Safety and Efficacy of a Low-Level Radiofrequency Thermal Treatment in an Animal Model of Obstructive Meibomian Gland Dysfunction

Jung Yeob Han
Asan Medical Center
So Young Park
Asan Medical Center
Jeong Hye Sunwoo
Asan Institute for Life Sciences
HUN LEE (yhun777@gmail.com)
Asan Medical Center https://orcid.org/0000-0001-7225-6580
Jae Yong Kim
Asan Medical Center
Hungwon Tchah
Asan Medical Center

Research Article

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Abstract

Purpose: To evaluate the safety and efficacy of a low-level radiofrequency thermal treatment in obstructive MGD rabbit model.

Materials and Methods: Meibomian gland orifices of central two-thirds of upper and lower eyelid margins were coagulated 2 times at 2-week intervals using a 5-MHz high-frequency electrosurgical unit. Sixteen eyes of 8 rabbits were treated with 1 session of radiofrequency thermal treatment (radiofrequency group) and 8 eyes of 4 rabbits were followed up without treatment (Control group). We evaluated lid margin abnormality and corneal staining scores, histologic examination of eyelids and meibomian gland, and meibography images before meibomian gland orifice closure, 4 weeks after meibomian gland orifice closure, and 4 weeks after radiofrequency thermal treatment.

Results: There were significant improvements in lid margin abnormality score for upper and lower eyelids after radiofrequency thermal treatment ($P<0.001$ for upper and lower eyelids). Corneal staining score remained unchanged in radiofrequency group. However, it increased at the final follow-up in control group. Mean area of secretory acini showed a significant improvement, almost to the baseline levels, in radiofrequency group ($P=0.004$). On meibography, an improvement was seen in meibomian gland loss rate in radiofrequency group.

Conclusions: Low-level of radiofrequency thermal treatment for heating the inner and outer eyelid surfaces is safe and effective for the treatment of obstructive MGD in a rabbit animal model of MGD.

Introduction

Meibomian gland dysfunction (MGD) is a chronic, diffuse abnormality of the meibomian gland, which is caused by changes in the glandular secretion due to terminal duct obstruction [1–4]. Consequently, abnormal meibum lipids can result in tear instability and eyelid inflammation[5–7]. For that reason, MGD is known as the main cause of evaporative dry eye disease.

MGD therapy aims to provide long-term symptom relief with improvement of the quality of meibum, increase of meibum flow, and reduction of ocular surface inflammation. Such treatment modalities include a forced meibum expression, systemic medications (minocycline and tetracycline), topical eyedrops (azithromycin, cyclosporine, loteprednol etabonate, diquafosol), intraductal probing, and intense pulsed light (IPL) treatment[8–21]. Recently, a report evaluating the efficacy of radiofrequency (RF) thermal treatment using the Pellevé Wrinkle Reduction System for treating dry eye due to MGD demonstrated that the thermal treatment of the eyelids via RF shows an efficacy similar to LipiFlow Thermal Pulsation System (Johnson & Johnson Vision, Jacksonville, USA) in terms of symptoms, meibomian gland orifice plugging and function, and conjunctival staining score[22]. The Pellevé Wrinkle Reduction System has been shown to tighten loose skin, to reduce fine wrinkles, and to improve the aesthetic appearance of the face and periorbital area[23]. During the treatment, the electrode is continuously moved by the physician over the gel-covered skin around the eyelid, to deliver the RF energy.

Since the treatment for MGD using RF, LipiFlow Thermal Pulsation System, or IPL treatment is considered to be effective due to the thermal effect on the eyelid, the clinical improvement could be attributed to the melting of the abnormal meibum lipids[24–27]. We hypothesized that heat transfer to both the outer and inner eyelid surface is required to melt the abnormal meibum lipids and designed an RF thermal treatment system that could efficiently deliver RF energy to both the outer and inner eyelid surfaces simultaneously.

