physiol Opt.* 30: 274–280.

Chen, J., He, J. C., Chen, Y., Xu, J., Wu, H., Wang, F., Lu, F., Jiang, J. (2016). Interocular difference of peripheral refraction in anisomyopic eyes of schoolchildren. *PLoS One.* 11:e0149110.

Chen, X., Sankaridurg, P., Donovan, L., Lin, Z., Li, L., Martinez, A., Holden, B., Ge, J. (2010). Characteristics of peripheral refractive errors of myopic and non-myopic Chinese eyes. *Vision Res.* 50: 31–35.

Chen, Z., Niu, L., Xue, F., Qu, X., Zhou, Z., Zhou, X., Chu, R. (2012). Impact of pupil diameter on axial growth in orthokeratology. *Optom Vis Sci.* 89: 1636–1640.

Chen, Z., Xue, F., Zhou, J., Qu, X., Zhou, X. (2016). Effects of Orthokeratology on Choroidal Thickness and Axial Length. *Optom Vis Sci.* 93: 1064–1071.

Cheung, S. W., Cho, P. (2000). Endothelial cells analysis with the TOPCON specular microscope SP-2000P and IMAGEnet system. *Curr Eye Res.* 21: 788–798.

Cheung, S. W., Cho, P. (2004). Subjective and objective assessments of the effect of orthokeratology – a cross-sectional study. *Curr Eye Res.* 28: 121–127.

Cheung, S. W., Cho, P. (2005). Longitudinal study on the ocular effect of overnight orthokeratology. *Optom Vis Sci.* 82: E-abstract 055074.

Cheung, S. W., Cho, P. (2013). Validity of axial length measurements for monitoring myopic progression in orthokeratology. *Invest Ophthalmol Vis Sci.* 54: 1613–1615.

Cheung, S. W., Cho, P., Bron, A. J., Chui, V., Chan, B. (2006).The occurrence of fibrillary lines during overnight orthokeratology. *Ophthalmic Physiol Opt.* 26: 525–531.

Cheung, S. W., Cho, P., Cheung, A. (2005). White lesion in the corneal pigmented ring associated with orthokeratology. *Ophthalmic Physiol Opt.* 25: 264–268.

Cheung, S. W., Cho, P., Fan, D. (2004) Asymmetrical increase in axial length in the two eyes of a monocular orthokeratology patient. *Optom Vis Sci.* 81: 653–656.

Chia, A., Lu, Q. S., Tan, D. (2016). Five-year clinical trial on atropine for the treatment of myopia 2: myopia control with atropine 0.01% eyedrops. *Ophthalmology.* 123: 391–399.

Cho, P., Boost, M. V., Cheng, R. (2009). Non-compliance and microbial contamination in orthokeratology. *Optom Vis Sci.* 86: 1227–1234.

Cho, P., Chan, B., Cheung, S. W., Mountford, J. (2012). Do fenestrations affect the performance of orthokeratology lenses? *Optom Vis Sci.* 89: 401–410.

Cho, P., Cheung, S. W. (2012). Retardation of myopia in Orthokeratology (ROMIO) study: a 2-year randomized clinical trial. *Invest Ophthalmol Vis Sci.* 53: 7077–7085.

Cho, P., Cheung, S. W. (2017). Discontinuation of orthokeratology on eyeball elongation (DOEE). *Cont Lens Anterior Eye.* 40: 82–88.

Cho, P., Cheung, S. W. (2017a). Protective role of orthokeratology in reducing risk of rapid axial elongation: a reanalysis of data from the ROMIO and TO-SEE Studies. *Invest Ophthalmol Vis Sci.* 58: 1411–1416.

Cho, P., Cheung, S. W., Edwards, M. (2005). The longitudinal orthokeratology research in children (LORIC) in Hong Kong. A pilot study on refractive changes and myopic control. *Curr Eye Res.* 30: 71–80.

Cho, P., Cheung, S. W., Edwards, M. H. (2002). Practice of Orthokeratology by a group of contact lens practitioners in Hong Kong. I. General overview. *Clin Exp Optom.* 85: 365–371.

Cho, P., Cheung, S. W., Edwards, M. H. (2003). Practice of Orthokeratology by a group of contact lens practitioners in Hong Kong. II. Orthokeratology lenses. *Clin Exp Optom.* 86: 42–46.

Cho, P., Cheung, S. W., Edwards, M. H., Fung, J. (2003a). An assessment of consecutively presenting orthokeratology patients in a Hong Kong based private practice. *Clin Exp Optom.* 86: 331–338.

Cho, P., Cheung, S. W., Mountford, J., Chui, W. S. (2005b). Incidence of corneal pigmented arc and factors associated with its appearance in orthokeratology. *Ophthalmic Physiol Opt.* 25: 478–484.

Cho, P., Cheung, S. W., Mountford, J., White, P. (2008). Good clinical practice in orthokeratology. *Cont Lens Anterior Eye.* 31: 17–28.

Cho, P., Chui, W. S., Cheung, S. W. (2003c). Reversibility of corneal pigmented arc associated with orthokeratology. *Optom Vis Sci.* 80:791–795.

Cho, P., Chui, W. S., Mountford, J., Cheung, SW. (2002). Corneal iron ring associated with orthokeratology lens wear. A case report. *Optom Vis Sci.* 79: 565–568.

Choi, K. Y., Yu, W. Y., Lam, C. H. I., Li, Z. C., Chin, M. P., Lakshmanan, Y., Wong, F. S. Y., Do, C. W., Lee, P. H., Chan, H. H. L. (2017). Childhood exposure to constricted living space: a possible environmental threat for myopia development. *Ophthalmic Physiol Opt*. 37: 568–575.

Choo, J. D., Caroline, P. J., Harlin, D. D., Papas, E. B., Holden, B. A. (2008). Morphologic changes in cat epithelium following continuous wear of orthokeratology lenses: a pilot study. *Cont Lens Anterior Eye.* 31: 29–37.

Chua, W. H., Balakrishnan, V., Chan, Y. H., Tong, L., Ling, Y., Quah, B. L., Tan, D. (2006). Atropine for the treatment of childhood myopia. *Ophthalmology.* 113: 2285–2291.

Chung, K., Mohidin, N., O'Leary, D. J. (2002). Undercorrection of myopia enhances rather than inhibits myopia progression. *Vision Res.* 42: 2555–2559.

Ciuffreda, K. J., Ordonez, X. (1998). Vision therapy to reduce abnormal nearwork-induced transient myopia. *Optom Vis Sci.* 75: 311–315.

Collins, F., Hampton, S. (2005). Hand-washing and methicillin-resistance staphylococcus aureus. *Br J Nursing.* 14: 703–707.

Correction of Myopia Evaluation Trial 2 Study Group for the Pediatric Eye Disease Investigator Group. (2011). Progressive-addition lenses versus single-vision lenses for slowing progression of myopia in children with high accommodative lag and near esophoria. *Invest Ophthalmol Vis Sci.* 25: 2749–2757.

Currie, B. J., Brewster, D. R. (2001). Childhood infections in the tropical north of Australia. *J Paediatr Child Health.* 37: 326–330.

Dart, J. K., Radford, C. F., Minassian, D., Verma, S., Stapleton, F. (2008). Risk factors for microbial keratitis with contemporary contact lenses: a case-control study. *Ophthalmology.* 115: 1647–1654.

Dart, J. K., Stapleton, F., Minassian, D. (1991). Contact lenses and other risk factors in microbial keratitis. *Lancet.* 338(8768): 650–653.

Delshad, S., Chun, J. M. (2013). Corneal endothelial cell density and morphology in low and moderate myopic Chinese eyes. *Int J Ophthalmol.* 6: 467–470.

Dirani, M., Chamberlain, M,, Shekar, S. N., Islam, A. F., Garoufalis, P., Chen, C. Y., Guymer, R. H., Baird, P. N. (2006). Heritability of refractive error and ocular biometrics: the Genes in Myopia (GEM) twin study. *Invest Ophthalmol Vis Sci.* 47: 4756–4761.

