Safety and efficacy of a low-level radiofrequency thermal treatment in an animal model of obstructive meibomian gland dysfunction

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Abstract
This study aimed to evaluate the safety and efficacy of a low-level radiofrequency thermal treatment in an obstructive MGD rabbit model. Meibomian gland orifices of the central two-thirds of the upper and lower eyelid margins were coagulated twice at 2-week intervals using a 5-MHz high-frequency electrosurgical unit. Sixteen eyes of eight rabbits were treated with one session of radiofrequency thermal treatment (radiofrequency group) and eight eyes of four rabbits were followed up without treatment (control group). Lid margin abnormality and corneal staining scores, histologic examination of the eyelids and meibomian gland, and meibography imaging were evaluated just before and 4 weeks after meibomian gland orifice closure and 4 weeks after radiofrequency thermal treatment. Lid margin abnormality score improved significantly for the upper and lower eyelids after radiofrequency thermal treatment ($P < 0.001$ for both eyelids). Corneal staining score remained unchanged in the radiofrequency group; however, the control group saw an increase at final follow-up. There was a significant improvement to almost baseline levels in the mean area of secretory acini in the radiofrequency group ($P = 0.004$). Additionally, meibography indicated an improvement in meibomian gland loss rate in the radiofrequency group. Low-level radiofrequency thermal treatment heating the inner and outer eyelid surfaces is safe and effective to treat obstructive MGD in a rabbit animal model of MGD.

Keywords Meibomian gland · Obstructive meibomian gland dysfunction · Radiofrequency thermal treatment · Dry eye · Animal model

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

MGD therapy aims to provide long-term symptom relief by improving meibum quality, increasing meibum flow, and reducing ocular surface inflammation. Such treatment modalities include forced meibum expression, systemic medications (minocycline and tetracycline), topical eye drops (azithromycin, cyclosporine, loteprednol etabonate, diquafoosol), intraductal probing, and intense pulsed light (IPL) treatment [8–21]. A recent report evaluating the efficacy of radiofrequency (RF) thermal treatment using the Pellevé Wrinkle Reduction System for treating dry eye due to MGD demonstrated that RF thermal treatment of the eyelids had similar efficacy 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, reduce fine wrinkles, and improve the aesthetics of the face and periorbital area [23]. The physician continuously moves the electrode over the gel-covered skin around the eyelid to deliver the RF energy during treatment. Since...
the treatment for MGD using RF, LipiFlow Thermal Pulsation System, or IPL treatment is considered effective due to the thermal effect on the eyelid, clinical improvement could be attributed to the melting of 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 subsequently designed an RF thermal treatment system that efficiently delivers RF energy to 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 strictly adhered 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 good environmental control. The ambient environment was maintained at 24 °C with a 12-h light/dark regimen. After 7 days of cage adaptation, the animals underwent the procedure 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 s) were maintained throughout the study. Sixteen eyes of eight randomly selected rabbits were treated with one session of the RF thermal treatment device (Ilooda, Suwon, South Korea) 4 weeks after the first meibomian gland orifice closure (RF treatment group).

The RF thermal treatment system was designed to transfer heat to the outer and inner eyelid surfaces (Fig. 1). The treatment settings (power, 2; duration, 20 min) were maintained throughout the study. Eight eyes of four rabbits were followed up without any treatment or intervention (control group). We also performed a safety evaluation after the first treatment session by checking for skin burns, conjunctival and corneal injection, and corneal 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]. The examiners were blinded to the two groups.
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, while excessive tear fluid and dye were wiped away. The examiners were blinded to both groups.

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 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. Serially cut sections were used for hematoxylin and eosin (H&E) staining. Meibomian gland changes and infiltration of polymorphonuclear neutrophil cells (PMNs) around the meibomian gland orifices were observed under light microscopy (Zeiss, Inc., Thornwood, NY, USA) at ×4 and ×40 magnification. The number of PMNs per unit area was counted and compared with the control and RF groups using images of the transverse eyelid tissue sections. The area of the secretory acini around a meibomian gland duct was measured using the polygon selection tool, and the selection brush tool in ImageJ (National Institutes of Health, Bethesda, MD) was used to compare 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. The examiners were blinded to both groups.

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 specifically designed to perform infrared meibography and measure the meibomian gland loss rate semi-automatically for animal experiments. The examiners were blinded to both groups.