This study aimed to investigate the effects of a low-level RF thermal treatment on the ocular surface parameters and the meibomian gland structure in a rabbit animal model of obstructive MGD.

Materials And Methods

This study was conducted in strict accordance with and adherence to the relevant national and international guidelines regarding animal handling as mandated by the Institutional Animal Care and Use Committee (IACUC) of the University of Ulsan College of Medicine. The committee has reviewed and approved the animal study protocol (2019-12-184). Eighteen New Zealand white rabbits (1.8 to 2.2 kg) were used in this study. They were placed in standard rabbit cages and housed with a good environmental control. The room temperature was kept at 24°C with a 12-hour light/dark regimen. After 7 days of cage adaptation, the animals underwent the procedure, which was performed by an experienced physician (HL).

Rabbit obstructive MGD Model and RF thermal treatment

After general anesthesia with intramuscular injections of 5 mg/kg Zoletil (Virbac Korea, Seoul, Korea) and 2 mg/kg Rompun (Bayer Korea, Seoul, Korea), the meibomian gland orifices of the central two-thirds of the upper and lower eyelid margins in both the eyes of 15 rabbits were coagulated twice at 2-week intervals using a 5-MHz high-frequency electrosurgical unit (COVE; Jejoong Medical Co.,Ltd., Gangwon-do, South Korea)[28]. The same coagulation settings (power, 3 W; duration, 3 seconds) were maintained throughout the study. Sixteen eyes of randomly selected 8 rabbits were treated with 1 session of the RF thermal treatment device (Ilooda, Suwon, South Korea) at 4 weeks after the first meibomian gland orifice closure (RF treatment group).

The RF thermal treatment system was designed to transfer heat to both the outer and inner eyelid surfaces. The treatment settings (power, 2; duration, 20 minutes) were maintained throughout the study. Eight eyes of 4 rabbits were followed up without any treatment or intervention (Control group). We also performed a safety evaluation after the first session of the treatment by checking for skin burns, conjunctival and corneal injection and burns.

Lid margin examinations

Before the meibomian gland orifice closure, 4 weeks after the first meibomian gland orifice closure, and at 4 weeks after the RF thermal treatment, lid margin findings were assessed and scored for the presence or absence of the following abnormalities on a scale of 0 (no finding) to 4 (all findings): lid margin irregularity and thickness, plugging of the meibomian gland orifices, and lid margin vascular engorgement (telangiectasia)[29].

Corneal Fluorescein Staining

The corneal staining score was measured under general anesthesia before the meibomian gland orifice closure, 4 weeks after the first meibomian gland orifice closure, and at 4 weeks after the RF thermal treatment according to the National Eye Institute/Industry (NEI) scoring scheme[30]. Fluorescein sodium-impregnated paper strips were wetted with normal saline and applied on the upper bulbar surface after retracting the upper lid. Slit-lamp examination under cobalt blue illumination was used to observe the corneal staining after the rabbit's eye was gently closed 5 times and excessive tear fluid and dye were wiped away.

Histologic Examination of Eyelids and Meibomian Glands

Both upper and lower eyelids (n = 3 for each group at each time point) were gently excised before the meibomian gland orifice closure, 4 weeks after the first meibomian gland orifice closure, and at 4 weeks after the RF thermal treatment. Eyelid tissue was embedded in ordinary paraffin. Paraffin-embedded tissue blocks were cut into 4-mm sections in sagittal or transverse planes using a microtome, and thin sections were placed on microscope slides. After deparaffinization of the sections with xylene, thin sections were immersed in a graded series of ethanol and phosphate-buffered saline, and serially cut sections were used for hematoxylin and eosin (H&E) staining. Meibomian gland changes and infiltration of polymorphonuclear neutrophil (PMN) cells around the meibomian gland orifices were observed under light microscopy (Zeiss, Inc., Thornwood, NY, USA) at x4 and x40 magnification. Using the images of transverse eyelid tissue sections, the number of PMN cells per unit area were counted and compared between the control and RF treatment groups. The area of the secretory acini, around a meibomian gland duct, which was measured using the polygon selection tool and the selection brush tool in ImageJ (National Institutes of Health, Bethesda, MD) was also compared between the two groups[28]. The unit of measurement of the digital images was changed from distance in pixels to millimeters based on the scale bar of the digital images using the set scale tool in ImageJ.