Donovan, L., Sankaridurg, P., Ho, A., Chen, X., Lin, Z., Thomas, V., Smith, EL III, Ge, J., Holden, B. (2012). Myopia progression in Chinese children is slower in summer than in winter. *Optom Vis Sci.* 89: 1196–1202.

Doughty, M. J., Aakre, B. M. (2007). Central versus paracentral endothelial cell density values in relation to duration of soft contact lens wear. *Eye Contact Lens.* 33: 180–184.

Downie, L. E., Lowe, R. (2013). Corneal reshaping influences myopic prescription stability (CRIMPS): an analysis of the effect of orthokeratology on childhood myopic refractive stability. *Eye Contact Lens.* 39: 303–310.

Edwards, M. H. (1999). The development of myopia in Hong Kong children between the ages of 7 and 12 years: a five-year longitudinal study. *Ophthalmic Physiol Opt.* 19: 286–294.

Edwards, M. H., Li, R. W., Lam, C. S., Lew, J. K., Yu, B. S. (2002). The Hong Kong progressive lens myopia control study: study design and main findings*. Invest Ophthalmol Vis Sci.* 43: 2852–2858.

El Hage, S., Leach, N. E., Miller, W., Prager, T. C., Marsack, J., Parker, K., Minavi, A., Gaume, A. (2007). Empirical advanced orthokeratology through corneal topography: the University of Houston clinical study. *Eye Contact Lens.* 33: 224–235.

Elbaz, U., Mireskandari, K., Tehrani, N., Shen, C., Khan, M. S., Williams, S., Ali, A. (2017). Corneal Endothelial Cell Density in Children: Normative Data From Birth to 5 Years Old. *Am J Ophthalmol.*173: 134–138.

Erdoğan, H., Kemal, M., Toker, M. I., Topalkara, A., Bakici, Z. (2002). Effect of frequent-replacement contact lenses on normal conjunctival flora. *CLAO J*. 28: 94–95.

Fan, D. S., Lam, D. S., Lam, R. F., Lau, J. T., Chong, K. S., Cheung, E. Y., Lai, R. Y., Chew, S. J. (2004). Prevalence, incidence, and progression of myopia of school children in Hong Kong. *Invest Ophthalmol Vis Sci.* 45: 1071–1075.

Fan, L., Jun, J., Jia, Q., Jin, W., Mao, X., Shen, Y. (1999). Clinical study of orthokeratology in young myopic adolescents. *Int Contact Lens Clin.* 26: 113–116.

Fang, P. C., Lo, J., Chang, T. C., Chien, C. C., Hsiao, C. C., Tseng, S. L., Lai, Y. H., Kuo, M. T. (2017). Bacterial bioburden decrease in orthokeratology lens storage cases after forewarning: assessment by the dna dot hybridization assay. *Eye Contact Lens.* 43: 174–180.

Faria-Ribeiro, M., Navarro, R., González-Méijome, J. M. (2016). Effect of pupil size on wavefront refraction during orthokeratology. *Optom Vis Sci.* 93: 1399–1408.

Fernández-Rubio, E., Urcelay, J. L., Cuesta-Rodriguez, T. (2009). The antibiotic resistance pattern of conjunctival bacteria: a key for designing a cataract surgery prophylaxis. *Eye.* 23: 1321–1328.

Fleiszig, S. M., Efron, N. (1992). Conjunctival flora in extended wear of rigid gas permeable contact lenses. *Optom Vis Sci.* 69: 354–357.

Fong, C. F., Tseng, C. H., Hu, F. R., Wang, I. J., Chen, W. L., Hou, Y. C. (2004). Clinical characteristics of microbial keratitis in a university hospital in Taiwan. *Am J Ophthalmol.* 137: 329-42.

Fu, A. C., Chen, X. L., Lv, Y., Wang, S. L., Shang, L. N., Li, X. H., Zhu, Y. (2016). Higher spherical equivalent refractive errors is associated with slower axial elongation wearing orthokeratology. *Cont Lens Anterior Eye.* 39: 62–66.

Fujiwara, M., Hasebe, S., Nakanishi, R., Tanigawa, K., Ohtsuki, H. (2012). Seasonal variation in myopia progression and axial elongation: an evaluation of Japanese children participating in a myopia control trial. *Jpn J Ophthalmol.* 56: 401–406.

Fulk, G. W., Cyert, L. A., Parker, D. E. (2000). A randomized trial of the effect of single-vision vs. bifocal lenses on myopia progression in children with esophoria. *Optom Vis Sci.* 77: 395–401.

Fulk, G. W., Cyert, L. A., Parker, D. A. (2002). Seasonal variation in myopia progression and ocular elongation. *Optom Vis Sci.* 79: 46–51.

Gambato, C., Longhin, E., Catania, A. G., Lazzarini, D., Parrozzani, R., Midena, E. (2015). Aging and corneal layers: an in vivo corneal confocal microscopy study. *Graefes Arch Clin Exp Ophthalmol.* 253: 267–275.

Gardner, D. J., Walline, J. J., Mutti, D. O. (2015). Choroidal Thickness and Peripheral Myopic Defocus during Orthokeratology. *Optom Vis Sci.* 92: 579–588.

Gifford, P., Gifford, K. L. (2016). The Future of Myopia Control Contact Lenses. *Optom Vis Sci.* 93: 336–343.

Gifford, P., Swarbrick, H. A. (2008). Time course of corneal topographic changes in the first week of overnight hyperopic orthokeratology. *Optom Vis Sci.* 85: 1165–1171.

Gifford, P., Swarbrick, H. A. (2013). Refractive changes from hyperopic orthokeratology monovision in presbyopes. *Optom Vis Sci.* 90: 306–313.

González-Mesa, A., Villa-Collar, C., Lorente-Velázquez, A., Nieto-Bona, A. (2013). Anterior segment changes produced in response to long-term overnight orthokeratology. *Curr Eye Res.* 38: 862–870.

Green, M., Apel, A., Stapleton, F. (2008). Risk factors and causative organisms in microbial keratitis. *Cornea.* 27: 22–27.

Grice, E. A., Segre, J. A. (2011). The skin microbiome. *Nat Revi Microbiol.* 9: 244–253.

Guggenheim, J. A., Northstone, K., McMahon, G., Ness, A. R., Deere, K., Mattocks, C., Pourcain, B. S., Williams, C. (2012). Time outdoors and physical activity as predictors of incident myopia in childhood: a prospective cohort study. *Invest Ophthalmol Vis Sci.* 53: 2856–2865.

Guo, X., Xie, P. (2014). Corneal thickness and endothelial observation for youth myopia patients fitted with ortho-k lens for seven years. *[Zhonghua Yan Ke Za Zhi].* 50: 9–13.

Guo, Y., Liu, L. J., Tang, P., Lv, Y. Y., Feng, Y., Xu, L., Jonas, J. B. (2017). Outdoor activity and myopia progression in 4-year follow-up of Chinese primary school children: The Beijing Children Eye Study. *PLoS One.* 12:e0175921.

Gwiazda, J. E., Hyman, L., Norton, T. T., Hussein, M. E., Marsh-Tootle, W., Manny, R., Wang, Y., Everett, D.; COMET Group. (2004). Accommodation and related risk factors associated with myopia progression and their interaction with treatment in COMET children. *Invest Ophthalmol Vis Sci.* 45: 2143–2151.

Gwiazda, J., Hyman, L., Hussein, M., Everett, D., Norton, T. T., Kurtz, D., Leske, M. C., Manny, R., Marsh-Tootle, W., Scheiman, M. (2003). A randomized clinical trial of progressive addition lenses versus single vision lenses on the progression of myopia in children. *Invest Ophthalmol Vis Sci.* 44: 1492–1500.

Gwiazda, J., Deng, L., Manny, R., Norton, T. T.; COMET Study Group. (2014). Seasonal variations in the progression of myopia in children enrolled in the correction of myopia evaluation trial. *Invest Ophthalmol Vis Sci.* 55: 752–758.

Haque, S., Fonn, D., Simpson, T., Jones, L. (2007). Corneal refractive therapy with different lens materials, part 1: corneal, stromal, and epithelial thickness changes. *Optom Vis Sci.* 84: 343–348.

