In vivo Slit Scanning Confocal Microscopic Observation in a Patient with Keratoconus Wearing Miniscleral Contact Lens for One Year: A Case Report

(Semerhan Pengimbas Celah Konfokus Mikroskop secara in vivo pada Pesakit dengan Keratokonus menggunakan Kanta Lekap Sklera Mini untuk Satu Tahun: Suatu Laporan Kes)

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ABSTRACT

The purpose of the present case report was to evaluate corneal microstructure in a patient with keratoconus wearing miniscleral contact lens (MS CL) for 1 year. A 38 years old patient diagnosed with stage III keratoconus in right eye and corneal graft (penetrating keratoplasty) in left eye was referred to optometry department of a public hospital in Malaysia. Based on the MS CLs fitting performance on the patient’s eye, MS CLs were prescribed in both eyes. However, patient opted for MS CL only in the right eye. In vivo slit scanning confocal microscopy was performed at baseline and after 1 year of contact lens wear in right eye. Qualitative analysis was done using a grading scale designed by Hollingsworth et al. (2005a), while quantitative analysis was performed using NAVIS software in a fixed frame of 0.06 mm². After 1 year of MS CL wear, qualitative observation of right cornea showed no change in stromal haze and architecture of nerve fibers. However, morphological alterations like dark bands were observed both in anterior and posterior stroma along with tendency of clustering keratocyte nuclei in posterior stroma. Quantitative analysis showed that anterior and posterior stroma keratocyte density and endothelium cell density were relatively low after 1 year of MS CL wear (817.3, 667.2, and 2577 cells/mm², respectively) compared to baseline observation (850.7, 705.0, and 2666 cells/mm², respectively). Mean cell area of anterior stroma, posterior stroma and endothelium were also different in post MS CL wear. Polymegathism and pleomorphism calculated by NAVIS software were varied after 1 year (49.6% and 37.5%, respectively) compared to baseline (67.1% and 29.6%, respectively). In the discussed case, noticeable changes were observed in corneal microstructure after 1 year of MS CL wear. Thereby, in conclusion we recommend future studies with more number of keratoconic eyes wearing MS CLs to emphasize the findings on microstructural changes in keratoconic cornea wearing MS CLs.

Keywords: Confocal microscope; corneal microstructure; keratoconus; miniscleral lens

ABSTRAK

Laporan kes ini adalah bertujuan untuk mengkaji struktur mikro kornea pesakit keratokonus yang memakai kanta lekap sklera mini (MS CL) selama 1 tahun. Seorang pesakit berumur 38 tahun telah didiagnosis dengan keratokonus tahap III pada mata kanan dan graf kornea (penetrating keratoplasty) pada mata kiri telah dirujuk ke Jabatan Optometri di hospital awam di Malaysia. Berdasarkan prestasi MS CL di atas mata pesakit beliau telah dipakai dengan MS CL pada kedua-dua matanya. Walau bagaimanapun, pesakit memilih untuk memakai MS CL hanya pada mata kanan sahaja. Pengimbas celah konfokus mikroskop in vivo telah dijalankan pada garis dasar dan selepas setahun memakai kanta lekap pada mata kanan. Analisis kualitatif telah dilakukan dengan menggunakan skala penggredan rekaan Hollingsworth et al. (2005a), sementara analisis kuantitatif dilakukan dengan menggunakan perisian NAVIS yang menggunakan bingkai tetap 0.06 mm². Selepas 1 tahun memakai MS CL, kornea kanan secara kualitatif menunjukkan tiada perubahan pada kekabutan stroma, lapisan Bowman dan rangkaian corak saraf. Walau bagaimanapun, perubahan morfologi seperti jatir gelap telah kelihatan di anterior dan posterior stroma bersama dengan pengumpulan nukleus keratosit di posterior stroma. Analisis kuantitatif menunjukkan keamatan keratosit di stroma anterior dan posterior dan sel endotelium adalah rendah secara relatif selepas setahun memakai MS CL (817.3, 667.2 dan 2577 cel/mm²) berbanding dengan nilai garis dasar (850.7, 705.0 dan 2666 cel/mm²). Luas sel purata stroma anterior, stroma posterior dan endotelium didapati berbeza selepas memakai MS CL. Polimegatisme dan pleomorfisme dihitung menggunakan perisian NAVIS selepas 1 tahun (49.6% dan 37.5%) menunjukkan penurunan berbanding dengan garis data (67.1% dan 29.6%). Seperti yang dibincangkan, perubahan ketara didapati pada struktur mikro kornea selepas setahun memakai MS CL. Sebagai kesimpulannya kami mengesyorkan kajian lanjut dengan mengambil lebih banyak mata keratokonus untuk dipasang dengan MS CL dan ini boleh menguatkan hasil penemuan perubahan struktur mikro mata keratokonus.

