

Impression cytology in rigid gas permeable contact lens wearers

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ABSTRACT

Background: Impression cytology is a technique used to evaluate alterations on conjunctival surface cells. However, the method is not popular among eye care practitioners and thus limited data is available in the literature.

Aims and Objectives: This study evaluates changes in the tarsal conjunctival cells following continuous wear rigid gas permeable contact lens (CWRGP) using impression cytology technique.

Methods: Twenty subjects aged between 19 to 23 years were fitted with CWRGP. Eleven (55%) of them succeeded in wearing the lens continuously till the third month. Thirty non-lens wearing subjects were recruited as controls. Impression cytology was performed at baseline and after 3 months of wearing CWRGP. The cytological changes were graded according to Saini Grading Scale.

Results: All 11 RGP wearing subjects showed Saini Grade 1 staining at baseline. At 3 months, 10 (91%) of them showed features of grade 1 and 1 demonstrated of grade 2 features with inflammatory cells.. Twenty-nine (29, 97%) of control subjects showed grade 1 at baseline and 3 months. No significant difference was found in the appearance of conjunctival cells between both groups at visit 1 and the 3 month visit (Fisher's exact test, $p = 0.470$).

Conclusion: The wearing of CWRGP for 3 months did not cause notable changes in the tarsal conjunctiva. Impression cytology should be considered as a diagnostic tool to investigate alterations on ocular surface and to improve the management of contact lens patients.

Keywords: contact lens, conjunctiva, tarsal, impression cytology

INTRODUCTION

Conjunctiva impression cytology is one of the most practical, fast, safe and economical method to examine the changes of conjunctiva cells. The method involves the process of obtaining superficial layer of the ocular surface especially the epithelial layers by applying cellulose acetate paper to the conjunctival surface. Various analyses can be conducted on the collected cells such as molecular, histological and immunohistological analysis for further investigation. Impression cytology was used earlier to study changes in the conjunctival epithelium in several diseases of the ocular surface including those induced by contact lenses (1, 2). The comparison between conjunctival biopsy findings and impression cytology confirm that impression cytology provides the same information as that of biopsy for diagnosis of dry eye syndrome (3).

Previous studies reported that symptomatic and asymptomatic contact lens wearers showed changes in conjunctival cytology (2, 4, 5, 6). These changes include reduction nucleus to cytoplasm ratio, increased epithelial cell sizes, decreased in goblet cells and the presence of snake-like chromatin. In a recent study, it was reported that the prevalence and severity of cytological alteration was higher among symptomatic contact lens wearers but severe changes were also noted among asymptomatic soft contact lens wearers (6). Their results also showed insignificant difference in the severity of cytological alteration in rigid gas permeable (RGP) and soft contact lens wearing eyes within 6 months of study and that the severity was found to change with duration of contact lens wear. The authors hypothesized that mechanical irritation by contact lens may be the cause of goblet cell damage thus leading to symptoms. However, another research study reported that the conjunctiva of asymptomatic contact lens wearers was histologically normal in spite of many claimed that contact lens can induced epithelial cell damage (4, 7). Therefore, mechanical stimulation is an unlikely explanation.

This study was planned to examine the changes in the upper tarsal conjunctiva in new continuous gas-permeable contact lens (CWRGP) wearers using impression cytology procedure. Due to the unpopularity of the technique, limited data in this area is available in the literature. This study used a relatively new CWRGP available in South-East Asian market (Menicon Z-alpha from Menicon Co. Ltd, Japan). This continuous wear hyper Dk (163) rigid gas permeable contact lens was approved by the United States Food and Drugs Administration (FDA) for 30 days continuous wear and has been shown to provide safe and full-time vision correction alternative for patients who can adapt to daily RGP wear (8). Studies on clinical performance showed the changes in papillary conjunctiva were less in wearers of Menicon Z-alpha RGP hyper Dk lens than those in Focus Night & Day soft hyper Dk continuous wear lens (9) and that no microbial keratitis was reported during a 24 months study (8).