Harvitt, D. M., Bonanno, J. A. (1999). Re-evaluation of the oxygen diffusion model for predicting minimum contact lens Dk/t values needed to avoid corneal anoxia. *Optom Vis Sci.* 76: 712–719.

Haug, S. J., Bhisitkul, R. B. (2012). Risk factors for retinal detachment following cataract surgery. *Curr Opin Ophthalmol.* 23: 7–11.

Hayashi, K., Ohno-Matsui, K., Shimada, N., Moriyama, M., Kojima, A,, Hayashi, W,, Yasuzumi, K., Nagaoka. N., Saka, N., Yoshida. T., Tokoro. T., Mochizuki, M. (2010). Long-term pattern of progression of myopic maculopathy: a natural history study. *Ophthalmology.* 117: 1595–1611.

He, M., Du, Y., Liu, Q., Ren, C., Liu, J., Wang, Q., Li, L., Yu, J. (2016). Effects of orthokeratology on the progression of low to moderate myopia in Chinese children. *BMC Ophthalmol.* 16: 126. doi:10.1186/s12886-016-0302-5.

He, M., Xiang, F., Zeng, Y., Mai, J., Chen, Q., Zhang, J., Smith, W., Rose, K., Morgan, I. G. (2015). Effect of time spent outdoors at school on the development of myopia among children in china: a randomized clinical trial. *JAMA.* 314: 1142–1148.

He, M., Xiang, F., Zeng, Y., Mai, J., Chen, Q., Zhang, J., Smith, W., Rose, K., Morgan, I. G. (2015). Effect of time spent outdoors at school on the development of myopia among children in china: a randomized clinical trial. *JAMA.* 314: 1142–1148.

He, M., Zeng, J., Liu, Y., Xu, J., Pokharel, G. P., Ellwein, L. B. (2004). Refractive error and visual impairment in urban children in southern china. *Invest Ophthalmol Vis Sci.* 45: 793–799.

Hickson-Curran, S., Chalmers, R. L., Riley, C. (2011). Patient attitudes and behavior regarding hygiene and replacement of soft contact lenses and storage cases. *Cont Lens Anterior Eye.* 34: 207–215.

Hiraoka, T., Furuya, A., Matsumoto, Y., Okamoto, F., Kakita, T., Oshika, T. (2004). Influence of overnight orthokeratology on corneal endothelium. *Cornea.* 23(8 Suppl): S82–86.

Hiraoka, T., Kaji, Y., Okamoto, F., Oshika, T. (2009). Corneal sensation after overnight orthokeratology. *Cornea.* 28: 891–895.

Hiraoka, T., Kakita, T., Okamoto, F., Oshika, T. (2015). Influence of ocular wavefront aberrations on axial length elongation in myopic children treated with overnight orthokeratology. *Ophthalmology.* 122: 93–100.

Hiraoka, T., Kakita, T., Okamoto, F., Takahashi, H., Oshika, T. (2012). Long-term effect of overnight orthokeratology on axial length elongation in childhood myopia: a 5-year follow-up study. *Invest Ophthalmol Vis Sci.* 53: 3913–3919.

Hiraoka, T., Okamoto, C., Ishii, Y., Kakita, T., Okamoto, F., Takahashi, H., Oshika, T. (2009a) Patient satisfaction and clinical outcomes after overnight orthokeratology. *Optom Vis Sci.* 86:875–882.

Ho, J., Boost, M. V., O'Donoghue, M. M. (2015). Sustainable reduction of nasal colonization and hand contamination with Staphylococcus aureus among food handlers, 2002–2011. *Epidemiol Infect.* 43: 1751–1760.

Holden, B. A., Fricke, T. R., Wilson, D. A., Jong, M., Naidoo, K. S., Sankaridurg, P., Wong, T. Y., Naduvilath, T. J., Resnikoff, S. (2016). Global Prevalence of Myopia and High Myopia and Temporal Trends from 2000 through 2050. Ophthalmology. 123: 1036–1042.

Holden, B. A., Mertz, G. W. (1984). Critical oxygen levels to avoid corneal edema for daily and extended wear contact lenses. *Invest Ophthalmol Vis Sci.* 25: 1161–1167.

Holden, B. A, Sweeney, D. F., Vannas, A., Nilsson, K. T., Efron N. (1985). Effects of long-term extended contact lens wear on the human cornea. *Invest Ophthalmol Vis Sci.* 26: 1489–1501.

Holley, G. P., Sugerman, P. E., Purohit, A. R., Edelhauser, H. F. (2002). Endothelial cell density is significantly increased in the far peripheral (limbal) region of the human cornea. *Invest Ophthalmol Vis Sci.* 43(13): 3176.

Hollingsworth, J., Perez-Gomez, I., Mutalib, H. A., Efron, N. (2001). A population study of the normal cornea using an in vivo, slit-scanning confocal microscope. *Optom Vis Sci.* 78: 706–711.

Hollingsworth, J., Perez-Gomez, I., Mutalib, H. A., Efron, N. (2001). A population study of the normal cornea using an in vivo, slit-scanning confocal microscope. *Optom Vis Sci.* 78: 706–711.

Huang, C., Ma, W., Stack, S. (2012). The hygienic efficacy of different hand-drying methods: a review of the evidence. *Mayo Clin Proc.* 87: 791–798.

Huang, J., Wen, D,, Wang, Q., McAlinden, C., Flitcroft, I., Chen, H., Saw, S. M., Chen, H., Bao, F., Zhao, Y., Hu, L., Li, X., Gao, R., Lu, W., Du, Y., Jinag, Z., Yu, A., Lian, H., Jiang, Q., Yu, Y., Qu, J. (2016). Efficacy comparison of 16 interventions for myopia control in children: a network meta-analysis. *Ophthalmology.* 123: 697–708.

Hyman, L., Gwiazda, J., Hussein, M., Norton, T. T., Wang, Y., Marsh-Tootle, W., Everett, D. (2005). Relationship of age, sex, and ethnicity with myopia progression and axial elongation in the correction of myopia evaluation trial. *Arch Ophthalmol.* 123: 977–987.

Ip, J. M., Huynh, S. C., Robaei, D., Kifley, A., Rose, K. A., Morgan, I. G., Wang, J. J., Mitchell, P. (2008). Ethnic differences in refraction and ocular biometry in a population-based sample of 11-15-year-old Australian children. *Eye.* 22: 649–656.

Ip, J. M., Saw, S. M., Rose, K. A., Morgan, I. G., Kifley, A., Wang, J. J., Mitchell, P. (2008). Role of near work in myopia: findings in a sample of Australian school children. *Invest Ophthalmol Vis Sci.* 49: 2903–2910.

Iskeleli, G., Bahar, H., Eroglu, E., Torun, M. M., Ozkan, S. (2005). Microbial changes in conjunctival flora with 30-day continuous-wear silicone hydrogel contact lenses. *Eye Contact Lens.* 31: 124–126.

Jayakumar, J., Swarbrick, H. A. (2005). The effect of age on short-term orthokeratology. *Optom Vis Sci.* 82: 505–511.

Jones, L. A., Sinnott, L. T., Mutti, D. O., Mitchell, G. L., Moeschberger, M. L., Zadnik, K. (2007). Parental history of myopia, sports and outdoor activities, and future myopia. *Invest Ophthalmol Vis Sci.* 48: 3524–3532.

Jones, L. A., Walline, J. J., Gaume, A., Rah, M. J., Manny, R. E., Berntsen, D. A., Chitkara, M., Kim, A., Quinn, N; CLIP Study Group. (2009). Purchase of contact lenses and contact-lenses-related symptoms following the Contact Lenses in Pediatrics (CLIP) Study. *Cont Lens Anterior Eye.* 32: 157–163.

Jones-Jordan, L. A., Sinnott, L. T., Cotter, S. A., Kleinstein, R. N., Manny, R. E., Mutti, D. O., Twelker, J. D., Zadnik, K.; CLEERE Study Group. (2012). Time outdoors, visual activity, and myopia progression in juvenile-onset myopes. *Invest Ophthalmol Vis Sci.* 53: 7169–7175.