Kata kunci: Kanta sklera mini; keratokonus; konfokus mikroskop; struktur mikro kornea
INTRODUCTION

Keratoconus is a non-inflammatory progressive disease. It adversely affects the corneal cell morphology (Rabinowitz et al. 1998). The management of keratoconus is done with spectacle, contact lens and surgical corrections based on the severity of the disease (Jhanji et al. 2011; Romero-Jimenez et al. 2010). Contact lenses are widely used in most of the patients as a primary treatment option in keratoconus. Several types of contact lenses are available for management of keratoconus such as rigid gas-permeable (RGP), soft, hybrid, piggyback, miniscleral, and scleral contact lenses. However, researches have reported that the prolong use of contact lenses alter the underlying corneal cell morphology in normal healthy as well as keratoconic corneas (Bitirgen et al. 2013; Efron 2007; Ghosh et al. 2017a; Yeniad et al. 2010). The morphological changes in keratoconic cornea with different types of contact lenses are rarely being observed. Confocal microscope plays the key role in evaluating the cellular morphology changes in keratoconic cornea (Efron & Hollingsworth 2008; Erie et al. 2002; Ghosh et al. 2017b; Ucakhan et al. 2006). The purpose of the present case study was to observe the corneal microstructure in a patient with keratoconus wearing miniscleral (MS) contact lens for 1 year.

CASE PRESENTATION

A 38 year old Indian male was referred to optometry clinic from ophthalmology department for the contact lens management with complaints of blurry vision in both eyes even with present spectacle. The patient was diagnosed with keratoconus in both eyes 12 years back. However, penetrating keratoplasty was done in left eye 5 years back. The patient had no history of any systemic disease, ocular infection or ocular trauma. On examination, the best corrected visual acuity with spectacle was 6/60 in the right eye and 6/36 in the left eye using Snellen visual acuity chart. The refractive power in the right and the left eye was -8.00 DS (Dioptre spherical) with -7.00 DC (Dioptre cylindrical) at 180 degree and +2.00 DS with -7.00 DC at 20 degree, respectively. On Slit lamp biomicroscopy, signs like Flesher ring, prominent corneal nerves, Munson’s sign and Vogt’s straie were observed in the right eye. Left eye showed an optically clear graft with better view of anterior segment and was graded as grade 4 following the corneal graft grading of Sihota et al. (1998). Corneal topography (Pentacam, Oculus, Optikgerate GmbH, Wetzlar, Germany) showed the central and paracentral steepening of the cornea in the right eye and steepening of central cornea in the left eye (Figure 1). The simulated keratometric readings, were K1/K2= 56.5D @ 3.1 / 63.5 D @ 93.1 and K1/K2 = 49.1 D @ 121.6 / 40.8 D (Dioptre) @ 31.6 in the right eye and left eye, respectively. Induced astigmatism detected by the corneal topography was 6.90 D in right eye and 8.30 D in left eye, respectively. Corneal thickness was 389 µm and 506 µm in the right eye and left eye, respectively, as measured with corneal topographer. The severity of the keratoconus of the patient was graded as stage 3 in the right eye based on the Amsler-Krumeich classification (Alio & Shahyek 2006; Krumeich et al. 1998).

