The meibomian glands are essential for the maintenance of tear film and the health of ocular surface. As a special type of sebaceous gland, the secretory acini also follow a holocrine secretion mode. Meibum is then delivered within acini as well as keratinization within ducts of mouse meibomian gland epithelial cell line (hMGEC) that has been widely used to evaluate the effects of hormones, fatty acids, and drugs on the regulation of meibocytes in vitro. To our knowledge, the investigations carried out by cell line were mainly limited to the secretion of acini; however, the function and pathology of the ducts was rarely involved.

Here, we reported a novel organotypic culture system of mouse meibomian gland. By providing different culture conditions and administering proinflammatory cytokine IL-1β to the explants, we observed the lipid accumulation within acini as well as keratinization within ducts of mouse meibomian glands, respectively. We proposed that our organotypic culture model might be a promising method for investigating pathological processes in meibomian glands and providing therapeutic strategies for MGD.

Purpose. Meibomian glands are essential in maintaining the integrity and health of the ocular surface. Meibomian gland dysfunction (MGD), mainly induced by ductal occlusion, is considered as the major cause of dry eye disease. In this study, a novel in vitro model was established for investigating the role of inflammation in the process of MGD.

Methods. Mouse tarsal plates were removed from eyelids after dissection and explants were cultured during various time ranging from 24 to 120 hours. Meibomian gland epithelial cells were further enzymatically digested and dissociated from tarsal plates before culturing. Both explants and cells were incubated in different media with or without serum or azithromycin (AZM). Furthermore, explants were treated with IL-1β or vehicle for 48 hours. Analyses for tissue viability, histology, biomarker expression, and lipid accumulation were performed with hematoxylin and eosin (H&E) staining, immunofluorescence staining, and Western blot.

Results. Higher viability was preserved when explants were cultured on Matrigel with immediate addition of culture medium. The viability, morphology, biomarker expression, and function of meibomian glands were preserved in explants cultured for up to 72 hours. Lipid accumulation and peroxisome proliferator-activated receptor γ (PPARγ) expression increased in both explants and cells cultured in media containing serum or AZM. Treatment with IL-1β induced overexpression of Keratin (Krt) 1 in meibomian gland ducts.

Conclusions. Intervention with pro-inflammatory cytokine IL-1β induces hyperkeratinization in meibomian gland ducts in vitro. This novel organotypic culture model can be used for investigating the mechanism of MGD.

Keywords: organotypic culture, meibomian gland, meibomian gland dysfunction, inflammation, hyperkeratinization
A Novel Meibomian Gland Culture System In Vitro

FIGURE 1. Culture of mouse meibomian gland explants. (A) Schematic of organotypic culture model. The tarsal plates were isolated from eyelid tissues of mouse by removing skin, subcutaneous tissue, muscle, and palpebral conjunctiva. Then, the dissected tarsi were carefully transferred to a 24-well culture plate with conjunctival side up and covered by a thin layer of culture medium. The explants were incubated at 37°C with 5% CO2/95% air. (B) MTT assay comparison of tissue viability in explants with different culture methods (n = 4 in each group). *Significantly different from the control group (P < 0.05). PC, Positive Control; MI, Matrigel-coated plates with Immediate addition of media; BI, Bare plates with Immediate addition of media; MD, Matrigel-coated plates with Delayed addition of media; NC, Negative Control.

MATERIALS AND METHODS

Animals

Four to eight-week-old C57BL/6 mice of either sex were obtained from the Laboratory Animal Center of Tongji Medical College of Huazhong University of Science and Technology (Wuhan, China). The design and implementation of animal-related activities were adherent to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Isolation and Culture of Mouse Meibomian Gland Explants

After the mice were euthanized, tarsal plates were isolated from eyelids under a dissecting microscope by removing skin, subcutaneous tissue, muscle, and palpebral conjunctiva. The tarsus was then cut into two segments of equal size, each containing approximately six glands. These segments were placed conjunctival side up in 24-well culture plates with defined keratinocyte serum free medium (DKSFM; 10744-019; ThermoFisherScientific, Waltham, MA) sufficient to submerge tissues (Fig. 1A).

