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ORIGINAL PAPER

Use of commercially available sodium hyaluronate 0.18% eye drops for corneal epithelial healing in diabetic patients

KietPhang Ling · Mae-Lynn Catherine Bastion

Abstract

Purpose To evaluate the effect of topical sodium hyaluronate (SH) 0.18% treatment on corneal epithelial healing after epithelial debridement in pars plana vitrectomy in diabetic patients.

Method This is prospective and randomized clinical trial. Our study population included 30 eyes undergoing pars plana vitrectomy that required near total corneal debridement intra-operatively for surgical view. We compared the residual wound and wound healing rate in between 3 groups: 10 diabetic eyes (DMV) on topical SH 0.18%; 10 diabetic eyes (DMC) and 10 non-diabetic eyes (NDM) not treated with topical SH 0.18%. The corneal epithelial wound was measured at 12, 24, 36, 48, 60, 72 and 120 h after the vitrectomy surgery.

Results DMC group had corneal wounds that reepithelialization significantly more slowly than in NDM and DMV groups at 12, 24, 36 and 48 h (Mann–Whitney test $p < 0.05$). The epithelial healing rate was significantly faster at 12 h in NDM and DMV group (Mann–Whitney test $p < 0.05$). No differences in the residual epithelial wound and wound healing were detected in between NDM and DMV groups. The mean for epithelial closure in DMC group was delayed 87.6 ± 28.31 h, compared with DMV group (64.8 ± 21.31) and NDM group (56.4 ± 9.88). All groups were followed up 1 month beyond completed wound closure. No recurrent corneal epithelial wound, corneal melting or corneal neovascularization was noted.

Conclusion Diabetic patients on SH 0.18% four times daily for epithelial defect had similar corneal wounds healing rate as non-diabetics. This treatment significantly improved corneal wound healing and accelerated complete corneal wound resurfacing in diabetic patients.

Keywords Sodium hyaluronate (SH) · Hyaluronan · Corneal epithelium · Wound healing · Vitrectomy · Diabetes

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Introduction

Vitreoretinal surgery may be associated with intra-operative corneal epithelial edema and endothelial decompensation in some cases [1]. The surgeon may elect to mechanically scrape away the epithelium with a blade in order to achieve better visualization of the retina if the corneal epithelium becomes hazy during the course of surgical intervention. These scraping results in a postoperative corneal epithelial defect, and

healing of such a lesion may be delayed, particularly in patients with diabetes [2]. Overall, the average corneal epithelial debridement rate among various surgeons in diabetic vitrectomies has been reported to be $17.4 \pm 19.0\%$ and to range from 0 to 90% [1]. Because of the increasing prevalence of diabetes in the general population and the associated increased incidence of eye disease requiring surgical intervention, it is important to investigate the treatment of corneal epithelial wound healing in patients with diabetes mellitus.

Sodium hyaluronate (SH) is a naturally occurring glycosaminoglycan of the extracellular matrix that plays an important role in development, wound healing and inflammation [3–6]. Its viscoelastic properties have rendered it ideally suitable for use in ophthalmic practice to protect the corneal endothelium and to maintain the anterior chamber depth during intraocular surgery [3, 7]. It has also been used in the treatment of dry eyes because of its long ocular surface residence time [8, 9].

Recent experiments in animals have shown that sodium hyaluronate promotes corneal epithelial wound healing by stimulating the migration, adhesion and proliferation of the corneal epithelium [3, 10–13]. The mechanism of action of sodium hyaluronate on these functions remains controversial. Human studies had been confined to the in vivo [14] and in vitro [4] where topical instillation of sodium hyaluronate drops in eyes with epithelial problems.

The effectiveness of sodium hyaluronate on corneal epithelial wound healing in patients has not been reported. In this study, we performed a prospective randomized study to determine the effectiveness of commercially available topical sodium hyaluronate 0.18% eye drops as a primary treatment for corneal epithelial defect in diabetic patients where epithelial debridement had been performed during pars plana vitrectomy.