The contact lens used for this study was KATT miniscleral contact lens (Capricornia Contact Lens Pty Ltd., Brisbane, Queensland, Australia). The diameter of KATT is 16.50 mm. The lens has got 4 distinct zones:
A spherical or aspheric central Optic Zone (10.00 mm), a Peripheral Clearance Zone (T1), a Limbal Clearance Zone (T2), and the final Peripheral Curve which starts at the sclera at a diameter of 15 mm. The material of KATT is Boston XO2 which is a high Dk material (Dk141). The fitting philosophy of KATT is based on the matching the sagittal depth of the eye with an apical clearance (John Mountford, KATT Fitting Instructions (2011), http://capcl.com.au/custom-rgp-designs). In both the eyes, MS CL vault was more than 400 µm with 80 to 100 microns of clearance at the limbus. MS CL rested on the sclera comfortably. Based on MS CLs fitting performance on the eyes, the MS CLs were prescribed in both eyes. The parameters of the MS CL were KATT 3 / 16.5 mm diameter / -11.75 D in right eye and KATT 1 / 16.5 mm diameter / -12.00 D in the left eye. The vision was improved by 6/9 and 6/6 with MS CLs for the right eye and left eye, respectively. The care and maintenance were explained to the patient. However, patient opted contact lens for the right eye only. Therefore, left eye was not considered for the study as operated and without contact lens.

After 1 year, the visual acuity was 6/9 in right with MS CL and 6/60 in left eye with spectacle lens. However, there was no change in MS CL fittings on patient’s eye. In vivo slit scanning confocal microscopy (ConfoScan4; Nidek Technologies Srl, Albignasego, Italy) was carried out to evaluate the corneal microstructure at baseline and after 1 year of contact lens wear for the right eye. The non-aplanatic water immersion 40X objective lens with a numerical aperture of 0.75 and corneal full thickness scan were used in the study. Standard operative procedure was strictly followed to carry out the confocal microscopy. Throughout the examination, a total of 350 images of all corneal layers were captured for right eye. Best three images of each corneal layer were taken for qualitative and quantitative investigation. In qualitative analysis, corneal edema was graded using the grading scale designed by Efron et al. (2002). Furthermore, stromal haze was graded based on the grading scale designed by Hollingsworth et al. (2005a) (Table 1). Quantitative analysis was performed using NAVIS (Nidek Advanced Visual Information System) software to analyse the cell area and density of corneal layers in a fixed frame of 0.06 mm². The stromal layers and endothelium were analysed using semi-automated and fully automated method, respectively.

| Grade | Severity | Description |
|-------|----------|-------------|
| 0     | Normal   | Keratocyte nuclei clearly defined. Accurate analysis possible in all cases |
| 1     | Trace    | Keratocyte nuclei visible; some background haze. Accurate analysis possible in most cases |
| 2     | Mild     | Some keratocyte nuclei visible; many keratocytes partially obscured by haze. Accurate analysis possible in some cases |
| 3     | Moderate | Keratocytes almost completely obscured by haze. Accurate analysis possible in few cases |
| 4     | Severe   | Keratocytes completely obscured by haze; extreme levels of hyper-reflectivity. Accurate analysis not possible |

Source: Hollingsworth and Efron (2005)

**CONFOCAL MICROSCOPIC OBSERVATION**

In the right eye, images of superficial, wing, basal epithelial cells, Bowman’s layer and Descemet’s membrane were captured with poor visibility at baseline and after 1 year of contact lens wear. Therefore, both qualitative and quantitative analysis was not performed to avoid the improper analysis. However, stroma and endothelium were visible clearly and analysis was performed at baseline and after 1 year of MS lens wear.

**Qualitative Observation**

At baseline examination, oedema of corneal layers including anterior and posterior stroma and endothelium...
of right eye was observed as grade I (1-4%). After one year of MS CL wear in right eye, oedema did not increase in any of the corneal layers. Furthermore, the anterior stroma and posterior stroma exhibited grade 2 haze and grade 1 haze, respectively. While, after 1 year of MS CL wear the stromal haze remain same as baseline. No alterations in corneal morphology were observed at baseline in anterior and posterior stroma. However, after 1 year of MS CL wear faint, thin, short and discontinuous dark bands were observed in anterior stroma (Figure 2) and prominent, thick, long and continuous dark bands were observed in posterior stroma (Figure 3). Tendency of clustering keratocyte nuclei were observed in posterior stroma after 1 year of MS lens wear (Figure 4).