Explants were divided into three groups according to different methods: (1) MI: culture plates were coated with 5% Matrigel (8.68 mg/mL, 356234; Corning, Corning, NY) in advance, with immediate addition of medium; (2) BI: culture plates were bare, with immediate addition of medium; and (3) MD: 5% Matrigel with delayed addition of medium (2 hours). Explants were cultured at 37°C with 5% CO2/95% air. The medium was changed every 2 days.

Culture of Primary Mouse Meibomian Gland Epithelial Cells

Using the culture method modified from Richards et al.,14 tarsal plates were excised from eyelids and further digested with 0.25% Collagenase I (C0130; Sigma-Aldrich, St. Louis, MO) at 37°C. After incubation for 40 minutes, the glands of segments were separated under a dissecting microscope. Single glands were then dissociated into a single-cell suspension by 0.25% Trypsin-EDTA (25200-056; Thermo Fisher Scientific) treatment for 5 minutes and cultured on 12-well culture plates coated with 5% Matrigel. Cells were cultured in DKSFM at 37°C with 5% CO2/95% air. The medium was changed every 2 days.

Incubation of Cells and Explants in Different Culture Conditions

After reaching 60% to 70% confluence, primary mouse meibomian gland epithelial cells as well as explants were cultured under 3 conditions for 48 hours separately: DKSFM (proliferation medium [PM]), serum-containing medium composed of 10% fetal bovine serum (10099-141C; Thermo Fisher Scientific) in equal volumes of Dulbecco’s modified Eagle’s medium and Ham’s F12 (SH30023.01; GE Life Science, Marlborough, MA; differentiation medium [DMI]), and DM added with 10 μg/mL azithromycin (AZM; T6401; TargetMol, Wellesley Hills, MA [AZM]).

Treatment of IL-1β on Explants

IL-1β (211-11B; PeproTech, Rocky Hill, NJ) was dissolved in DKSFM to prepare 10 mg/mL stocks. Explants of mouse meibomian gland were exposed to medium with IL-1β (50 ng/mL) or vehicle for 48 hours, according to a study on mouse skin explants.15

MTT Assay

Viability of explants was evaluated by the MTT assay using a technique modified from Gaucher et al.16 Solution of MTT (M2128; Sigma-Aldrich) was prepared at 1 mg/mL in DKSFM medium. After various time of culture, explants were incubated with 500 μl MTT solution for 4 hours under standard culture condition. Then, the supernatant was removed and replaced by 500 μl DMSO and formazan was solubilized for 10 minutes. Eluate was transferred in triplicate to a 96-well plate before optical density (OD) readings were taken at 490 nm (ELX800; BioTech Instruments, Winooski, VT). Fresh meibomian gland tissues were used as the positive control and negative control were prepared by boiling tissues in PBS for 10 minutes.

Histology

Fresh tarsal plates and explants were fixed in 4% paraformaldehyde for 1 hour at room temperature and dehydrated through a series of alcohols before embedded into paraffin for tissue sectioning (8 μm). Then sections were hematoxylin and eosin (H&E) stained for morphological observation on a Leica DFC550 microscope.
**Immunofluorescence Staining**

Fresh tarsal plates and explants were fixed in 4% paraformaldehyde for 1 hour and dehydrated with a series of sucrose before embedded in O.C.T. (4583; SAKURA, Chiba Prefecture, Japan) for sectioning (10 μm).

The sections as well as fixed cells were blocked in 10% donkey serum (ANT051; AntGene, China) for 1 hour, and incubated with rabbit polyclonal antibodies against keratin14 (Krt14; 1:500, ab181595; Abcam, UK), Krt6 (1:300, 905701; BioLegend, San Diego, CA), Krt1 (1:300, 905601’ BioLegend), and peroxisome proliferator-activated receptor γ (PPARγ; 1:100, ARG55241; Arigo) overnight at 4°C. Then samples were stained by Alexa Fluor 488 donkey anti-rabbit IgG antibody (1:5000, ANT020; AntGene) and visualized with ECL reaction kit (P0018; Sigma-Aldrich) were used for nucleus and lipid staining, respectively. Images were obtained with a laser scanning confocal microscope (Nikon, Japan).

**Lipid Quantification**

Lipid quantification was conducted with ImageJ 1.52a software. Immunofluorescence images were transformed into an eight-bit format and inverted. Next, artifacts were eliminated from the images by thresholding. The same threshold setting was used strictly within different groups. Finally, the integrated densities of lipid particles were evaluated.