Methodology

This is a prospective, randomized, comparative case–control hospital-based study involving patients with and without diabetes mellitus with postoperative corneal epithelial defect who fulfilled the inclusion criteria. In this study, 30 patients who underwent vitrectomy surgery with intra-operative corneal debridement causing near total corneal epithelial defect in Universiti Kebangsaan Malaysia Medical Centre were involved. It was conducted from February 2010 to May 2012. Patients who fulfilled the inclusion criteria were selected for this study. Randomized sampling method (draw paper from the box) was used to select from diabetic patients with topical SH 0.18% or without topical SH 0.18%. All non-diabetic patients who fulfilled the inclusion and exclusion criteria were treated with standard regimes without topical SH 0.18%.

Sample size was calculated using manual calculation from Snedecor and Cochran 1989 based on the study by Nakamura [12], the dependence variable is the healing rate (mm^2/h) and the non-diabetic group had a mean rate of reepithelialization of $0.61 \pm 0.02\ mm^2/h$ in comparison with the diabetic group with hyaluronan 0.1% ($0.65 \pm 0.02\ mm^2/h$). Comparison of the rates of wound healing between the non-diabetic and the diabetic group differed at the $p < 0.05$ level. Thus, sample size required in this study was minimal 9 subjects in each group. The inclusion criteria of this study were patients with almost total corneal epithelial defect after pars plana vitrectomy; age ranging from 40 to 80 year old; preoperative control blood sugar HbA1c < 10%; not known of ocular surface disease or previous corneal epithelial disease and no preexisting glaucoma or previous eye surgery. The exclusion criteria were patient with postoperative infection and patient with incomplete eyelid closure or pregnant patient.

Measurements of the area of corneal epithelial defect from the anterior segment photographs taken at regular intervals were done by using Quick Imaging Measurement Software (QIMS). This software was invented to calculate the area of polygon with regular and irregular shapes in photographic images, with error less than 1% [15]. All the photographs were taken with scale and uploaded in computer for analysis (Fig. 1).

All consecutive patients with corneal epithelial defect following pars plana vitrectomy operation in the UKMMC were recruited for this study. Intra-operatively, balanced salt solution was used as a corneal lubricant, and EIBOS (Erect Indirect Binocular Microscope; Haag-Streit Surgical) non-contact viewing system was used. All surgeries were performed by the same surgeon, and epithelial defect was unintentionally performed to obtain surgical view.