Jones-Jordan, L. A., Sinnott, L. T., Cotter, S. A., Kleinstein, R. N., Manny, R. E., Mutti, D. O., Twelker, J. D., Zadnik, K.; CLEERE Study Group. (2012). Time outdoors, visual activity, and myopia progression in juvenile-onset myopes. *Invest Ophthalmol Vis Sci.* 53: 7169–7175.

Joyce, N. C. (2003). Proliferative capacity of the corneal endothelium. *Prog Retin Eye Res.* 22: 359–389.

Kakita, T., Hiraoka, T., Oshika, T. (2011). Influence of overnight orthokeratology on axial elongation in childhood myopia. *Invest Ophthalmol Vis Sci.* 52: 2170–2174.

Kam, K. W., Yung, W., Li, G. K. H., Chen, L. J., Yong, A. L. (2017). Infectious keratitis and orthokeratology lens use: a systematic review. *Infection.* doi:10.1007/s15010-017-1023-2.

Kang, P., Swarbrick, H. (2011). Peripheral refraction in myopic children wearing orthokeratology and gas-permeable lenses. *Optom Vis Sci.* 88: 476–482.

Keay, L., Edwards, K., Naduvilath, T., Taylor, H. R., Snibson, G. R., Forde, K., Stapleton, F. (2006). Microbial keratitis predisposing factors and morbidity. *Ophthalmology.* 113: 109–116.

Kerns RL (1976a). Research in orthokeratology. Part III: results and observations. *J Am Optom Assoc.* 47: 1505–1515.

Kerns, R. L. (1976). Research in orthokeratology. Part I: Introduction and background. *J Am Optom Assoc.* 47: 1047–1051.

Koh, V., Yang, A., Saw, S. M., Chan, Y. H., Lin, S. T., Tan, M. M., Tey, F., Nah, G., Ikram, M. K. (2014). Differences in prevalence of refractive errors in young Asian males in Singapore between 1996-1997 and 2009-2010. *Ophthalmic Epidemiol*. 21: 247–255.

Kuehnert, M. J., Kruszon-Moran, D., Hill, H. A., McQuillan, G., McAllister, S. K., Fosheim, G., McDougal, L. K., Chaitram, J., Jensen, B., Fridkin, S. K., Killgore, G., Tenover, F. C. (2006). Prevalence of Staphylococcus aureus nasal colonization in the United States, 2001–2002. *J Infect Dis.* 193: 172–179.

Kwan, J. (2010). Can children wear silicone hydrogel contact lenses? Silicone Hydrogel Website, 2010 October. Available at: http://www.siliconehydrogels.org/editorials/oct_10.asp. Accessed on Oct 18, 2017.

Lam, C. S. Y., Goh, W. S. H. (1991). The incidence of refractive errors among school children in Hong Kong and its relationship with the optical components. *Clin Exp Optom.* 74: 97–103.

Lam, C. S., Lam, C. H., Cheng, S. C., Chan, L. Y. (2012). Prevalence of myopia among Hong Kong Chinese schoolchildren: changes over two decades. *Ophthalmic Physiol Opt.* 32: 17–24.

Larkin, D. F., Leeming, J. P. (1991). Quantitative alterations of the commensal eye bacteria in contact lens wear. *Eye* 5: 70–74.

Lee, T. T., Cho, P. (2013). Relative peripheral refraction in children: twelve-month changes in eyes with different ametropias. *Ophthalmic Physiol Opt.* 33: 283–293.

Lee, Y. S., Tan, H. Y., Yeh, L. K., Lin, H. C., Ma, D. H., Chen, H. C., Chen, S. Y., Chen, P. Y., Hsiao, C. H. (2014). Pediatric microbial keratitis in Taiwan: clinical and microbiological profiles, 1998–2002 versus 2008–2012. *Am J Ophthalmol.* 157: 1090–1096.

Levy, S. G., Moss, J., Sawada, H., Dopping-Hepenstal, P. J., McCartney, A. C. (1996). The composition of wide-spaced collagen in normal and diseased Descemet's membrane. *Curr Eye Res.*15: 45–52.

Li, L., Moody, K., Tan, D. T., Yew, K. C., Ming, P. Y., Long, Q. B. (2009). Contact lenses in pediatrics study in Singapore. *Eye Contact Lens.* 35: 188–195.

Li, S. M., Kang, M. T., Wu, S. S., Liu, L. R., Li, H., Chen, Z., Wang, N. (2016). Efficacy, safety and acceptability of orthokeratology on slowing axial elongation in myopic children by meta-analysis. *Curr Eye Res.* 41: 600–608.

Li, S. M., Li, H., Li, S. Y., Liu, L. R., Kang, M. T., Wang, Y. P., Zhang, F., Zhan, S. Y., Gopinath, B., Mitchell, P., Wang, N.; Anyang Childhood Eye Study Group. (2015). Time outdoors and myopia progression over 2 years in chinese children: the Anyang Childhood Eye Study. *Invest Ophthalmol Vis Sci.* 56: 4734–4740.

Lim, K. L., Fam, H. B. (2006). NeuroVision treatment for low myopia following LASIK regression. *J Refract Surg.* 22: 406–408.

Lin, L. L., Shih, Y. F., Hsiao, C. K., Chen, C. J. (2004). Prevalence of myopia in Taiwanese schoolchildren: 1983 to 2000. *Ann Acad Med Singapore.* 33: 27–33.

Lin, L. L., Shih, Y. F., Hsiao, C. K., Chen, C. J. (2004). Prevalence of myopia in Taiwanese schoolchildren: 1983 to 2000. *Ann Acad Med Singapore.* 33: 27–33.

Liu, Y. M., Xie, P. (2016). The safety of orthokeratology – a systematic review. *Eye Contact Lens.* 42: 35–42.

Low, W., Dirani, M., Gazzard, G., Chan, Y. H., Zhou, H. J., Selvaraj, P., Au Eong, K. G., Young, T. L., Mitchell, P., Wong, T. Y., Saw, S. M. (2010). Family history, near work, outdoor activity, and myopia in Singapore Chinese preschool children. *Br J Ophthalmol.* 94: 1012–1016.

Lum, E., Golebiowski, B., Gunn, R., Babhoota, M., Swarbrick, H. (2011). Corneal sensitivity with contact lenses of different mechanical properties. *Optom Vis Sci.* 90: 954–960.

Lum, E., Golebiowski, B., Swarbrick, H. (2012). Mapping the corneal sub-basal nerve plexus in orthokeratology lens wear using in vivo laser scanning confocal microscopy. *Invest Ophthalmol Vis Sci.* 53: 1803–1809.

Lum, E., Golebiowski, B., Swarbrick, H. (2017). Changes in corneal subbasal nerve morphology and sensitivity during orthokeratology: onset of change. *Ocul Surf.* 15: 227–235.

Lum, E., Golebiowski, B., Swarbrick, H. (2017a). Changes in corneal subbasal nerve morphology and sensitivity during orthokeratology: recovery of change. *Ocul Surf.* 15: 236–241.

Lum, E., Golebiowski, B., Swarbrick, H. (2017b). Reduced corneal sensitivity and sub-basal nerve density in long-term orthokeratology lens wear. *Eye Contact Lens.* 43: 218–224.

Lum, E., Swarbrick, H. (2007). Fibrillary lines in overnight orthokeratology. *Clin Exp Optom.* 90: 299–302.

Lum, E., Swarbrick, H. (2011). Lens Dk/t influences the clinical response in overnight orthokeratology. *Optom Vis Sci.* 88: 469–475.

Luo, M., Ma, S., Liang, N. (2014). Clinical efficacy of toric orthokeratology in myopic adolescent with moderate to high astigmatism. *Eye Sci.* 29: 209–213.

Lyu, B., Hwang, K. Y., Kim, S. Y., Kim, S. Y., Na, K. S. (2016). Effectiveness of Toric Orthokeratology in the Treatment of Patients with Combined Myopia and Astigmatism. *Korean J Ophthalmol.* 30: 434–442.