**Western Blot**

Explants were processed in RIPA lysis buffer (P0013B; Beyotime Biotechnology, Shanghai, China). Proteins were separated by electrophoresis on 12% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. The samples were blocked in 5% milk for 1 hour and then incubated with rabbit polyclonal antibodies against PPARγ (1:1000), Krt1 (1:1000), and β-actin (1:1000, 04-1116; Millipore, USA). Immunodetection was performed with HRP-conjugated goat anti-rabbit IgG antibody (1:5000, ANT020; AntGene) and visualized with ECL reaction kit (P0018; Beyotime Biotechnology). Bands were analyzed by normalizing to the corresponding β-actin bands using ImageJ 1.52a software.

**Statistical Analysis**

All data were presented as means ± SD. One-way ANOVA with post hoc Dunnett t-test or Bonferroni’s test for multiple groups and t-test for two groups were performed for data analyses in SPSS 22.0.0 software. Any P < 0.05 was considered as statistically significant.

**RESULTS**

**Culture of Mouse Meibomian Gland Explants and Epithelial Cells**

To explore a suitable solution for in vitro culture of mouse meibomian glands, we performed 3 different methods on explants for 24 hours. Tissue viability was detected by MTT assay, which showed the relative OD values for the MI group and the BI group were 0.97 ± 0.05 and 0.86 ± 0.08, both had no significant difference compared to that for the positive control group (P > 0.05). The relative OD value was 0.70 ± 0.17 for the MD group and 0.03 ± 0.01 for the negative control group, which were significantly lower than that for fresh tissues (P < 0.05; Fig. 1B, n = 4 in each group).

Thus, higher viability, as determined by the MTT assay, was found when glands were cultured on Matrigel with immediate addition of the medium. This method was selected for explants culturing in our following studies.

As for epithelial cells, approximately (8.07 ± 0.97) × 10^4 viable cells were isolated per mouse (n = 4). Cells reached 70% confluence about 1 week after initial culture and reached 80% confluence in 4 to 5 days following the first passage. The trypan blue assay showed that the viability of cells at the end of each generation were 94% and 91%, respectively (data not shown).

**The Morphology, Biomarker Expression, and Lipid Distribution of Mouse Meibomian Gland Explants**

To confirm that meibomian gland explants still retain their histomorphology and secretory function, we performed paraffin and frozen sections on explants cultured for 24 hours in vitro. Paraffin sections were H&E stained and imaged, demonstrating that explants (Fig. 2A) and fresh tissues (Fig. 3B) had comparable morphology. Furthermore, immunofluorescence staining was performed on cryosections with Krt14, 6, and 1. As shown in Fig. 2, Krt14 was present in all stratified epithelia, including the conjunctiva, the acini, and ducts of meibomian glands (Fig. 2B). Krt6 expression was limited to meibomian gland ductal cells (Fig. 2C), whereas the expression of Krt1 was absent except for the epidermal cells and the orifice of meibomian glands (Fig. 2D). To observe the lipid distribution in explants, Nile Red was also stained on cryosections. The results showed that lipid was not only present in the cytoplasm of acinar cells (Fig. 2E) but also distributed evenly in the lumen of the ductal system (Fig. 2F). The above results indicated that explants cultured for 24 hours can maintain histomorphology and function comparable to fresh tissues.

**Viability of Mouse Meibomian Gland Explants During Different Culture Time**

After detecting the viability of explants cultured for 24 hours, in order to observe whether the tissue viability decreases over time, explants cultured in vitro for 24 to 120 hours were measured by MTT assay. Compared to the positive control group (0 hour), the relative OD value of explants at 24 hours, 48 hours, 72 hours, and 120 hours were 0.97 ± 0.03, 0.94 ± 0.14, 0.89 ± 0.03, and 0.58 ± 0.11 (n = 4 in each group), respectively. No significant differences (P > 0.05) in relative OD value were found between the former three groups and fresh tissues (Fig. 3A). At 120 hours, the viability decreased significantly compared to the control group (P < 0.05; Fig. 3A).