Fig. 1 We manually demarcate the epithelial defect (yellow line) with scale in the photograph and the size of the defect will be calculated by using Quick Imaging Measurement Software (QIMS)
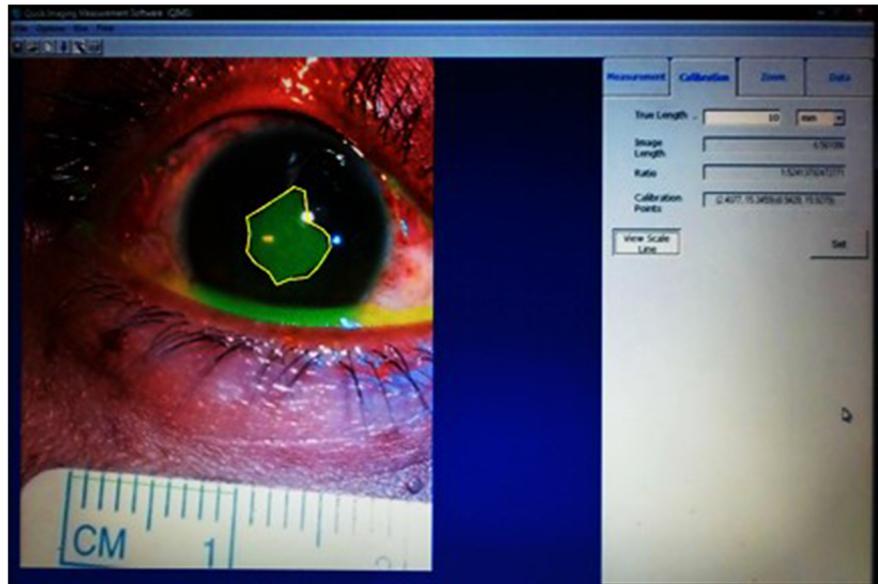
Postoperatively, eye patching was done in operation room and was removed after 2 h in the ward. Only patients who fulfilled the inclusion and exclusion criteria were included in this study. Those included met the following criteria: almost total epithelial defect after pars plana vitrectomy; age 40 to 80 years; preoperative HbA1c $<$ 10%; not known of ocular surface diseases, no pre-existing glaucoma and no previous ocular surgery. Patients with post-operative infection, incomplete eyelid closure and pregnant patients were excluded from this study. Purpose and details of the study were explained to the patients. Patients who were agreeable had to sign a consent form, and information of their demographic data, past medical history, ocular history and present ocular surgery was obtained. All the patients were divided into 3 groups. Group 1 and group 2 consisted of non-diabetic patients (NDM) and diabetic patients (DMC) who were treated without topical SH 0.18% eye drops. Group 3 were the diabetic patients (DMV) who were treated with topical SH 0.18% eye drops. Group 1 was the control group, and group 2 and 3 were the study group. Diabetic patients were randomly assigned using the simple random sample method. A piece of paper was drawn from a box to decide the treatment of sodium hyaluronate 0.18% for diabetic patient. All the study eyes received similar post-operative topical steroid (dexamethasone 0.1%) 2 hourly and topical antibiotic (ciprofloxacin 0.3%) 2 hourly. Some patients required topical anti-glaucoma (Timolol 0.5% and Alphagan P 0.15%) if there was high IOP, and some required oral acetaminophen 1000 mg for pain relief. For diabetic patients who were assigned to SH 0.18%, eye drops were given to the patients 6 hourly. The compound was given as a single drop to the central cornea of the study eye, with the lower eyelid held away from the eye to avoid overflow.

After obtaining patient consent, the study eye was stained with fluorescein to visualize the area of epithelial defect. The epithelial defect was photographed at baseline by using a CASIO digital camera EX-Z90 with magnification factor of 1.0 (Fig. 3). Serial photographs were taken at regular intervals at $t = 0$, $t = 12$, $t = 24$, $t = 36$, $t = 48$, $t = 60$ $t = 72$ h, $t = 120$ and day 5 ($t =$ hour from the first photograph). The topical SH 0.18% eye drops were instilled into the study eye 4 times daily at 6 a.m., 12 p.m., 6 p.m., and 12 a.m. by the ward staff nurses. The nurses were brief by the investigator for proper technique of eye drops instillation. The compliance of the eye drops was written in the medications chart when each eye drop was given. The SH 0.18% eye drops and photographs were stopped after the corneal epithelial defect complete closure during the examinations. All the patients were hospitalized until complete closure of corneal epithelial defect. All patients were followed up for 1 month, noting corneal surface and recurrence of epithelial defect.

Data were analyzed using Statistical Package for Social Science (SPSS) version 20.0. Kruskal–Wallis test was used for the nonparametricsamples to find the differences in all three groups. Mann–Whitney test was used to compare the two independent samples in between groups. Statistical significance (p) was defined as a probability of error equal to or less than 0.05. The approval from Research and Ethics Committee, Faculty of Medicine, University Kebangsaan Malaysia and Ministry of Health Malaysia, was obtained (Project code: FF-245-2010). The purpose of the study was explained to the patients in detail. Written consent was obtained from patients at study entry.