Marcus, M. W., de Vries, M. M., Junoy Montolio, F. G., Jansonius, N. M. (2011). Myopia as a risk factor for open-angle glaucoma: a systematic review and meta-analysis. *Ophthalmology.* 118: 1989–1994.

Maseedupally, V., Gifford, P., Lum, E., Swarbrick, H. (2013). Central and paracentral corneal curvature changes during orthokeratology. *Optom Vis Sci.* 90: 1249–1258.

Matsubara, M., Kamei, Y., Takeda, S., Mukai, K., Ishii, Y., Ito, S. (2004). Histologic and histochemical changes in rabbit cornea produced by an orthokeratology lens. *Eye Contact Lens.* 30: 198–204.

Matsumura, H., Hirai, H. (1999). Prevalence of myopia and refractive changes in students from 3 to 17 years of age. *Surv Ophthalmol.* 44(Suppl1): S109–115.

McCullough, S. J., O'Donoghue, L., Saunders, K. J. (2016). Six year refractive change among white children and young adults: evidence for significant increase in myopia among white UK children. *PLoS One.* 11: e0146332.

Merry, A. F., Miller, T. E., Findon, G., Webster, C. S., Neff, S. P. (2001). Touch contamination levels during anaesthetic procedures and their relationship to hand hygiene procedures: a clinical audit. *Br J Anaesth.* 87: 291–294.

Miedziak, A. I., Miller, M. R., Rapuano, C. J., Laibson, P. R., Cohen, E. J. (1999). Risk factors in microbial keratitis leading to penetrating keratoplasty. *Ophthalmology.* 106: 1166–1170.

Mohle-Boetani, J. C., Ajello, G., Breneman, E., Deaver, K. A., Harvey, C., Plikaytis, B. D., Farley, M. M., Stephens, D. S., Wenger, J. D. (1993). Carriage of Haemophilus influenzae type b in children after widespread vaccination with conjugate Haemophilus influenzae type b vaccines. *Pediatr Infect Dis J.* 12: 589–593.

Mok, A. K. H., Chung, C. S. T. (2011). Seven-year retrospective analysis of the myopic control effect of orthokeratology in children: a pilot study. *Clin Optom.* 3: 1–4.

Morgan, I. G., Ohno-Matsui, K., Saw, S. M. (2012). Myopia. *Lancet.* 379(9827): 1739–1748.

Morgan, P. B., Woods, C. A., Tranoudis, I. G., Helland, M., Efron, N., Orihuela, G. C., Grupcheva, C. N., Jones, D., Tan, K., Pesinova, A., Ravn, O., Santodomingo, J., Malet, F., Sze, L., Cheng, P., Végh, M., Erdinest, N., Ragnarsdóttir, J. B., Montani, G., Davila-Garcia, E., Itoi, M., Chu, B. S., Bendoriene, J., van der Worp, E., Awasthi, S., Lam, W., Casablanca, J., González-Méijome, J. M., Johansson, O., Silih, M. S., Hsiao, J., Nichols, J. J. (2013). International Contact Lens Prescribing in 2012. *Cont Lens Spectrum.* Available at https://www.clspectrum.com/issues/2013/january-2013/international-contact-lens-prescribing-in-2012. Accessed on October 18, 2017.

Mountford, J. (1997). An analysis of the changes in corneal shape and refractive error induced by accelerated orthokeratology. *Int Contact Lens Clin.* 24: 128–143.

Mountford, J. (2004). History and general principles, Orthokeratology - Principles and Practice. Mountford J, Dave T and Ruston D, Eds. Butterworth-Heinemann, London. pp 1–16.

Mountford, J. (2004a). Trial lens fitting, Orthokeratology - Principles and Practice. Mountford J, Dave T and Ruston D, Eds. Butterworth-Heinemann, London. pp 139–173.

Mountford, J. (2004b). A model of forces acting in orthokeratology, Orthokeratology - Principles and Practice. Mountford J, Dave T and Ruston D, Eds. Butterworth- Heinemann, London. pp 269–301.

Mutti, D. O., Mitchell, G. L., Moeschberger, M. L., Jones, L. A., Zadnik, K. (2002). Parental myopia, near work, school achievement, and children's refractive error. *Invest Ophthalmol Vis Sci.* 43: 3633–3640.

Mutti, D. O., Mitchell, G. L., Moeschberger, M. L., Jones, L. A., Zadnik, K. (2002). Parental myopia, near work, school achievement, and children's refractive error. *Invest Ophthalmol Vis Sci.* 43:3633–3640.

Mutti, D. O., Sinnott, L. T., Mitchell, G. L., Jones-Jordan, L. A., Moeschberger, M. L., Cotter, S. A., Kleinstein, R. N., Manny, R. E., Twelker, J. D., Zadnik, K.; CLEERE Study Group. (2011). Relative peripheral refractive error and the risk of onset and progression of myopia in children. *Invest Ophthalmol Vis Sci.* 52: 199–205.

Ngo, C. S., Pan, C. W., Finkelstein, E. A., Lee, C. F., Wong, I. B., Ong, J., Ang, M., Wong, T. Y., Saw, S. M. (2014). A cluster randomised controlled trial evaluating an incentive-based outdoor physical activity programme to increase outdoor time and prevent myopia in children. *Ophthalmic Physiol Opt.* 34: 362–368.

Nichols, J. J., Marsich, M. M., Nguyen, M., Barr, J. T., Bullimore, M. A. (2000). Overnight orthokeratology. *Optom Vis Sci.* 77: 252–259.

Nieto-Bona, A., González-Mesa, A., Nieto-Bona, M. P., Villa-Collar, C., Lorente-Velázquez, A. (2011). Long-term changes in corneal morphology induced by overnight orthokeratology. *Curr Eye Res.* 36: 895–904.

Nieto-Bona, A., González-Mesa, A., Nieto-Bona, M. P., Villa-Collar, C., Lorente-Velázquez, A. (2011a). Short-term effects of overnight orthokeratology on corneal cell morphology and corneal thickness. *Cornea.*30: 646–54.

Nieto-Bona, A., Nombela-Palomo, M., Felipe-Márquez, G., Teus, M. A. (2017). Tear film osmolarity in response to long-term orthokeratology treatment. *Eye Contact Lens.* doi:10.1097/ICL.0000000000000347.

Nieuwendaal, C. P., Odenthal, M. T. P., Kok, J. H. C., Venema, H. W., Oosting, J., Reimslag, F. C. C., Kijlstra, A. (1994). Morphology and function of the corneal endothelium after long-term contact lens wear. *Invest Ophthalmol Vis Sci.* 35: 3071–3077.

Nombela-Palomo, M., Felipe-Marquez, G., Hernandez-Verdejo, J. L., Nieto-Bona, A. (2016). Short-term effects of overnight orthokeratology on corneal sub-basal nerve plexus morphology and corneal sensitivity. *Eye Contact Lens.* doi:10.1097/ICL.0000000000000282.

Nombela-Palomo, M., Felipe-Marquez, G., Hernandez-Verdejo, J. L., Nieto-Bona, A. (2017). Long-term impacts of orthokeratology treatment on sub-basal nerve plexus and corneal sensitivity responses and their reversibility. *Eye Contact Lens.* doi:10.1097/ICL.0000000000000386.

Nucci, P., Brancato, R., Mets, M. B., Shevell, S. K. (1990). Normal endothelial cell density range in childhood. *Arch Ophthalmol.* 108: 247–248.

Owens, H., Garner, L. F., Craig, J. P., Gamble, G. (2004). Posterior corneal changes with orthokeratology. *Optom Vis Sci.* 81:421–426.

Pan, C. W., Cheng, C. Y., Saw, S. M., Wang, J. J., Wong, T. Y. (2013). Myopia and age-related cataract: a systematic review and meta-analysis. *Am J Ophthalmol.* 156: 1021–1033.

Paragon Vision Sciences. (2002). Premarket approval application supplement P870024/S43; June 13, 2002. Available at: https://www.accessdata.fda.gov/cdrh_docs/pdf/P870024S043b.pdf. Accessed on October 18, 2017.