Meibography

The integrity of the meibomian gland was assessed using an ICP MGD meibography device (SBM Sistemi, Turin, Italy) before the meibomian gland orifice closure, 4 weeks after the first meibomian gland orifice closure, and at 4 weeks after the RF thermal treatment. This device is specially designed to perform infrared meibography and to measure meibomian gland loss rate semi-automatically for animal experiments.

Statistical Analyses

Statistical analyses were performed using the repeated measures analysis of variance with a Bonferroni correction for multiple comparisons in SPSS software version 22.0 (IBM, Armonk, NY, USA). *P* value of less than 0.05 was considered as statistically significant.

Results

There was a significant increase in the lid margin abnormality score for both the upper and lower lids at 4 weeks after the first meibomian gland orifice closure in both the groups (upper lid, $P = 0.006$ for control group and $P < 0.001$ for RF treatment group, and lower lid, $P = 0.003$ for control group and $P < 0.001$ for RF treatment group; Table 1 and Figure 1). After the RF thermal treatment, there was a significant improvement in the score for both the upper and lower eyelids in the RF treatment group ($P < 0.001$ for upper and lower eyelids). However, there was no significant improvement in the control group ($P = 0.682$ for upper and $P = 0.511$ for lower eyelids). There was a significant difference in the score for the upper ($P = 0.006$) and the lower eyelid ($P = 0.002$) between both the groups at 4 weeks after the RF treatment.

Table 1
Changes in lid margin abnormality score and corneal staining score between control and radiofrequecy treatment groups

	Control group			**RF treatment group**		
	Before MG orifices closure	4 wks after MG orifices closure	4 wks after without treatment	Before MG orifices closure	4 wks after MG orifices closure	4 wks after RF treatment
Lid margin abnormality score (UL) †	0.25 (0.46)	2.00 (0.76)	1.50 (1.07)	0.28 (0.46)	2.22 (0.81)	0.44 (0.71)
Lid margin abnormality score (LL) †	0.00 (0.001)	1.88 (0.99)	1.38 (0.74)	0.00 (0.001)	1.72 (0.67)	0.39 (0.61)
Corneal fluorescein staining score	0.00 (0.001)	0.00 (0.001)	1.50 (1.07)	0.00 (0.001)	0.11 (0.47)	0.22 (0.65)

Results are expressed as mean and standard deviation.

†Lid margin abnormality score was scored for the presence or absence of the following lid margin abnormalities: lid margin irregularity, plugging of the meibomian orifices, lid margin vascular engorgement (telangiectasia), and lid margin thickness (on a scale of 0 to 4).

RF = radiofrequecy; MG = meibomian gland; UL = upper lid; LL = lower lid.

There was no significant change in the corneal staining score before and after the meibomian gland orifice closure in both the groups (Table 1). In the control group, the scores increased at the final follow-up ($P = 0.016$), whereas they remained unchanged in the RF treatment group ($P = 0.489$). There was a significant difference in the scores between both the groups at 4 weeks after the RF thermal treatment (1.50 ± 1.07 versus 0.22 ± 0.65; $P = 0.011$).