To the best of our knowledge, there is no reported data available on subjects wearing CWRGP using impression cytology methods. It is possible that CWRGP contact lens may cause some alterations to the tarsal conjunctival cells due to mechanical irritation. This study evaluates changes in the tarsal conjunctival cells of young adults before and after wearing CWRGP for 3 months.

METHODOLOGY

A group of 20 Universiti Kebangsaan Malaysia (UKM) students aged between 19 to 23 years were recruited based on purposive sampling for a clinical trial study. They were selected based on the pre-set criteria: never worn contact lenses or had stopped wearing contact lenses for at least 6 months before participating in this study, myopes with refractive error of not more than 5.50DS and astigmatism of not more than 1.25DC and able to attain visual acuity of 6/6 at distance and N5 at near after correction. The myopia criterion of 5.50DS was adopted for this study to reduce complications that could arise from higher myopia which could have pathological implications (10). No history of systemic and ocular disease that requires medication that would contra-indicate contact lens wear. A total of 30 non-wearing subjects with same pre-set criteria were selected as a control group.

Informed consent was obtained from each of the subject and prior ethical approval was obtained from the Medical Ethics Committee of UKM and adhered to the tenets of the Declaration of Helsinki (UKM 1.5.3.5/244/PPP2). The selected subjects were prescribed with Menicon Z-alpha RGP continuous wear lens and requested to continuously wear the lens for at least four to five nights in a week, after one week of adaptation. Subjects were asked to report for any adverse events such as discomfort, dryness, blurring of vision, variable vision, itching, photophobia and secretion during the follow up examination. Follow up examinations were conducted at 24 hours, 1 month and 3 months after continuous contact lens wear. However, samples of conjunctiva cells were collected only at baseline and after 3 months of wearing the lenses using impression cytology.

Subjects were prescribed Menicon O₂[®] care multipurpose solution (Menicon Co. Ltd, Japan) for cleaning and disinfection of the contact lens and rewetting eye drops (Boston, USA) for instillation before sleeping at night time and upon awakening to manage the possibility of lens adherence.

Impression cytology

The subjects were educated regarding the procedure before taking the samples. Only samples from the right eye were used in this study. The eye was anaesthetized with 1% amethocaine and lid everted. Small cellulose acetate papers (Sartorius AG, Germany 11200-25-150-N) with size 3 x 4mm were pressed for 3-5 seconds on the everted tarsal conjunctiva, and later massaged with the tip of a cotton bud. The cellulose acetate papers were then gently removed in a peeling motion with a blunt forcep which was earlier sterilized/cleaned with 70% ethyl alcohol. The above procedures were conducted by the first author.

The impression was immediately transferred to a clean glass slide by uniform and gentle pressure of a cotton bud. The slides were then fixed in 95% ethyl alcohol and stained with Giemsa, Hematoxylin-Eosin and Periodic Acid Schiff and mounted on slides cover-slipped using DPX. After the cells were appropriately stained, the slides were examined under light microscopy and were photo documented. The cytology was graded according to the classification of Saini Grading Scale (4). The grading scale was as follows:

Grade 1: small round epithelial cells with a nucleus to cytoplasm ratio of 1:2. Large number of deeply stained goblet cells (PAS positive). A good confluent sheet of cells was usually obtained.

Grade 2: a good cell sheet consisting of larger polygonal epithelial cells with decreased nucleus to cytoplasm ratio 1:3. Goblet cells were slightly reduced in number but still deeply stained with PAS.

Grade 3: larger polygonal epithelial cells with a further decrease in nucleus to cytoplasm ratio. A reduced number of goblet cells, often with reduced staining.

Grade 4: larger polygonal epithelial basophilic cells with pyknotic nuclei. Intracellular keratin often demonstrable. Absent goblet cells. Often only few loose clumps of cells were obtainable.