Results

The mean age of the study population was 61.2 ± 7.9 years. The youngest patient was 43 years old, and the oldest patient was 79 years old. The age among each group was similar and not statistically significant (Kruskal–Wallis test $p = 0.462$). There were 10 eyes in the non-diabetic group treated without SH (NDM group), 10 eyes in diabetic group treated without SH (DMC group) and 10 eyes in diabetic group treated with SH (DMV group). The mean duration of diabetes in this study was 14.1 ± 7.13 years (Table 1). There was a small disparity in the mean duration of diabetes where the mean in DMC was longer than DMV group. However, this was not statistically significant ($p = 0.190$). The glycemic control was referring to the preoperative HbA1c where the mean in this study was $7.09 \pm 1.10\%$. The highest HbA1c in DMC was 8.3% and in DMV was 9.5% which was not statistically significant ($p = 0.739$). The initial area of

corneal epithelial wound ranged from 50.54 to 86.65 mm^2 and corresponded to near total corneal epithelial defect. Figure 2 and Table 2 show the residual epithelial wound over 120 h. There was a small disparity in the initial corneal epithelial wound size, but there were no statistical differences in between groups ($p = 0.205$) at the start of the study. However, there were asymptotic significances (Kruskal–Wallis test) in residual epithelial wound size commencing at 12 h, 24 h, 36 h and 48 h between the groups.

From Table 2 and Fig. 3, residual epithelial wound in DMC is around twofold larger than wound defect in NDM and DMV groups at 24–48 h which were statistically significant ($p < 0.05$ Mann–Whitney test). On the other hand, the residual epithelial wound size in NDM and DMV exhibited similar pattern of reduced size over 120 h.

The epithelial wound size over 120 h was converted to healing rate over 12 h for analysis. The healing rate is the amount of reduction in defect size over time and was evaluated in areas (mm^2) and percentage (%). Table 3 and Fig. 4 show that DMC group had corneal epithelial wound that reepithelialized more slowly than that in NDM and DMV group in the first 36 h. Furthermore, the epithelial wound reepithelialized significantly faster in DMV compared to DMC group in the first 12 h ($p = 0.019$) (Table 4). No differences in the rate of the reepithelialization were detected between NDM and DMV groups. The healing rate after the 48 h was not considered because some of the patients achieved completed wound closure after 48 h.

Figure 4 shows the total amount of healing in mm^2 in each group. We found that the maximum reepithelialization in NDM group and DMC group occurred in 36 h and declined after that, whereas in DMV group, the maximum reepithelialization was in the first 12 h.

Table 1 Demographic characteristics of the study population

Characteristic	Total	NDM	DMC	DMV	p value*
Age (years), mean $\pm$ SD	61.2 ± 7.9	62.2 ± 6.0	58.6 ± 7.9	62.8 ± 9.6	0.462
Duration of diabetes (years), mean $\pm$ SD		14 ± 7	17 ± 5	12 ± 8	0.190
Preoperative HbA1c (%), mean $\pm$ SD		7.1 ± 1.1	7.1 ± 0.8	7.0 ± 1.4	0.739

DMC diabetic group treated without sodium hyaluronate; *DMV* diabetic group treated with sodium hyaluronate; *NDM* non-diabetic group treated without sodium hyaluronate; *SD* standard deviation

*Statistically significant p value < 0.05 (One-way ANOVA test)

Times (Hours)	0	12	24	36	48	60	72	120
NDM								
DMC								
DMV								

DMC= diabetic group treated without sodium hyaluronate; DMV= diabetic group treated with sodium hyaluronate; NDM= non-diabetic group treated without sodium hyaluronate.
(completed closure of epithelial defect noted after 48 hours in NDM and 60 hours in DMV)

Fig. 2 Photographs of the residual wound in each group. The photograph was stopped after the completed closure of the epithelial defect. One eye from the NDM had completed wound closure after 48 h and one eye from the DMV completed wound closure after 60 h. In contrary, one eye from the DMC only noted completed wound healing at 120 h

Table 2 Mean residual corneal epithelial wound over 120 h

Mean residual corneal epithelial wound (mm^2)	Total	NDM	DMC	DMV	Kruskal–Wallis test (Asymp.Sig)
0 h	68.16	67.96	72.37	64.13	0.205
12 h	51.99	50.98	61.99	43.04	*0.005**
24 h	38.24	36.08	50.46	28.17	*0.004**
36 h	21.66	16.90	34.45	13.61	*0.002**
48 h	12.10	9.44	20.62	6.23	*0.015**
60 h	5.24	3.09	9.97	2.65	0.109
72 h	2.52	0	6.68	0.88	0.051
120 h	0.60	0	1.80	0	0.368