Light microscopy images of the sagittal and transverse eyelid tissue sections stained with H&E showed obstructed orifices, dilated ductules and central ducts, and distinctly smaller secretory acini after the meibomian gland orifice closure in both the groups (Figure 2A and 2B). At 4 weeks after the RF thermal treatment, the dilated ductules and central ducts showed some improvement, but not up to the baseline condition. There was no difference observed between both the groups at 4 weeks after the RF thermal treatment (Figure 2A and 2B). The mean area of the secretory acini around 1 meibomian gland duct significantly decreased after the meibomian gland orifice closure in both the groups ($P = 0.002$ for control group and $P < 0.001$ for RF treatment group; Table 2). At 4 weeks after the RF thermal treatment, the mean area showed significant improvement, almost to the baseline levels especially in the RF treatment group ($P = 0.004$). There was significant difference between both the groups at 4 weeks after the RF thermal treatment (0.09 ± 0.03 mm^2 for RF treatment group versus 0.05 ± 0.02 mm^2 for control group; $P = 0.022$; Table 2).

Table 2
Changes in area of the secretory acini and numbers of polymorphonuclear neutrophil cells between control and radiofrequecy treatment groups

	Control group			**RF treatment group**		
	Before MG orifices closure	4 wks after MG orifices closure	4 wks after without treatment	Before MG orifices closure	4 wks after MG orifices closure	4 wks after RF treatment
Secretory acini area	0.11 (0.03)	0.03 (0.01)	0.05 (0.02)	0.09 (0.02)	0.01 (0.004)	0.09 (0.03)
PMN	0.13 (0.35)	21.50 (0.54)	2.00 (3.67)	0.20 (0.42)	21.50 (0.53)	0.70 (1.49)

Results are expressed as mean and standard deviation.

RF = radiofrequecy; MG = meibomian gland; PMN = polymorphonuclear neutrophil.

The mean number of PMN cells significantly increased at 4 weeks after the first meibomian gland orifice closure in both the groups (all $P < 0.001$; Table 2). However, the numbers significantly decreased at 4 weeks after the RF thermal treatment in both the groups (all $P < 0.001$; Table 2). The mean number of PMN cells at 4 weeks after the RF thermal treatment in the RF treatment group were not significantly different from those in the control group (0.70 ± 1.49 for RF treatment group versus 2.00 ± 3.67 for control group; $P = 0.371$).

According to the results obtained from the meibography device, there was a significant increase in the meibomian gland loss rate for the upper and lower eyelids at 4 weeks after the first meibomian gland orifice closure in both the groups. There was no significant change observed between 4 weeks after the meibomian gland orifices closure and 4 weeks after the RF thermal treatment in the control group (Table 3 and Figure 3). However, an improvement was noted for the upper and lower eyelids after 1 session of the RF thermal treatment in the RF treatment group, which was similar to the baseline value ($P = 0.001$ for upper eyelid and $P < 0.001$ for lower eyelid, Table 3 and Figure 3). Moreover, there was a significant difference in the meibomian gland loss rate for the upper and lower eyelids at 4 weeks after the RF thermal treatment between both the groups ($P = 0.001$ for upper eyelid, and $P < 0.001$ for lower eyelid). There was no sign of skin burn and conjunctival or corneal injection and burn after 1 session of the RF thermal treatment under the same treatment settings (power, 2; duration, 20 min)

Table 3
Changes in meibomian gland loss rate between control and radiofrequecy treatment groups

	Control group			**RF treatment group**		
	Before MG orifices closure	4 wks after MG orifices closure	4 wks after without treatment	Before MG orifices closure	4 wks after MG orifices closure	4 wks after RF treatment
MG loss rate (UL) †	25.83 (10.72)	50.17 (7.63)	51.83 (4.96)	25.00 (10.77)	53.75 (15.53)	34.63 (11.89)
MG loss rate (LL) †	19.50 (7.87)	42.67 (13.71)	55.00 (8.94)	17.63 (7.67)	44.75 (8.10)	15.25 (5.75)

Results are expressed as mean and standard deviation.

†Meibomian gland loss rate was assessed using an ICP MGD meibography device.

RF = radiofrequecy; MG = meibomian gland; UL = upper lid; LL = lower lid.