RESULTS

Symptoms

After 3 months of contact lens wear, only 11 (55%) subjects with mean age 21.36 ± 1.21 years succeeded in wearing the lenses continuously for at least 4 nights in a week. The mean age for control group was 22.07 ± 1.41 years. There was no significant difference in mean age between the two groups (Mann Whitney, $p=0.075$). Nine (45%) of the subjects had stopped wearing the lenses due to non-compliance and intolerance to RGP continuous wear lenses.

The majority of the subjects (82%) did not complain of any adverse encounters except for lens adhesion in 2 subjects during first month of contact lens wear. Changing their rewetting drops to that of a non-preserved type (Alcon, USA) and reevaluating their handling techniques successfully managed the problems. None of the subjects used artificial tears during the study. None of the subjects presented with corneal keratitis or ischemia. At the end of 3 months, all subjects adapted well to the contact lens wear and claimed that they had no symptoms of discomfort, dryness, blurring of vision, variable vision, itching, photophobia and secretion.

Impression cytology

Samples of conjunctival cells were collected at baseline and the end of 3 months of contact lens wear. All the 11 subjects showed Saini's Grade 1 at the baseline. After 3 months of RGP contact lens wear, the results showed that 10 (91%) subjects had the features of Grade 1 (small round epithelial cells with a nucleus to cytoplasm ratio 1:2), with large number of deeply stained goblet cells and a good confluent sheet of cells was obtained at baseline based on Saini Grading Scale (4). One (9%) subject showed the feature of Grade 2 with the presence of inflammatory cells. In control group, 29 (97%) subjects showed Grade 1 at baseline and 3 months except for one (3%) subject who showed the presence of Grade 2. There was no significant difference in the appearance of conjunctival cells between the RGP continuous wearers and control group (Fisher's exact test, $p = 0.470$).

Figure 1 showed an example of a good confluent sheet of conjunctival cells stained with hematoxylin-eosin in one of the subject. An asymptomatic subject showed the presence of inflammatory cells (neutrophil) after 3 months of wearing the lenses. Inflammatory cells can be easily detected with the used of giemsa stain (Figure 2).

DISCUSSION

Impression cytology has been used widely to evaluate several ocular surface disorders, monitoring the effect of treatment such as contact lens wear and documenting sequential changes in the conjunctival and corneal surface over time (1, 11-13). This procedure can also be used to detect increasing squamous metaplasia and identify those at imminent risk of developing symptoms (14). The technique is simple, non-invasive and can be repeated a number of times in an individual (6). Multiple cell samples can be obtained in one time procedure with minimal discomfort to the patients. It is an ideal procedure to perform in order to examine the changes of ocular surface when diagnosis cannot be ascertained due to normal clinical manifestation (2). Unfortunately, this technique has not gained popularity as a routine diagnostic tool in majority of the eye clinics due to the fact that it is more time consuming for both clinicians and pathologists.

The results of this study showed no significant difference in the appearance of conjunctival cells at baseline and after 3 months between the control group and Menicon Z-alpha RGP continuous wear subjects. With the exception of one subject, all the asymptomatic continuous RGP contact lens wearing eyes did not show any cytological alterations. We are aware that the number of subjects in this study was quite small to support any conclusions made from the observation. However, the observation is in accordance with the results of an earlier study which reported no abnormal cytological changes in the conjunctival cells of asymptomatic daily RGP contact lens wearers within 3-6 months of contact lens wear (6). Their results also showed that 33.4% of the asymptomatic soft contact lens wearing eyes showed abnormal conjunctiva impression cytology changes within the same period (6).

For symptomatic contact lens wearers, cytological changes were seen to occur within 3-6 months and the prevalence and severity of cytological alteration was found to be significantly greater in the symptomatic than asymptomatic contact lens wearers (6, 14). In this study we used contact lenses made of material with high oxygen permeability value (Dk 189) and also prescribed rewetting eye drops to all subjects which may help to alleviate the symptoms of lens intolerance during continuous wear of contact lens.