H&E staining of paraffin sections at different time points showed that the general morphology of meibomian glands was maintained until 72 hours without disintegration, despite some acinar cells missing and the arrangement slightly disordered over time (Figs. 3B–3E). More cells appeared vacuole degeneration at 120 hours (Fig. 3F). We also performed immunofluorescence staining on frozen sections of explants at various time points. The meibomian gland epithelial cell marker Krt14 retained strong intensity.
FIGURE 2. Morphology, biomarker expression, and lipid distribution of mouse meibomian gland explants cultured for 24 hours. (A) Morphology of the cultured explants by H&E staining. (B–D) Immunofluorescence of biomarkers in cultured explants. The expression of Krt14 (green, B), Krt6 (green, C), and Krt1 (green, D), with nuclei labeled by DAPI (blue) were examined separately in cryosections. (E, F) Immunofluorescence of Krt14 (green), with Nile Red staining (red) for lipid and nuclei labeled by DAPI (blue) in acini (E) and ducts (F) of cultured explants. Du, duct; Ac, acinus.

until 72 hours (Figs. 3G–3J). At 120 hours, staining of partial acinar cells weakened or even disappeared (Fig. 3K). As expected, tissue viability decreased with time, with higher viability maintained within 72 hours of in vitro culture.

Effects of Serum and AZM on Lipid Accumulation of Mouse Meibomian Gland Explants

Lipid secretion is an important indicator of the maturation of acini in meibomian glands.17 To study the influence of serum and AZM on lipid secretion in mouse meibocytes, explants and epithelial cells were cultured under PM, DM, and AZM separately for 48 hours. MTT assay indicated no significant difference in tissue viability among different media (P > 0.05, n = 4 in each group; Fig. 4A).

For both explants and cells, Nile Red staining showed lowest strength in the PM group (Figs. 4B, 4E) and an apparent increase in size and intensity in the DM group (Figs. 4C, 4F) and the AZM group (Figs. 4D, 4G). We quantified the lipid staining, which confirmed the intensity of the DM and AZM groups were significantly higher than that of the PM group (P < 0.05 for both, n = 6 in each group). Further, the AZM group was also higher than the DM group (P < 0.05; Figs. 4H, 4I).

PPARγ mainly acts as a regulator of lipid synthesis in meibocytes.18 Similar to Nile Red, the fluorescence intensities of PPARγ in the DM group (Figs. 5B, 5E) and the AZM group (Figs. 5C, 5F) were enhanced sharply relative to the PM group (Figs. 5A, 5D) both in explants and cells. PPARγ expressions in explants cultured in DM and AZM were increased by 6.36-fold and 6.33-fold compared to the PM group (P < 0.05, n = 3 in each group; Figs. 5G, 5H). This data, combined with above results, demonstrated that serum and AZM can promote PPARγ expression as well as lipid accumulation in mouse meibomian gland explants.

Impact of IL-1β Treatment on Keratinization of Mouse Meibomian Gland Explants

Hyperkeratinization of the ductal orifice is the main pathological change for obstructive MGD.4 To investigate the influence of inflammation on keratinization of ducts in meibomian glands, mouse meibomian gland explants were treated with 50 ng/mL IL-1β or vehicle as control for 48 hours. MTT assay showed that this proinflammatory cytokine had no significant effect on tissue viability (P > 0.05, n = 3 in each group; Fig. 6A).

As a biomarker for epithelial keratinization,19 the expression of Krt1 was examined in above groups. Compared to vehicle, IL-1β led to an increase by 1.45-fold in the expression of Krt1 of the whole explants (P < 0.05, n = 3 in each group; Figs. 6B, 6C). As illustrated in Fig. 6D, the control group showed no Krt1 staining except the orifice of meibomian glands. While exposed to IL-1β, Krt1 staining intensity was reinforced, along with an expanded range from the lid margin to the central duct (Fig. 6E). Therefore, we found that
A Novel Meibomian Gland Culture System In Vitro

**FIGURE 3.** Viability of mouse meibomian gland explants during different culture time. (A) MTT assay comparison of tissue viability in explants with different culture time (n = 4 in each group). The relative OD values of explants (each performed in triplicate) at 24 hours, 48 hours, 72 hours, and 120 hours compared to that of fresh tissues (0 hour) assessed by MTT assay are shown. (B–F) Morphology of fresh tissues (B) and explants at 24 hours (C), 48 hours (D), 72 hours (E), and 120 hours (F) by H&E staining. (G–K) Immunofluorescence of Krt14 (green), with nuclei labeled by DAPI (blue) in fresh tissues (G) and explants at 24 hours (H), 48 hours (I), 72 hours (J), and 120 hours (K). *Significantly different from the control group (P < 0.05).