Discussion

The present study demonstrated that one session of the RF thermal treatment was safe and effective for the treatment of obstructive MGD with respect to lid margin abnormality, corneal staining score, and meibomian gland structure. In cases of obstructive MGD, abnormal meibum secretion by the primary obstructive hyperkeratinization of the meibomian gland and changes in glandular secretion result in tear film instability and eyelid and corneal inflammation[31, 32]. It can damage the ocular surface directly or indirectly by initiating an inflammatory cascade that generates immunological responses and microbiological changes[31, 32]. The blockage in obstructive MGD is caused by the inspissated secretions resulting from the hyperkeratinization, and an elevated melting point of the solidified oils from the stagnation and bacterial activity[32].

LipiFlow Thermal Pulsation System is currently the only FDA approved treatment, which works by direct eyelid heating and meibomian gland massage to express the waxy blockage and restore the meibomian gland function. Here, sufficient and direct eyelid heating is important to melt the modified or deficient meibum lipids for unblocking the obstructed meibomian gland[33]. Compared with this system, the use of

RF energy for heating via the Pellevé Wrinkle Reduction System showed similar results with regard to improvements in subjective symptoms, meibomian gland expression grade and plugging score, and conjunctival staining score[22]. Pellevé Wrinkle Reduction System has been utilized to decrease the skin laxity and improve rhytids of the face, including the periocular area, by heating the dermis and achieving a higher temperature[34]. High-frequency electron flow RF generates heat in the surrounding tissues as a result of the difference in impedance between the tissue types, which turns the kinetic energy into thermal energy[35]. It not only eliminates the problem of heating the unwanted target chromophores in the skin, such as melanin, as seen with the IPL treatment, but also allows heating of the deep dermis. A recent clinical study conducted by Jaccoma *et al.* only evaluated 10 participants that were recruited from a diverse age group, thus necessitating further studies to elucidate the mechanisms of the RF treatment for the management of MGD[22]. However, we assumed that the RF energy via the Pellevé Wrinkle Reduction System might be insufficient to achieve complete melting of the abnormal meibum lipids because it was designed to apply the heat only externally to the outer eyelid surface. Furthermore, considering that the electrode should be continuously moved by the physician over the eyelid to deliver the RF energy, there could be an uneven distribution of the RF energy over the eyelid. Thus, we hypothesized that transfer of heat to both the outer and inner eyelid surfaces was important for the treatment of obstructive MGD.

Based on our results, we demonstrated that the lid margin abnormality score significantly improved after the RF thermal treatment in the treatment group as compared with the control group, with the former having a significantly lower score. Hence, the RF thermal treatment could be effective for obstructive MGD through its heating of the eyelid and opening of the meibomian gland orifice.

Corneal staining scores increased at 4 weeks after without the RF thermal treatment in the control group, when compared with baseline and 4 weeks after meibomian gland orifice closure. Gilbard et al have shown an abnormal rose bengal staining of the cornea 4 weeks after the meibomian gland closure, which is in contrast with the results of our study[36] Possible explanations would be incomplete coagulation of the meibomian gland orifices of the central two-thirds of eyelids, sparing the nasal and temporal meibomian glands or an inherent homeostatic system providing a compensatory tear fluid production[37]. The corneal staining score in our study remained unchanged throughout the experiment in the RF treatment group. We considered this to be due to the ocular surface stabilization via the RF thermal treatment accompanied with the compensatory tear fluid production in obstructive MGD.

The histologic sections showed that the sizes of the secretory acini were reduced with the widening of the central ducts after the meibomian gland orifice closure in both the groups. Accordingly, a significant increase in the meibomian gland loss rate was noted at 4 weeks after the meibomian gland orifice closure in both the groups. After 1 session of the RF thermal treatment, there was an improvement in the meibomian gland loss rate for the upper and lower eyelids, which was almost normalized to baseline condition. Moreover, there were improvements in the mean area of the secretory acini and the dilated central ducts as well. Moreover, there was a significant difference in the mean area of the secretory acini between both the groups at 4 weeks after the RF thermal treatment.