According to past literature, the Menicon Z (tisilfocon A) rigid gas-permeable (RGP) contact lens is a hyper Dk (163) lens material which provides safe continuous wear for a duration exceeding 7-day and 6-night standard (15). In this prospective study conducted for 3 months, no changes were observed in the severity of cytological alteration in continuous wear RGP contact lens wearing eyes. However, we were unable to make any conclusions from these observations due to the small number of subjects who successfully wore the contact lens continuously for 3 months. Further long term studies with larger sample size are needed in order to investigate the effect of different types and materials of contact lens on conjunctival cytology.

Interestingly, in recent times, impression cytology has proved to be very effective in the diagnosis of ocular surface diseases, better understanding of the pathophysiology of the diseases related to ocular surface and provides biomarkers which could be used as outcome measures (7). Impression cytology is a painless procedure that should be considered by contact lens practitioners to examine the changes in conjunctival surface as results of contact lens wear. The changes in the conjunctiva have been attributed to the presence of inflammatory cells (16).

Limitations of the study

Small number of subjects is the main limitation in this study. Majority of the subjects dropped out from the experiment due to difficulties in adapting to RGP contact lens wear. Future larger longitudinal studies should be administered to confirm our findings.

CONCLUSION

This study concludes that well adapted and asymptomatic continuous RGP wearers for 3 months show no adverse effects to their conjunctival epithelia as measured by impression cytology. This technique may prove useful in understanding ocular surface health and disease in contact lens wear.

List of abbreviations (if any)

CWRGP: Continuous wear rigid gas permeable contact lens

IRB permissions

This study was approved by the Medical ethics committee of Universiti Kebangsaan Malaysia. (UKM 1.5.3.5/244/PPP2).

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Competing interest

None to declare

Authors information

The first author of this manuscript is a PhD candidate at Universiti Kebangsaan Malaysia.

Authors' contributions

On behalf of all the authors, I certify that we have all participated sufficiently in the conception and design of this work and the interpretation of the data (when applicable), as well as in the writing of the manuscript, to take public responsibility for it. Neither this manuscript nor one with substantially similar content under our authorship has been published or is being considered for publication elsewhere.

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Conflict of interest

None to declare

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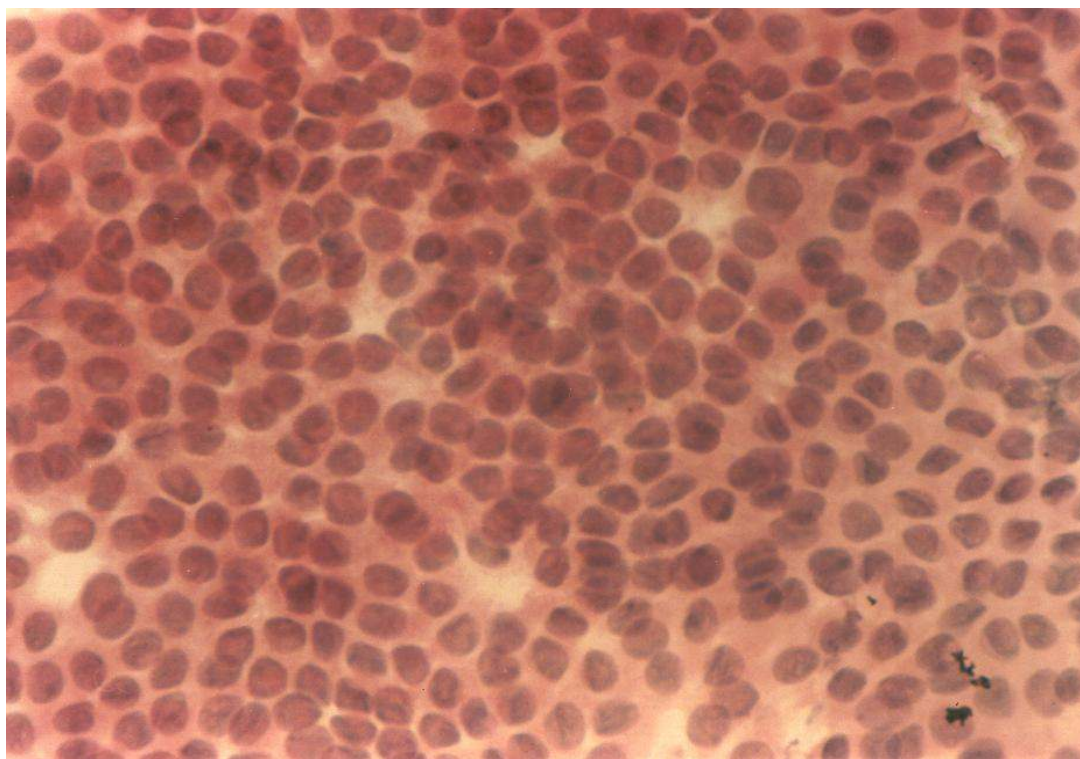