Asymp.Sig Asympotic Significance; *DMC* diabetic group treated without sodium hyaluronate; *DMV* diabetic group treated with sodium hyaluronate; *NDM* non-diabetic group treated without sodium hyaluronate

*Statistically significant *p* value < 0.05

Fig. 3 Histogram of the comparison of the mean residual wound over 120 h. The residual epithelial wound in DMC exhibited around twofold larger than wound defect in NDM and DMV groups at 24–48 h which were statistically significance ($p < 0.05$ Mann–Whitney test)
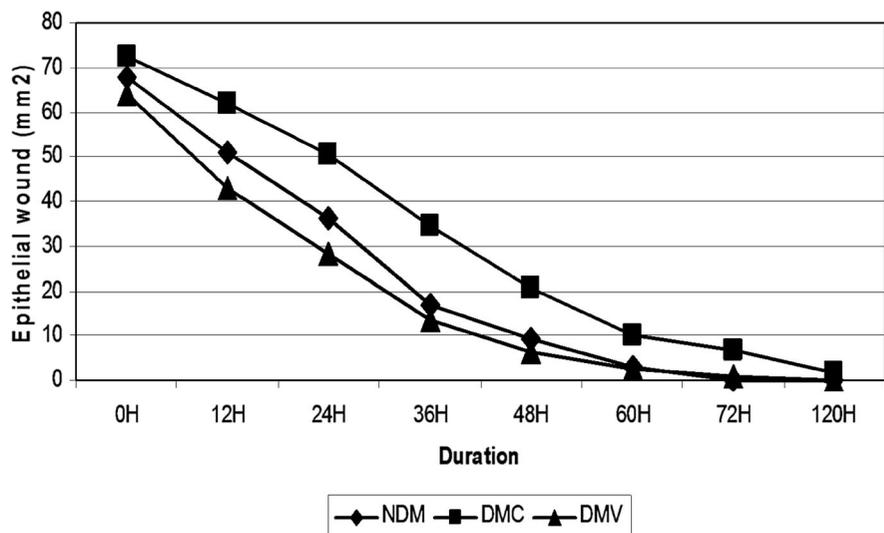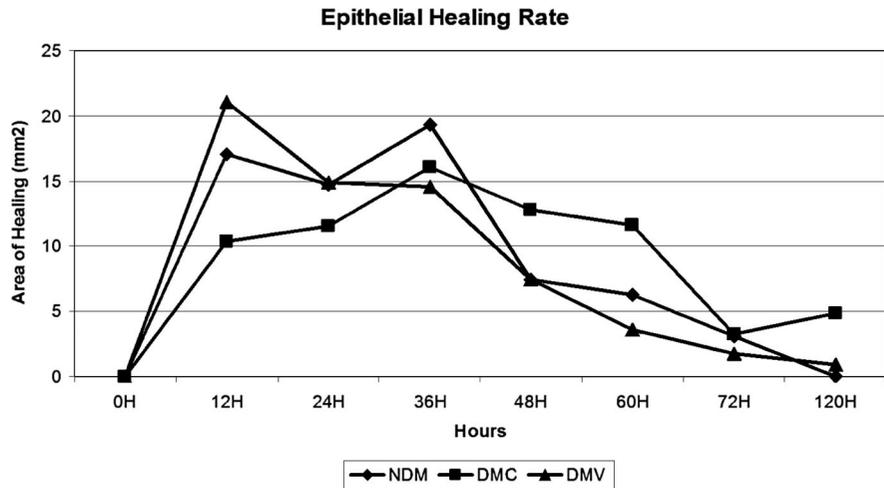
Table 3 Mean corneal epithelial healing rate over first 36 h