Pärssinen, O., Kauppinen, M. (2016). What is the influence of parents' myopia on their children's myopic progression? A 22-year follow-up study. *Acta Ophthalmol.* 94: 579–585.

Patrick, D. R., Findon, G., Miller, T. E. (1997). Residual moisture determines the level of touch-contact-associated bacterial transfer following hand washing. *Epidemiol Infect.* 119: 319–325.

Pauné, J., Cardona, G., Quevedo, L. (2012). Toric double tear reservoir contact lens in orthokeratology for astigmatism. *Eye Contact Lens.* 38: 245–251.

Pauné, J., Morales, H., Armengol, J., Quevedo, L., Faria-Ribeiro, M., González-Méijome, J.,M. (2015). Myopia control with a novel peripheral gradient soft lens and orthokeratology: a 2-year clinical trial. *Biomed Res Int.* 2015:507572.

Pauné, J., Morales, H., Armengol, J., Quevedo, L., Faria-Ribeiro, M., González-Méijome, J. M. (2015). Myopia control with a novel peripheral gradient soft lens and orthokeratology: a 2-year clinical trial. *Biomed Res Int.* 2015:507572. doi:10.1155/2015/507572.

Queirós, A,. Villa-Collar, C., Gutiérrez, A. R., Jorge, J., González-Méijome, J. M. (2012). Quality of life of myopic subjects with different methods of visual correction using the NEI RQL-42 questionnaire. *Eye Contact Lens.* 38: 116–121.

Queirós, A., González-Méijome, J. M., Jorge, J., Villa-Collar, C., Gutiérrez, A. R. (2010). Peripheral refraction in myopic patients after orthokeratology. *Optom Vis Sci.* 87: 323–329.

Rah, M. J., Jackson, J. M., Jones, L. A., Marsden, H. J., Bailey, M. D., Barr, J. T. (2002). Overnight orthokeratology: preliminary results of the Lenses and Overnight Orthokeratology (LOOK) study. *Optom Vis Sci.* 79: 598–605.

Rah, M. J., Walline. J. J., Jones-Jordan, L. A., Sinnott, L. T., Jackson, J. M., Manny, R. E., Coffey, B., Lyons, S.; ACHIEVE Study Group. (2010). Vision specific quality of life of pediatric contact lens wearers. *Optom Vis Sci.* 87: 560–566.

Read, S. A., Alonso-Caneiro, D., Vincent, S. J., Collins, M. J. (2015). Longitudinal changes in choroidal thickness and eye growth in childhood. *Invest Ophthalmol Vis Sci.* 56: 3103–3112.

Read, S. A., Collins, M. J., Vincent, S. J. (2015). Light Exposure and Eye Growth in Childhood. *Invest Ophthalmol Vis Sci.* 56: 6779–6787.

Regev-Yochay, G., Raz, M., Dagan, R., Porat, N., Shainberg, B., Pinco, E., Keller, N., Rubinstein, E. (2004). Nasopharyngeal carriage of *Streptococcus pneumoniae* by adults and children in community and family settings. *Clin Infect Dis.* 38: 632–639.

Robertson, D. M., McCulley, J. P., Cavanagh, H. D. (2007). Severe acanthamoeba keratitis after overnight orthokeratology. *Eye Contact Lens.* 33: 121–123.

Rose, K. A., Morgan, I. G., Ip, J., Kifley, A., Huynh, S., Smith, W., Mitchell, P. (2008). Outdoor activity reduces the prevalence of myopia in children. *Ophthalmology.* 115: 1279–1285.

Rudnicka, A. R., Kapetanakis, V. V., Wathern, A. K., Logan, N. S., Gilmartin, B., Whincup, P. H., Cook, D. G., Owen, C. G. (2016). Global variations and time trends in the prevalence of childhood myopia, a systematic review and quantitative meta-analysis: implications for aetiology and early prevention. *Br J Ophthalmol.* 100: 882–890.

Rudnicka, A. R., Owen, C. G., Nightingale, C. M., Cook, D. G., Whincup, P. H. (2010). Ethnic differences in the prevalence of myopia and ocular biometry in 10- and 11-year-old children: the Child Heart and Health Study in England (CHASE). *Invest Ophthalmol Vis Sci.* 51: 6270–6276.

Ruiz-Medrano, J., Flores-Moreno, I., Peña-García, P., Montero, J. A., Duker, J. S., Ruiz-Moreno, J. M. (2014). Macular choroidal thickness profile in a healthy population measured by swept-source optical coherence tomography. *Invest Ophthalmol Vis Sci.* 55: 3532–3542.

Sankaridurg, P., Donovan, L., Varnas, S., Ho, A., Chen, X., Martinez, A., Fisher, S., Lin, Z., Smith, EL III, Ge, J., Holden, B. (2010). Spectacle lenses designed to reduce progression of myopia: 12-month results. *Optom Vis Sci.* 87: 631–641.

Sankaridurg. P., Holden, B., Smith, EL III, Naduvilath, T., Chen, X., de la Jara, P. L., Martinez, A., Kwan, J., Ho, A., Frick, K., Ge, J. (2011). Decrease in rate of myopia progression with a contact lens designed to reduce relative peripheral hyperopia: one-year results. *Invest Ophthalmol Vis Sci.* 52: 9362–9367.

Santodomingo-Rubido, J., Villa-Collar, C., Gilmartin, B., Gutiérrez-Ortega, R. (2012). Myopia control with orthokeratology contact lenses in Spain: refractive and biometric changes. *Invest Ophthalmol Vis Sci.* 53: 5060–5065.

Santodomingo-Rubido, J., Villa-Collar, C., Gilmartin, B., Gutiérrez-Ortega, R. (2012a). Orthokeratology vs. spectacles: adverse events and discontinuations. *Optom Vis Sci.* 89: 1133–1139.

Santodomingo-Rubido, J., Villa-Collar, C., Gilmartin, B., Gutiérrez-Ortega, R. (2013). Myopia control with orthokeratology contact lenses in Spain: a comparison of vision-related quality-of-life measures between orthokeratology contact lenses and single-vision spectacles. *Eye Contact Lens*. 39: 153–157.

Santodomingo-Rubido, J., Villa-Collar, C., Gilmartin, B., Gutiérrez-Ortega, R., Suzaki, A. (2015). The effects of entrance pupil centration and coma aberrations on myopic progression following orthokeratology. *Clin Exp Optom.* 98: 534–540.

Saw, S. M., Gazzard, G., Shih-Yen, E. C., Chua, W. H. (2005). Myopia and associated pathological complications. *Ophthalmic Physiol Opt*. 25: 381–391.

Saw, S. M., Shankar, A., Tan, S. B., Taylor, H., Tan, D. T., Stone, R. A., Wong, T. Y. (2006). A cohort study of incident myopia in Singaporean children. *Invest Ophthalmol Vis Sci.* 47: 1839–1844.

Saw, S. M., Tong, L., Chua, W. H., Chia, K. S., Koh, D., Tan, D. T., Katz, J. (2005a). Incidence and progression of myopia in Singaporean school children. *Invest Ophthalmol Vis Sci.* 46: 51–57.

Shao, Y., Huang, Y., Liu, Y. P., Zhang, M. Z., Lam, D. S. C., Rao, S. K. (2007). Corneal endothelial cell density and morphology in healthy Chinese eyes. *Cornea.* 26: 130–132.

Sheng, H., Bullimore, M. A. (2007). Factors affecting corneal endothelial morphology. *Cornea.* 26: 520–525.

Sherwin, J. C., Reacher, M. H., Keogh, R. H., Khawaja, A. P., Mackey, D. A., Foster, P. J. (2012). The association between time spent outdoors and myopia in children and adolescents: a systematic review and meta-analysis. *Ophthalmology.* 119: 2141–2151.

Si, J. K., Tang, K., Bi, H. S., Guo, D. D., Guo, J. G., Wang, X. R. (2015). Orthokeratology for myopia control: a meta-analysis. *Optom Vis Sci.* 92: 252–257.