Figure 1: Good confluent sheet of conjunctival cells, Grade I (H&E x 400).

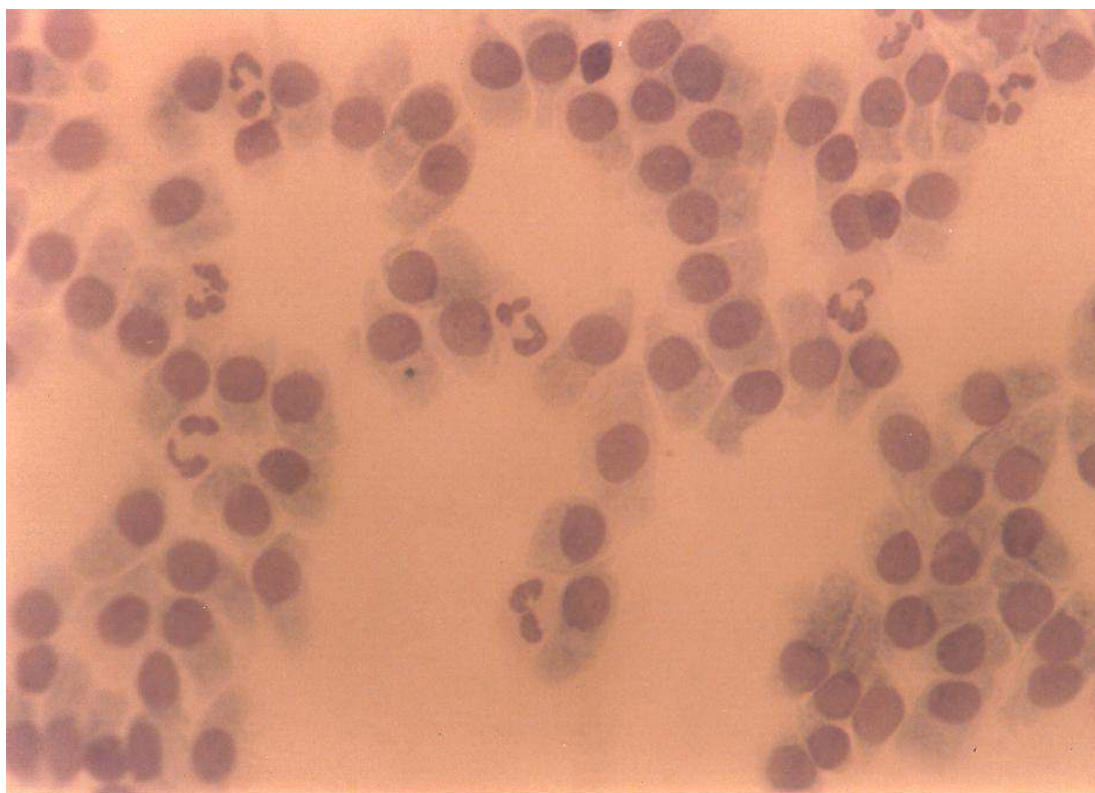


Figure 2: Inflammatory cells-neutrophil in one of the subjects (Giemsa x 400)