There was no difference in the number of PMN cells between the groups at 4 weeks after the RF thermal treatment. According to previous studies, inflammatory cells around the meibomian gland ducts were normalized to a baseline condition in less than 4 weeks after the meibomian gland orifice closure. Hence a long-term observation is needed to detect the differences in the number of PMN cells between the control and RF treatment groups[28, 36].

In the present study, the use of ICP MGD meibography device was helpful to evaluate the integrity of the meibomian gland before and after the MGD treatment in an animal MGD model because it could detect relatively early changes in the meibomian gland acini after the closure of the orifice or the RF thermal treatment. In a previous study, the meibography obtained from a Meiboviewer for animal experiments (Visual Optics, Chuncheon, South Korea) could not detect early changes in the meibomian gland acini after the closure of the orifice[28]. There might be some functional differences between the instruments, although both use the infrared light for the detection of the meibomian gland structure. Therefore, in animal MGD studies, considerable observation and comparison between the histologic examination and meibography is essential to detect subtle changes in the meibomian gland structure.

The coagulation of the meibomian gland orifices of the central two-thirds of the upper and lower eyelid margins was performed twice, at 2-week intervals using a 5-MHz high-frequency electrosurgical unit. In a previous study evaluating the effects of one-time electrosurgical coagulation of the meibomian gland orifices on the meibomian gland structure and ocular surface changes in rabbits, meibography showed a normal morphology, although the mean area of the secretory acini of the coagulation group was significantly smaller than that of the non-coagulation group[28]. Additionally, the effects of the thermal damage of the meibomian gland caused by the coagulation, resolve within 14 days. This is based on the normalization in the numbers of PMN cells, CD-11b-positive cells, and apoptotic cells around the meibomian gland and meibomian gland orifice[28]. Hence, the electrosurgical coagulation of the meibomian gland orifices was performed twice to guarantee an improved and sophisticated version of the obstructive MGD animal model.

This study had several limitations, including a short duration of follow-up period and a small sample size. Since the duration of follow-up after 1 session of the RF thermal treatment was limited to 4 weeks, it is thus recommended to observe the differences over a longer period for any meaningful conclusions. Further, the choice of rabbits for the animal experiments to study the biochemistry and biophysics of tear films, could be a shortcoming because of the differences between the rabbit and human meibum[38]. Nevertheless, a low-level RF thermal treatment was found to be safe and effective for the treatment of obstructive MGD, although the exact mechanism and significance of the RF thermal treatment remains uncertain. Further human studies to investigate the effects of the RF thermal treatment device, used in this study, for the treatment of MGD are needed.

In summary, the use of a low-level of RF energy for heating the inner and outer eyelid surfaces is safe and effective for the treatment of obstructive MGD in a rabbit animal model of MGD.

Declarations

Statements and Declaration: The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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Figures

Figure 1

Morphology of eyelid before the meibomian gland orifice closure, 4 weeks after the meibomian gland orifice closure, and at 4 weeks after the radiofrequency thermal treatment or without the radiofrequency treatment. (A) Control group. (B) Radiofrequency thermal treatment group. MG = meibomian gland, RF = radiofrequency.

Figure 2

H&E staining in rabbit eyelid sections in the sagittal and transverse planes at x 40 magnification. (A) Control group. (B) Radiofrequency thermal treatment group. MG = meibomian gland, RF = radiofrequency.