FIGURE 2. A) Baseline anterior stroma and B) Thin dark bands observed in anterior stroma after 1 year of MS lens wear viewed through confocal microscope

FIGURE 3. A) Baseline posterior stroma and B) Thick dark bands observed in posterior stroma after 1 year of MS lens wear viewed through confocal microscope
Quantitative observation

The keratocyte density of anterior and posterior stroma were less after 1 year of MS CL wear (817.3 cells/mm² and 667.2 cells/mm², respectively) compared with baseline observation (850.7 cells/mm² and 705.0 cells/mm², respectively). The endothelial cell density was also less after 1 year of MS lens wear (2577 cells/mm²) compared with baseline observation (2666 cells/mm²). The mean cell area of anterior stroma, posterior stroma and endothelium were also different in post MS CL wear from baseline, as shown in Table 2. The polymegathism and pleomorphism calculated by the Nidek Advanced Visual Information System (NAVIS) software (Version 3.6.6, Nidek Technologies) were relatively increased after 1 year of MS CL wear (67.1% and 37.5%, respectively) compared with baseline observation (49.6% and 29.6%, respectively).

| Corneal layers                          | Baseline | After contact lens wear | Change of cell density (%) |
|-----------------------------------------|----------|-------------------------|---------------------------|
| Anterior stroma keratocyte cell density (cells/mm²) | 850.7    | 817.3                   | -3.93%                    |
| Anterior stroma cell area (µm²)         | 1175.6   | 1223.5                  | 4.07%                     |
| Posterior stroma keratocyte cell density (cells/mm²) | 705.0    | 667.2                   | -5.37%                    |
| Posterior stroma cell area (µm²)        | 1427.5   | 1498.8                  | 4.99%                     |
| Endothelial cell density (cells/mm²)    | 2666     | 2577                    | -3.34%                    |
| Endothelial cell area (µm²)             | 375.1    | 388.0                   | 3.43%                     |
**DISCUSSION**

Contact lens wear is reported to alter the morphology of normal corneas (Efron 2007). While morphological alteration in keratoconic corneas are of research interest for past two decades (Efron & Hollingsworth 2008; Efron et al. 2002; Erie et al. 2002; Ghosh et al. 2017a; Hollingsworth et al. 2005a), literature on cellular alterations in keratoconic cornea post contact lens wear remain Limited. To the best of our knowledge, no previous study reported alterations of corneal cell morphology after scleral and mini scleral lenses. In the present case study, confocal microscopic evaluation showed that the various qualitative and quantitative parameters of corneal cell morphology altered after 1 year of MS CL wear in a keratoconic eye. In the study eye, tendency of accumulation of keratocyte nuclei was observed in posterior stroma after 1 year of MS CL wear. Similar findings were reported by Ghosh et al. (2017b) in keratoconic eyes after one year of RGP CL wear. The reason behind accumulation of cells could be because of the break in Bowman’s layer (Hollingsworth et al. 2005b; Savaguchi et al. 1998). The break in Bowman’s layer is probably due to the microtrauma caused by the contact lens wear in keratoconic cornea.

Furthermore, in the present case study, dark bands were only observed in anterior stroma and posterior stroma after 1 year of MS CL wear. Dark bands reported as Vogt’s striae in posterior stroma are often seen in keratoconic corneas (Ghosh et al. 2017b; Hollingsworth & Efron 2005; Somodi et al. 1996; Weed et al. 2007). Abnormal parting of collagen fibrils within stromal lamellae is thought to be the reason (Hollingsworth & Efron 2005; Weed et al. 2007) behind dark bands. Hayes et al. (2007) have reported that the alteration of collagen fibrils in stromal lamellae is related with the progression of the disease whereas Efron et al. (2002) have reported that the folds occur in the posterior stroma when the level of corneal oedema is more than 5%. In the present study, neither significant increase in oedema was observed post lens wear nor there was any confirmatory sign of disease progression as the visual acuity remained same during evaluation after 1 year of contact lens wear. Another recent theory behind stromal fold is related to biomechanical and viscoelastic properties of cornea. In a recent study, while assessing 118 human corneas using both *in vivo* and ex vivo imaging techniques with different hydration and pressure, Grieve et al. (2017) have hypothesized association between striae and corneal elasticity. Besides, CL wear is proved to change the viscoelastic and biomechanical properties of cornea (Radaie-Moghadam et al. 2016). Probably, MS CL wear for 1 year had some effect of the viscoelastic and biomechanical properties of the study cornea leading to appearance of the dark bands in both anterior and posterior stroma. However, further research has to be conducted for a proper explanation of the visibility of dark bands in the keratoconic cornea after CL wear.