**IL-1β mediated increased expression of Krt1 and hyperkeratinization of ducts in mouse meibomian gland explants.**

**DISCUSSION**

Herein, our results demonstrated that the viability, morphology, biomarker expression, and function of meibomian glands were preserved in mouse explants cultured in vitro for up to 72 hours. Lipid accumulation increased in explants cultured in medium containing serum or AZM, which is consistent with the results of cell experiments. In addition, this study also provided evidence for the first time that intervention with proinflammatory cytokine IL-1β induced overexpression of Krt1 in ducts of explants. Thus, we have established an in vitro model of mouse meibomian gland to investigate the mechanism of MGD.

Previous researchers have already shown that various types of organs could be cultured in vitro for days. We have established a similar in vitro model of meibomian gland, which was able to maintain tissue viability higher than 85% until 72 hours. In addition to viability, the morphology, biomarker expression, and function of explants were also maintained to a level comparable to fresh tissues. These results suggested that explants in our study retained considerable metabolic activity and it could be considered as substitute for meibomian gland in vitro. However, we have not studied the impacts of age and sex on the viability, morphology, and biomarker expression of explants, which needs further investigation in our future researches.

Several cell culture systems have been developed for meibomian glands derived from rabbit, mouse, and human. In comparison, organotypic culture of mouse meibomian gland in our study permits one to observe the morphological and functional changes in acini and ducts separately. Previous research has also shown that the culture of mouse meibomian gland epithelial cells may be enhanced and/or dependent on defined concentrations of multiple factors, including insulin, transferrin, epidermal growth factor, keratinocyte growth factor, endothelial cell growth supplement, cholera toxin, hydrocortisone, gentamicin, amphotericin B, bovine serum albumin, and calcium. We have yet to optimize the culture conditions for the meibomian gland explants and cells. Recently, another in vitro slice culture model of the human eyelid was established for studying the function of E-cadherin on meibomian glands. However, this method is not suitable for studying ductal system because it may damage the integrity of meibomian gland ducts.

In hMGECs, both serum and AZM can stimulate lipid synthesis of cells, which is paralleled by cellular maturation and decreased proliferation. To verify these results in our model, we incubated explants and primary epithelial cells in different media for 48 hours. Serum and
AZM significantly promoted lipid accumulation as well as PPARγ expression in acini. PPARγ is a lipid-activated nuclear hormone receptor that regulates lipid synthesis and plays a critical role in both fat and sebaceous gland development.27 In terms of meibomian gland, cell differentiation and lipid synthesis are promoted by PPARγ agonist both in mouse meibocytes18 and hMGECs.28 Therefore, serum and AZM might enhance lipid synthesis through regulating PPARγ signaling pathway. It is worth noting that the staining of PPARγ is primarily suprabasal in fresh tissues,18,28 which is inconsistent with our study. Because meibomian gland explants were examined after culture in vitro for 48 hours, it is possible that basal cells also differentiated during this process. In addition, serum and AZM further promoted maturation of meibocytes, which led to disintegration of the nucleus in some suprabasal cells.

MGD is a group of disorders linked by functional abnormalities of the meibomian glands.1 Sections from human eyelids showed variable amounts of CD45+ leukocyte infiltration within acini significantly associated with the severity of meibomian gland expression grade and meibomian gland dropout.5 In addition, epithelial immune cells and intraglandular immune cells were increased in patients with highly symptomatic MGD indicated by in vivo confocal microscopy.6 Furthermore, compared to healthy participants, the concentration of multiple proinflammatory...
FIGURE 5. Effects of serum and AZM on PPARγ expression of mouse meibomian gland explants and cells. (A–F) Immunofluorescence of PPARγ (green), with nuclei labeled by DAPI (blue) in explants (A–C) and cells (D–F) cultured under PM (A, D), DM (B, E), and AZM (C, F). (G) Representative Western blot result for explants under different media. β-actin was used as the control. (H) Relative PPARγ expression (n = 3 in each group) in explants under different media. *Significantly different from the control group (P < 0.05).