Siatkowski, R. M., Cotter, S. A., Crockett, R. S., Miller, J. M., Novack, G. D., Zadnik, K.; U.S. Pirenzepine Study Group. (2008). Two-year multicenter, randomized, double-masked, placebo-controlled, parallel safety and efficacy study of 2% pirenzepine ophthalmic gel in children with myopia. *J AAPOS.* 12: 332–339.

Singer, T. R., Isenberg, S. J., Apt, L. (1988). Conjunctival anaerobic and aerobic bacterial flora in paediatric versus adult subjects. *Br J Ophthalmol.* 72: 448–451.

Smith, E. L. III, Hung, L. F., Huang, J. (2009). Relative peripheral hyperopic defocus alters central refractive development in infant monkeys. *Vision Res.* 49: 2386–2392.

Smith, E. L. III, Kee, C. S., Ramamirtham, R., Qiao-Grider, Y., Hung, L. F. (2005). Peripheral vision can influence eye growth and refractive development in infant monkeys. *Invest Ophthalmol Vis Sci.* 46: 3965–3972.

Smith, EL III. (2013). Optical treatment strategies to slow myopia progression: effects of the visual extent of the optical treatment zone. *Exp Eye Res.* 114: 77–88.

Smith, M. J., Walline, J. J. (2015). Controlling myopia progression in children and adolescents. *Adolesc Health Med Ther.* 6: 133–140.

Snelling, A. M., Saville, T., Stevens, D., Beggs, C. B. (2011). Comparative evaluation of the hygienic efficacy of an ultra-rapid hand dryer vs conventional warm air hand dryers. *J Appl Microbiol.* 110: 19–26.

Sng, C. C., Lin, X. Y., Gazzard, G., Chang, B., Dirani, M., Chia, A., Selvaraj, P., Ian, K., Drobe, B., Wong, T. Y., Saw, S. M. (2011). Change in peripheral refraction over time in Singapore Chinese children. *Invest Ophthalmol Vis Sci.* 52: 7880–7887.

Sng, C. C., Lin, X. Y., Gazzard, G., Chang, B., Dirani, M., Chia, A., Selvaraj, P., Ian, K., Drobe, B., Wong, T. Y., Saw, S. M. (2011a). Peripheral refraction and refractive error in singapore chinese children. *Invest Ophthalmol Vis Sci.* 52: 1181–1190.

Soni, P. S., Nguyen, T. T. Bonanno, J. A. (2003). Overnight orthokeratology: visual and corneal changes. *Eye Contact Lens.* 29: 137–145.

Sorbara, L., Fonn, D., Simpson, T., Lu, F., Kort, R. (2005). Reduction of myopia from corneal refractive therapy. *Optom Vis Sci.* 82: 512–518.

Stapleton, F., Carnt, N. (2012). Contact lens-related microbial keratitis: how have epidemiology and genetics helped us with pathogenesis and prophylaxis. *Eye*. 26: 185–193.

Sthapit, P. R., Tuladhar, N. R. (2014). Conjunctival flora of normal human eye. *JSM Ophthalmol.* 2: 1021–1025.

Stillitano, I. G., Chalita, M. R., Schor, P., Maidana, E., Lui, M. M., Lipener, C., Hofling-Lima, A. L. (2007). Corneal changes and wavefront analysis after orthokeratology fitting test. *Am J Ophthalmol.* 144: 378–386.

Sun, J., Zhou, J., Zhao, P., Lian, J., Zhu, H., Zhou, Y., Sun, Y., Wang, Y., Zhao, L., Wei, Y., Wang, L., Cun, B., Ge, S., Fan, X. (2012). High prevalence of myopia and high myopia in 5060 Chinese university students in Shanghai. *Invest Ophthalmol Vis Sci.* 53: 7504–7509.

Sun, X., Chen, L., Zhang, Y., Wang, Z., Li, R. B. S., Luo, S., Jin, X. (2003). Acanthamoeba keratitis as a complication of orthokeratology. *Am J Ophthalmol.* 136: 1159–1161.

Sun, X., Zhao, H., Deng, S., Zhang, Y., Wang, Z., Li, R., Luo, S., Jin, X. (2006). Infectious keratitis related to orthokeratology. *Ophthalmic Physiol Opt.* 26: 133–136.

Sun, Y., Hong, J., Zhang, P., Peng, R., Xiao, G. (2013). Pathological characteristics of the different stages of *Acanthamoeba* keratitis. *Histopathology.* 63: 862–868.

Sun, Y., Xu, F., Zhang, T., Liu, M., Wang, D., Chen, Y., Liu, Q. (2015). Orthokeratology to control myopia progression: a meta-analysis. *PLoS One* 2015:10:e0124535.

Swarbrick, H. A. (2006). Orthokeratology review and update. *Clin Exp Optom.* 89: 124–143.

Swarbrick, H. A., Alharbi, A., Watt, K., Lum, E., Kang, P. (2015). Myopia control during orthokeratology lens wear in children using a novel study design. *Ophthalmology.* 122: 620–630.

Swarbrick, H. A., Wong, G., O'Leary, D. J. (1998). Corneal response to orthokeratology. *Optom Vis Sci.* 75: 791–799.

Tahhan, N., Du Toit, R., Papas, E., Chung, H., La Hood, D., Holden, A. B. (2003). Comparison of reverse-geometry lens designs for overnight orthokeratology. *Optom Vis Sci.* 80: 796–804.

Tan, D. T., Fong, A. (2008). Efficacy of neural vision therapy to enhance contrast sensitivity function and visual acuity in low myopia. *J Cataract Refract Surg.* 34: 570–577.

Tan, N. W., Saw, S. M., Lam, D. S., Cheng, H. M., Rajan, U., Chew, S. J. (2000). Temporal variations in myopia progression in Singaporean children within an academic year. *Optom Vis Sci.* 77: 465–472.

Tanaka, H., Okumura, N., Koizumi, N., Sotozono, C., Sumii, Y., Kinoshita, S. (2017). Panoramic view of human corneal endothelial cell layer observed by a prototype slit-scanning wide-field contact specular microscope. *Br J Ophthalmol.* 101: 655–659.

Tepelus, T. C., Vazquez, D., Seidemann, A., Uttenweiler, D., Schaeffel, F. (2012). Effects of lenses with different power profiles on eye shape in chickens. *Vision Res.* 54: 12–19.

Tilia, D., Lazon de la Jara, P., Zhu, H., Naduvilath, T. J., Holden, B. A. (2014). The effect of compliance on contact lens case contamination. *Optom Vis Sci.* 91: 262–271.

Tse, D. Y., Lam, C. S., Guggenheim, J. A., Lam, C., Li, K. K., Liu, Q., To, C. H. (2007). Simultaneous defocus integration during refractive development. *Invest Ophthalmol Vis Sci.* 48: 5352–5359.

Tsukahara, Y., Yamamoto, M. (1989). Postnatal development of corneal endothelial cells in normal children. *[Nippon Ganka Gakkai Zasshi].* 93: 763–768.

Tsukiyama, J., Miyamoto, Y., Higaki, S., Fukuda, M., Shimomura, Y. (2008). Changes in the anterior and posterior radii of the corneal curvature and anterior chamber depth by orthokeratology. *Eye Contact Lens.* 34: 17–20.

Tuft, S. J., Coster, D. J. (1990). The corneal endothelium. *Eye.* 4: 389–424.

Urban, B., BakunowiczŁazarczyk, A., Kretowska, M. (2002). Corneal endothelium in children and adolescents with myopia. *[Klin Oczna].* 2002;104:381–383.

Üstüntürk, M., Zeybek, Z. (2012). Microbial contamination of contact lens storage cases and domestic tap water of contact lens wearers. *[Wien Klin Wochenschr].* 124(Suppl 3): 17–22.

Van Meter, W. S., Musch, D. C., Jacobs, D. S., Kaufman, S. C., Reinhart, W. J., Udell, I. J.; American Academy of Ophthalmology. (2008). Safety of overnight orthokeratology for myopia: a report by the American Academy of Ophthalmology. *Ophthalmology.* 115: 2301–2313.

Vasudevan, B., Ciuffreda, K. J., Ludlam, D. P. (2009). Accommodative training to reduce nearwork-induced transient myopia. *Optom Vis Sci.* 86: 1287–1294.