Furthermore, in the study eye, noticeable amount of stromal haze was present in both pre and post MS CL wear. Several previous studies reported stromal haze in keratoconic cornea (Efron & Hollingsworth 2008; Hollingsworth et al. 2005b; Uçakhan et al. 2006). Ghosh et al. (2017) have reported about the increased stromal haze after 1 year of RGP contact lens wear in keratoconic cornea. However, in the present study there was no change in the haze after 1 year of MS CL wear in the keratoconic study eye. This could be because of the fact that RGP and MS CL might show different interaction with cornea for their different design and fitting philosophy.

In the quantitative evaluation of corneal cellular morphology, loss of stromal keratocyte density in keratoconus patients wearing contact lenses have been reported in many studies (Bitirgen et al. 2013; Edmonds et al. 2004; Erie et al. 2002; Ghosh et al. 2017a; Yenaid et al. 2010). In the present study, anterior and posterior stromal keratocyte density was 3.93% and 5.37% less in post contact lens wear compared with baseline. Reason behind the keratocyte cell loss may be associated with release of cytokines in pathological process of the disease that causes apoptosis of keratocyte (Bitirgen et al. 2015). Studies have also reported that the mechanical injuries due to eye rubbing or contact lens wear in epithelium releases interleukin-6 (IL-6) that also causes apoptosis of keratocyte (Bureau et al. 1993; Wilson et al. 1996). Erie et al. (2002) and Uçakhan et al. (2006) have reported that the contact lens wear in keratoconic cornea causes micro-trauma in corneal epithelium that results in release of apoptotic cytokines which causes apoptosis of keratocyte. In the present study, although contact lens used was of bigger diameter (16.5 mm) MS CL which rested on the sclera with apical clearance fitting technique, had minimum interaction with cornea, probably still induced some micro trauma to cornea that eventually resulted into cellular changes in stroma.

Furthermore, in the present study, percentage of polymegathism and pleomorphism were different between pre and post MS CL wear. Endothelial cell density was lower after 1 year of MS CL wear compared with baseline. Similar finding were reported by Ghosh et al. (2017) with 1 year of RGP contact lens wear. Contact lens wear have been reported to alter the hexagonality of the endothelial cells. The reason behind the endothelial cell loss is not yet completely understood. However, Kaldawy et al. (2002) have stated that the apoptosis is the only process of cell death in keratoconus. Therefore, in the present study, contact lens induced micro trauma or mechanical stress causing apoptosis could have been the reason behind the changes endothelial cell morphology observed in the keratoconic eye.

**CONCLUSION AND RECOMMENDATION**

In the discussed case, noticeable changes were observed in corneal microstructure after 1 year of MS CL wear.
Furthermore, when compared with previous researches on keratoconus wearing RGP contact lenses, cellular changes were almost similar in present case. However, interaction of different contact lenses with keratoconic corneas is expected to vary depending on the contact lens design and parameters which may lead to different morphological states of cornea. Thereby, in conclusion, we recommend future studies with more number of keratoconic eyes wearing MS CL with suitable control group to investigate the morphological changes with MS CL on Keratoconus and to further compare these morphological alterations with other types of contact lenses worn corneas.

ACKNOWLEDGEMENT

I express my deepest gratitude to Dr. Shamala Retnasabapathy and Ms Erni Fadhilah Norazmi Ophthalmology Department of Hospital Sungai Buloh, Malaysia for the guidance and support.

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Received: 25 October 2018
Accepted: 6 December 2019