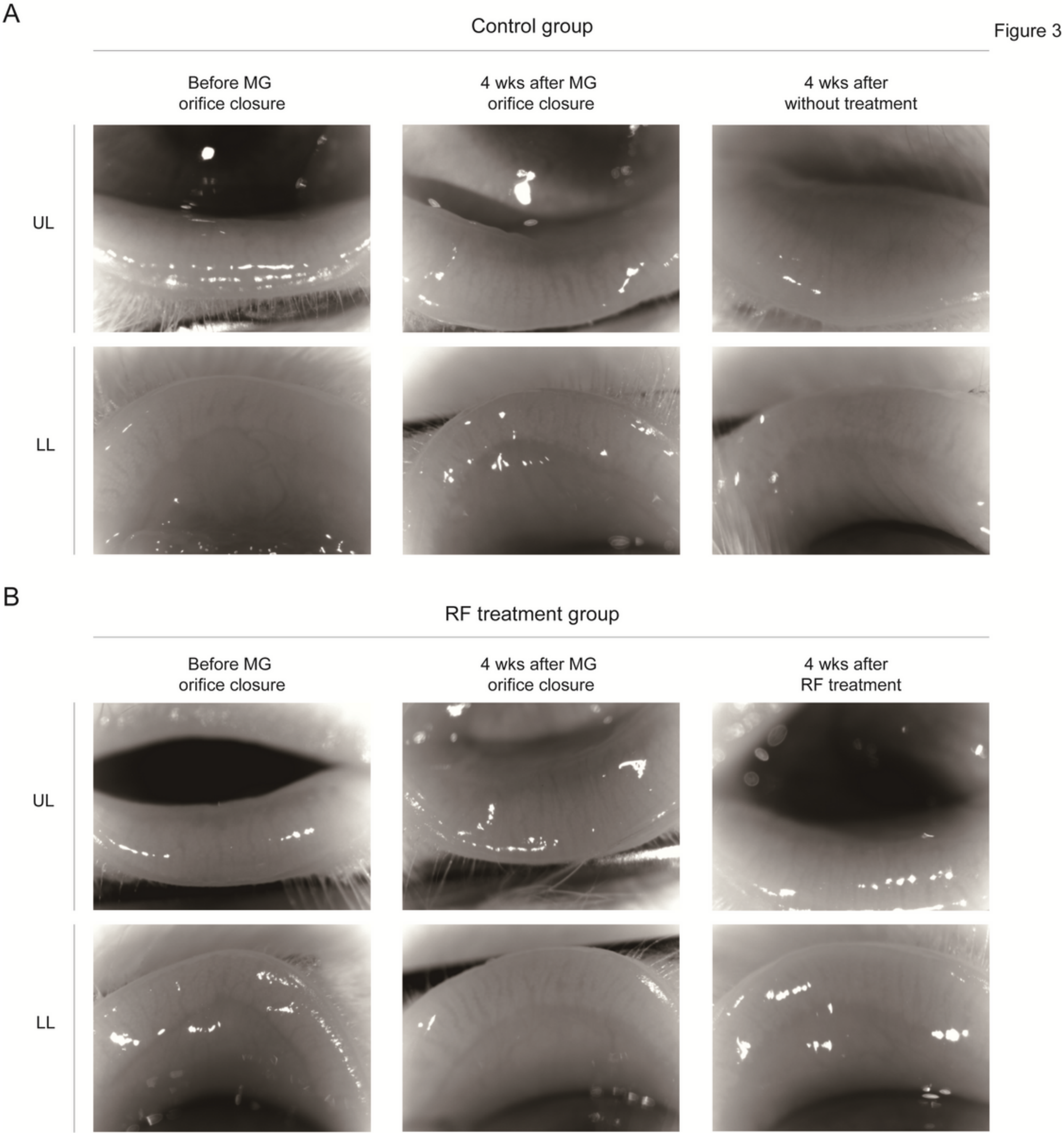

Figure 3

Meibography of rabbits before the meibomian gland orifice closure, 4 weeks after the meibomian gland orifice closure, and at 4 weeks after the radiofrequency thermal treatment or without the radiofrequency treatment. (A) Control group. (B) Radiofrequency thermal treatment group. MG = meibomian gland, RF = radiofrequency, UL = upper eyelid, LL = lower eyelid.