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). A P-value of < 0.05 was considered 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 groups (upper lid: P = 0.006 vs. P < 0.001; lower lid: P = 0.003 vs. P < 0.001 for the control and RF treatment groups, respectively; Table 1 and Fig. 2). There was a significant difference in the score for the upper (P = 0.006) and the lower eyelid (P = 0.002) between both groups at 4 weeks after RF treatment. After the RF thermal treatment, a significant improvement in the upper and lower eyelid score was noted 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).

No significant change was observed in the corneal staining score in either group before and after the meibomian gland orifice closure (Table 1). Although scores increased in the control group at the final follow-up (P = 0.016), they remained unchanged in the RF treatment group (P = 0.489). There was a significant difference in the scores between both groups at 4 weeks after RF 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 (Fig. 3A and B). Although the dilated ductules and central ducts improved at 4 weeks after RF thermal treatment, they did not reach baseline levels. No difference was observed between groups at 4 weeks after the RF thermal treatment (Fig. 3A and B). The mean area of the secretory acini around 1 meibomian gland duct significantly decreased after the meibomian gland orifice closure in both groups (P = 0.002 for the control group and P < 0.001 for RF treatment group; Table 2). The mean area significantly improved to almost baseline levels 4 weeks after RF treatment, particularly in the RF treatment group (P = 0.004). A significant difference was observed between the groups 4 weeks after RF thermal treatment (0.09 ± 0.03 mm² vs.
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The mean number of PMNs increased significantly at 4 weeks after the first meibomian gland orifice closure in both groups (all \( P < 0.001 \); Table 2). However, the numbers significantly decreased at 4 weeks after treatment or without treatment in both the groups (all \( P < 0.001 \); Table 2). There was no significant difference in the mean number of PMNs at 4 weeks after RF treatment between the two groups (RF group: 0.70 ± 1.49 vs. control group: 2.00 ± 3.67; \( P = 0.371 \)).

The results obtained from the meibography device indicated 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 groups. No significant change was observed between 4 weeks after meibomian gland orifices closure and 4 weeks after without treatment in the control group (Table 3 and Fig. 4). However, an improvement was noted for the upper and lower eyelids after one session of RF treatment in the treatment group, similar to the baseline value (upper eyelid: \( P = 0.001 \) and lower eyelid: \( P < 0.001 \), Table 3 and Fig. 4). 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.
treatment between both groups ($P = 0.001$ for the upper eyelid, and $P < 0.001$ for lower eyelid). There was no sign of skin burn and conjunctival or corneal injection and corneal burn after one session of the RF thermal treatment under the same treatment settings (power, 2; duration, 20 min).

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

|                | Control group | RF treatment group |
|----------------|---------------|--------------------|
|                | Before MG orifice closure | 4 weeks after MG orifice closure | 4 weeks 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 (0.54) | 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

$RF$, radiofrequency; $MG$, meibomian gland; $PMN$, polymorphonuclear neutrophil cell
### Table 3 Changes in meibomian gland loss rate between control and radiofrequency treatment groups

|                      | Control group                  | RF treatment group          |
|----------------------|--------------------------------|-----------------------------|
|                      | Before MG orifice closure      | Before MG orifice closure   |
| MG loss rate (UL) a  | 25.83 (10.72)                  | 25.00 (10.77)               |
|                      | 50.17 (7.63)                   | 53.75 (15.53)               |
|                      | 51.83 (4.96)                   | 53.75 (15.53)               |
|                      | 4 weeks after MG orifice closure| 4 weeks after RF treatment |
| MG loss rate (LL) a  | 19.50 (7.87)                   | 17.63 (7.67)                |
|                      | 42.67 (13.71)                  | 44.75 (8.10)                |
|                      | 55.00 (8.94)                   | 15.25 (5.75)                |

Results are expressed as mean and standard deviation

*RF*, radiofrequency; *MG*, meibomian gland; *UL*, upper lid; *LL*, lower lid

*a Meibomian gland loss rate was assessed using an ICP MGD meibography device

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**Fig. 4** 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
Discussion

Our study demonstrated that one session of RF thermal treatment was safe and effective for treating obstructive MGD concerning lid margin abnormality, corneal staining score, and meibomian gland structure. In cases of obstructive MGD, abnormal meibum secretion by primary obstructive hyperkeratinization of the meibomian gland and changes in glandular secretion result in tear film instability and eyelid and corneal inflammation [31, 32]. Damage to the ocular surface can occur directly or indirectly through the initiation of 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].