Healing rate in area and percentage	Total mm^2 (%)	NDM mm^2 (%)	DMC mm^2 (%)	DMV mm^2 (%)	Kruskal–Wallis test (Asymp.Sig)
0 h	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	1.000
12 h	16.18 (24.1)	17.08 (26.2)	10.37 (14.2)	21.10 (31.7)	0.033*
24 h	13.70 (20.5)	14.68 (21.5)	11.53 (15.8)	14.87 (24.4)	0.668
36 h	16.63 (25.1)	19.33 (28.2)	16.01 (24.7)	14.56 (22.5)	0.443

Asymp.Sig Asympotic Significance; *DMC* diabetic group treated without sodium hyaluronate; *DMV* diabetic group treated with sodium hyaluronate; *NDM* non-diabetic group treated without sodium hyaluronate

*Statistically significant p value < 0.05

Fig. 4 Histogram of the epithelial healing rate every 12 hourly in mm^2. We found that the maximum reepithelialization in NDM group and DMC group occurred in 36 h and declined after that, whereas in DMV group, the maximum reepithelialization was in the first 12 h

Table 4 Comparison of the corneal epithelial healing rate at 12 h and duration for completed wound closure in between group

Comparison in between groups	NDM and DMC	NDM and DMV	DMC and DMV
Epithelial healing rate at 12 h (mm^2)			
NDM = 50.93 ± 4.97			
DMC = 62.00 ± 2.71			
DMV = 43.01 ± 2.27	0.063	0.315	0.019*
Duration of completed epithelial wound closure (hours ± SD)			
NDM = 56.4 ± 9.88			
DMC = 87.6 ± 28.31			
DMV = 64.8 ± 21.31	0.004*	0.393	0.043

DMV diabetic group treated with sodium hyaluronate; *NDM* non-diabetic group treated without sodium hyaluronate; *DMC* diabetic group treated without sodium hyaluronate; *SD* standard deviation

*Statistically significant p value < 0.05 (Mann–Whitney test exact significance)

For comparison between NDM and DMV group, the healing amount in DMV was faster initially compared to NDM group in first 24 h. After 24 h, the NDM group had the faster healing amount.

The duration for complete wound closure was found to be 48 h in NDM and DMV group. The complete wound closure in DMC occurred in 60 h. The mean time for wound closure in NDM was 56.4 ± 9.88 h compared to NDM of 87.6 ± 28.31 h and DMV of 64.8 ± 21.31 h. There was a difference in each group in the mean time for complete corneal epithelial wound closure (Kruskal–Wallis test, $p = 0.01$). For comparison in between groups, the NDM ($p = 0.004$) and DMV ($p = 0.043$) had significantly faster completed wound closure compared to DMV group (Table 4).

Discussion

The major finding of this study was SH 0.18% eye drops facilitated corneal epithelial wound healing induced in diabetic patients undergoing vitrectomy surgery. The DMV group had a significantly smaller residual wound in first 48 h and faster wound healing rate in first 12 h compared with DMC group. This finding was similar to the animal study done by Nakamura [12], and he suggested one possible mechanism of binding of SH to provisional fibronectin in the wounded area. During the first 24 h, DMV group had accelerated phase of wound healing slightly faster than NDM group. After 24 h, the healing rate was slower in DMV group. It shows that possibly SH only aids in improving cell adhesion and migration rather than proliferation. A study by Gomes [4] also found that SH promotes migration but not proliferation on human corneal epithelial cells in vitro.

In this study, the residual epithelial wound size was different between groups especially from 12 to 48 h postoperatively. The residual wound size in DMV group declined in a similar pattern like NDM group. Therefore, SH 0.18% was effective in restoring normal reepithelialization in diabetic patients. The effectiveness of SH in normalizing the epithelial wound healing can be explained by the SH 0.18% eye drops interacting with the provisional fibronectin in diabetic patients and forming the hyaluronan–fibronectin complexes which act as a temporary matrix, substituting for abnormal basement membrane in diabetic corneas. Therefore, the DMV group patients had increased cell migration and adhesion and normalized epithelial wound healing similar to NDM group. However, the DMC group with possible abnormal basement membrane required more time for epithelial cells to migrate and cover the abnormal surface of the basement membrane.