Vitale, S., Sperduto, R. D., Ferris, FL III. (2009). Increased prevalence of myopia in the United States between 1971–1972 and 1999–2004. *Arch Ophthalmol.* 127: 1632–1639.

Walline, J. J., Greiner, K. L., McVey, M. E., Jones-Jordan, L. A. (2013). Multifocal contact lens myopia control. *Optom Vis Sci.* 90: 1207–1214.

Walline, J. J., Jones, L. A., Mutti, D., Zadnik, K. (2004). A randomized trial of the effects of rigid contact lenses on myopia progression. *Arch Ophthalmol.* 122: 1760–1766.

Walline, J. J., Jones, L. A., Sinnott, L. T. (2009). Corneal reshaping and myopia progression. *Br J Ophthalmol.* 93: 1181–1185.

Walline, J. J., Jones, L. A., Sinnott, L., Manny, R. E., Gaume, A., Rah, M. J., Chitkara, M., Lyons, S.; ACHIEVE Study Group. (2008). A randomized trial of the effect of soft contact lenses on myopia progression in children. *Invest Ophthalmol Vis Sci.* 49: 4702–4706.

Walline, J. J., Rah, M. J., Jones, L. A. (2004a). The Children's Overnight Orthokeratology Investigation (COOKI) pilot study. *Optom Vis Sci.* 81: 407–413.

Wang, B., Naidu, R. K., Qu, X. (2017). Factors related to axial length elongation and myopia progression in orthokeratology practice. *PLoS One.* 12:e0175913.

Wang, J., Fonn, D., Simpson, T. L., Sorbara, L., Kort, R., Jones, L. (2003). Topographical thickness of the epithelium and total cornea after overnight wear of reverse-geometry rigid contact lenses for myopia reduction. *Invest Ophthalmol Vis Sci.* 44: 4742–4746.

Watt, K. G., Swarbrick, H. A. (2005). Microbial keratitis in overnight orthokeratology: review of the first 50 cases. *Eye Contact Lens.* 31: 201–208.

Watt, K. G., Swarbrick, H. A. (2007). Trends in microbial keratitis associated with orthokeratology. *Eye Contact Lens.* 33: 373–377.

Wei, M. L., Liu, J. P., Li, N., Liu, M. (2011). Acupuncture for slowing the progression of myopia in children and adolescents. *Cochrane Database Syst Rev.* 2011 Sep 7;(9):CD007842; doi:10.1002/14651858.CD007842.pub2.

Weiss, A., Brinser, J. H., Nazar-Stewart, V. (1993). Acute conjunctivitis in childhood. *J Pediatr.* 122: 10–14.

Wen, D., Huang, J., Chen, H., Bao, F., Savini, G., Calossi, A., Chen, H., Li, X., Wang, Q. (2015). Efficacy and acceptability of orthokeratology for slowing myopic progression in children: a systematic review and meta-analysis. *J Ophthalmol.* 2015:360806. doi:10.1155/2015/360806.

Wertheim, H. F., Melles, D. C., Vos, M. C., van Leeuwen, W., van Belkum, A., Verbrugh, H. A., Nouwen, J. L. (2005). The role of nasal carriage in Staphylococcus aureus infections. *Lancet Infect Dis.* 5: 751–762.

Wiffen, S. J., Hodge, D. O., Bourne, W. M. (2000). The effect of contact lens wear on the central and peripheral corneal endothelium. *Cornea.* 19: 47–51.

Williams, K. M., Hysi, P. G., Nag, A., Yonova-Doing, E., Venturini, C., Hammond, C. J. (2013). Age of myopia onset in a British population-based twin cohort. *Ophthalmic Physiol Opt.* 33: 339–345.

Wolffsohn, J. S., Calossi, A., Cho, P., Gifford, K., Jones, L., Li, M., Lipener, C., Logan, N. S., Malet, F., Matos, S., Meijome, J. M., Nichols, J. J., Orr, J. B., Santodomingo-Rubido, J., Schaefer, T., Thite, N., van der Worp, E., Zvirgzdina, M. (2016). Global trends in myopia management attitudes and strategies in clinical practice. *Cont Lens Anterior Eye*. 39: 106–116.

Wong, Y. L., Saw, S. M. (2016). Epidemiology of Pathologic Myopia in Asia and Worldwide. *Asia Pac J Ophthalmol (Phila).* 5: 394–402.

Xiong, S., Sankaridurg, P., Naduvilath, T., Zang, J., Zou, H., Zhu, J., Lv, M., He, X., Xu, X. (2017). Time spent in outdoor activities in relation to myopia prevention and control: a meta-analysis and systematic review. *Acta Ophthalmol.* 95: 551–566.

Xu, L., Wang, Y., Wang, S., Wang, Y., Jonas, J. B. (2007). High myopia and glaucoma susceptibility the Beijing Eye Study. *Ophthalmology.* 114: 216–220.

Yamamoto, Y., Ugai, K., Takahashi, Y. (2005). Efficiency of hand drying for removing bacteria from washed hands: comparison of paper towel drying with warm air drying. *Infect Control Hosp Epidemiol.* 26: 316–320.

Yang, X., Xu, L., Zhong, F., Zhu, Y. (2012). Data mining-based detection of acupuncture treatment on juvenile myopia. *J Tradit Chin Med.* 32: 372–376.

Yoon, J. H., Swarbrick, H. A. (2013). Posterior corneal shape changes in myopic overnight orthokeratology. *Optom Vis Sci.* 90: 196–204.

Yoon, K. C., Mun, G. H., Kim, S. D., Kim, S. H., Kim, C. Y., Park, K. H., Park, Y. J., Baek, S. H., Song, S. J., Shin, J. P., Yang, S. W., Yu, S. Y., Lee, J. S., Lim, K. H., Park, H. J., Pyo, E. Y., Yang, J. E., Kim, Y. T., Oh, K. W., Kang, S. W. (2011). Prevalence of eye diseases in South Korea: data from the Korea National Health and Nutrition Examination Survey 2008-2009. *Korean J Ophthalmol.* 25: 421–433.

Young, A. L., Leung, K. S., Tsim, N,, Hui, M., Jhanji, V. (2013). Risk factors, microbiological profile, and treatment outcomes of pediatric microbial keratitis in a tertiary care hospital in Hong Kong. *Am J Ophthalmol.* 156: 1040–1044.

Yung, A. M., Cho, P., Yap, M. (2005). A market survey of contact lens practice in Hong Kong. *Clin Exp Optom.* 88: 165–175.

Zhang, M., O'Donoghue, M. M., Ito, T., Hiramatsu, K., Boost, M. V. (2011). Prevalence of antiseptic-resistance genes in Staphylococcus aureus and coagulase-negative staphylococci colonising nurses and the general population in Hong Kong. *J Hosp Infect.* 78: 113–117.

Zhao, J., Pan, X., Sui, R., Munoz, S. R., Sperduto, R. D., Ellwein, L. B. (2000). Refractive Error Study in Children: results from Shunyi District, China. *Am J Ophthalmol.* 129: 427–435.

Zheng, T., Le, Q., Hong, J., Xu, J. (2016). Comparison of human corneal cell density by age and corneal location: an in vivo confocal microscopy study. *BMC Ophthalmol.* 16:109. doi:10.1186/s12886-016-0290-5

Zhong, X., Chen, X., Xie, R. Z., Yang, J., Li, S., Yang, X., Gong, X. (2009). Differences between overnight and long-term wear of orthokeratology contact lenses in corneal contour, thickness, and cell density. *Cornea.* 28: 271–279.

Zhong, Y., Chen, Z., Xue, F., Miao, H., Zhou, X. (2015). Central and peripheral corneal power change in myopic orthokeratology and its relationship with 2-year axial length change. *Invest Ophthalmol Vis Sci.* 56: 4514–4519.

Zhu, M. J., Feng, H. Y., He, X. G., Zou, H. D., Zhu, J. F. (2014). The control effect of orthokeratology on axial length elongation in Chinese children with myopia. *BMC Ophthalmology.* 14:141. doi:10.1186/1471-2415-14-141.