The LipiFlow Thermal Pulsation System is currently the only FDA-approved treatment for MGD, which works by directly heating the eyelid and massaging the meibomian gland 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 and unblock the obstructed meibomian gland [33]. RF energy used for heating via the Pellevé Wrinkle Reduction System showed similar results in improving subjective symptoms, meibomian gland expression grade and plugging score, and conjunctival staining score [22]. The Pellevé Wrinkle Reduction System has been utilized to decrease skin laxity and improve rhytids of the face, including the periorcular area, by heating the dermis and achieving higher temperatures [34]. High-frequency electron flow RF generates heat in the surrounding tissues due to the difference in impedance between the tissue types, transforming kinetic energy into thermal energy [35]. The problem of heating the unwanted target chromophores in the skin, such as melanin, is eliminated, as noted with IPL treatment, and allows heating of the deep dermis. A recent clinical study conducted by Jaccoma et al. evaluated 10 participants recruited from a small but diverse age group, thus necessitating further studies to elucidate the mechanisms of the RF treatment for the management of MGD [22]. 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 external heat only to the outer eyelid surface. Furthermore, because the physician must continuously move the electrode over the eyelid to deliver the RF energy, there could be an uneven distribution of energy over the area. Thus, we hypothesized that heat transfer to both the outer and inner eyelid surfaces was important for treating obstructive MGD.

Our results demonstrated that the lid margin abnormality score significantly improved for patients in the treatment group compared with the control group, with the former having a significantly lower score. Hence, RF thermal treatment may be effective for obstructive MGD by heating the eyelid and opening the meibomian gland orifice.

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

The histologic sections indicated a reduction in the size of the secretory acini, with widening of the central ducts after the meibomian gland orifice closure in both groups. Accordingly, a significant increase in the meibomian gland loss rate was noted at 4 weeks after orifice closure in both groups. After one session of the RF thermal treatment, an improvement was observed in the upper and lower eyelid meibomian gland loss rate, which was almost normalized to the baseline condition. Moreover, there were improvements in the mean area of the secretory acini and the dilated central ducts. We noted a significant difference in the mean area of the secretory acini between both groups at 4 weeks after the RF thermal treatment.

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

In the present study, the ICP MGD meibography device helped evaluate the integrity of the meibomian gland before and after the MGD treatment in the animal MGD model, given its ability to detect relatively early changes in the meibomian gland acini after closure of the orifice or 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]. Although both instruments use infrared light to detect the meibomian gland structure, some functional differences may have been present. Therefore, considerable observation...
and comparison between the histologic examination and meibography are essential in animal MGD studies to detect subtle changes in the meibomian gland structure.

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. Normal morphology on meibography was reported in a study that evaluated the effects of one-time electrosurgical coagulation of the meibomian gland orifices on the meibomian gland structure and ocular surface changes in rabbits. However, the mean area of the secretory acini of the coagulation group was significantly smaller than that of the non-coagulation group [28]. Additionally, normalization in the number of PMNs, CD-11b-positive cells, and apoptotic cells around the meibomian gland and gland orifice indicates that the effects of the thermal damage on the meibomian gland caused by the coagulation resolve within 14 days [28]. Hence, 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 follow-up period and small sample size. Given the limited follow-up duration after one session of 4 weeks, we recommend observing the differences over a long period to draw more meaningful conclusions. Furthermore, studying the biochemistry and biophysics of tear films in the rabbit model creates limitations for applicability to human meibum given their differences [38]. Nevertheless, a low-level RF thermal treatment was found to be safe and effective for treating 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, using a low level of RF energy for heating the inner and outer eyelid surfaces can be safe and effective treatment modality for treating the obstructive MGD in a rabbit animal model of MGD.

Author contribution JYH: study supervision, concept and study design, data collection, data interpretation, data analysis and statistics, drafting of manuscript.
SYP: concept and study design, data collection, data interpretation, data analysis and statistics, drafting of manuscript.
JHS: data collection, data interpretation, data analysis and statistics.
JYK: study supervision, revision and final approval of manuscript.
HT: study supervision, revision and final approval of manuscript.
HL: study supervision, concept and study design, data interpretation, revision and final approval of manuscript.

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Data availability The data used to support the findings of this study are available from the corresponding author upon request.

Code availability Not applicable.

Declarations

Ethics approval 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.

Consent to participate Written informed consent was waived for this study given its animal study design.

Consent for publication Written informed consent was waived for this study given its animal study design.

Competing interests The authors declare no competing interests.

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