The overall epithelial healing amount was larger in NDM and DMV group compared to DMC group. However, the only statistical significance was at 12 h. The first 12 h after the injury is the lag phase for cellular reorganization and protein synthesis, and the wound healing is normally slow. But, in the comparison between groups, the DMC group showed much slower wound healing in the first 12 h or lag phase. This is possibly because of protein glycosylation in DMC group which affected the fibronectin activation and also other signaling molecules. In DMV group, SH may be able to shorten the lag phase of wound healing. One important phenomenon was observed in the DMC group. The initial wound size in DMC group was larger, but the healing rate was small compared to NDM and DMV group, which was not consistent with previous studies whereby larger epithelial wounds had a faster healing rate compared to smaller initial wounds at first 24 h [16, 17]. This may be explained by larger epithelial wound which approximate to the limbus or periphery of the cornea having a larger capacity of epithelial stem cell proliferation and healing rate. In this study, all the patients had near total epithelium debridement and the difference in the initial corneal epithelial wound size was not statistically significant in between groups. The slow healing rate in diabetic corneas with near total epithelial wounds suggests again that diabetic corneas lack cell migration, possibly lack adherents molecules and have abnormal signaling receptors to stimulate the migration of the cells. This phenomenon is unlikely due to a decrease in mitosis activity or cells proliferation because proliferation phase occurs later in wound healing.

Nakamura et al. [12] studied the effect of hyaluronan in corneal epithelial wound healing in diabetic rats. He found that the accelerated phase of wound healing was between 12 and 48 h in both diabetic and non-diabetic rats. However, the wound size in diabetic rats was larger than non-diabetic rats. This finding was quite similar to the present study whereby the accelerated phase of healing rate in NDM and DMV group was between 0 and 36 h. The accelerated phase was earlier than the animal study because the present study photographed the epithelial wound a few hours later after the corneal debridement from the operation

theater compared to immediately photographed epithelial wound in the animal study. Interestingly, the DMC group showed accelerated phase later between 24 and 60 h. This difference could have been caused by many possibilities. The diabetic rats were induced using streptozotocin and diagnosed if hyperglycemia was over 400 mg/dl after 2 weeks. In this study, all the diabetic patients have long duration of diabetes with eye complications such as retinopathy. Based on this finding, we postulate that DMC group had slower wound healing because of decrease in cells migration and proliferation was inhibited until the basal wound was covered.

The mean duration for completed epithelial closure in NDM was 56.4 h and DMV was 64.8 h. However, the DMC group had mean duration of 87.6 h (up to day 4 post-operation) for completed epithelial wound closure. In clinical practice, most postoperative endophthalmitis occurs within day 3 to day 5. Therefore, it seems necessary to induce the quickest epithelial wound closure because there may be additional sources of infection, especially in diabetic patients.

The limitations of the study include the small sample size in each group. The sample size calculation was based on the previous animal study since there were limited clinical data on the similar study. Other limitation of the study includes the presence of heterogeneous patients such as differences in age group, ethnic group, vitrectomy indications, types of surgery and duration of surgery. However, corneal epithelial debridement should never be deliberate. Hence, patient recruitment is likely to be too slow for the time frame available for this study. Although the debridement was performed by a single surgeon, a more standardized technique of de-epithelialization can be performed such as with microkeratome or femtosecond laser to produce uniform size of epithelial defect. However, this is an expensive tool not available in this center.

Conclusion

In conclusion, this study suggests that commercially available SH 0.18% eye drops hasten the closure of postoperative corneal epithelial wound induced in diabetic patients who underwent vitrectomy surgery. SH seems to help normalize the delayed corneal epithelial healing in diabetic patients. Our findings suggest a possible clinical use of SH 0.18% eye drops in the treatment of surgical or trauma-induced epithelial defects in diabetics.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was conducted in accordance with the principles of the Declaration of Helsinki. The approval from Research and Ethics Committee, Faculty of Medicine, University Kebangsaan Malaysia and Ministry of Health Malaysia, was obtained (Project code: FF-245-2010). The purpose of the study was explained to the patients in detail. Written consent was obtained from patients at study entry.

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