Mediators, such as IL-1β increased in the tear fluid, conjunctiva as well as eyelid margin of patients with MGD. However, the role of inflammation in the pathophysiology of MGD is not entirely understood.

In our study, treatment with IL-1β induced a significant elevation of Krt1 expression at the terminal part of central ducts. Krt1 is one of the best-known markers of fully keratinized epithelium and physiologically detected only in the excretory duct that is lined by an ingrowth of the corneified epidermis from lid margin. When incubation with IL-1β, not only the expression level but also the range of Krt1 has increased in explants, may indicate the hyperkeratinization of ducts. Hyperkeratinization is a major reason for obstructive MGD and causes obstruction of orifice, stasis of meibum, and cystic dilation of the duct that leads to a secondary disuse acinar atrophy and gland dropout. This is the first time that hyperkeratinization of ducts has been induced in meibomian glands in vitro. Hence, the organotypic culture system can be an effective model for studying MGD.
A Novel Meibomian Gland Culture System In Vitro

**FIGURE 6.** Impact of IL-1β on keratinization of mouse meibomian gland explants. (A) MTT assay comparison of tissue viability in explants treated with IL-1β (50 ng/mL) or vehicle as control (n = 3 in each group). (B) Representative Western blot result for explants treated with vehicle or IL-1β. β-actin was used as the control. (C) Relative Krt1 expression (n = 3 in each group) in explants exposed to vehicle or IL-1β. (D, E) Immunofluorescence of Krt1 (green), with nuclei labeled by DAPI (blue) in explants exposed to vehicle (D) or IL-1β (E). *Significantly different from the control group (P < 0.05).

In conclusion, we have established a novel organotypic culture model of mouse meibomian gland, which simulated the hyperkeratinization of ducts. This model might be used for investigating the mechanism of MGD and provide a new approach for exploring future therapeutic strategies on MGD.

**Acknowledgments**

Supported by National Key Research and Development Program of China (2017YFE0103500), National Natural Science Foundation of China (81670824 and 81600708), the Fundamental Research Funds for the Central Universities (HUST: 2019kfyXMBZ065), and Research Foundation of Union Hospital (02.03.2018-221).

Disclosure: **K.K. Xu,** None; **Y.K. Huang,** None; **X. Liu,** None; **M.C. Zhang,** None; **H.T. Xie,** None

**References**

1. Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. Invest Ophthalmol Vis Sci. 2011;52:1938–1978.
2. Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. Exp Eye Res. 2004;78:347–360.
3. Chhadva P, Goldhardt R, Galor A. Meibomian gland disease: the role of gland dysfunction in dry eye disease. Ophthalmology. 2017;124:S20–S26.
4. Gutgesell VJ, Stern GA, Hood CJ. Histopathology of meibomian gland dysfunction. Am J Ophthalmol. 1982;94:383–387.
5. Nien CJ, Massei S, Lin G, et al. Effects of age and dysfunction on human meibomian glands. Arch Ophthalmol. 2011;129:462–469.
6. Qazi Y, Kheirkhah A, Blackie C, et al. Clinically relevant immune-cellular metrics of inflammation in meibomian
A Novel Meibomian Gland Culture System In Vitro

7. Liu S, Hatton MP, Khandelwal P, Sullivan DA. Culture, immortalization, and characterization of human meibomian gland epithelial cells. Invest Ophthalmol Vis Sci. 2010;51:3993–4005.
8. Khandelwal P, Liu S, Sullivan DA. Androgen regulation of gene expression in human meibomian gland and conjunctival epithelial cells. Mol Vis. 2012;18:1055–1067.
9. Ding J, Liu Y, Sullivan DA. Effects of insulin and high glucose on human meibomian gland epithelial cells. Invest Ophthalmol Vis Sci. 2015;56:7814–7820.
10. Liu Y, Kam WR, Sullivan DA. Influence of omega 3 and 6 fatty acids on human meibomian gland epithelial cells. Cornea. 2016;35:1122–1126.
11. Hampel U, Kruger M, Kunnen C, Garreis F, Willcox M, Paulsen F. In vitro effects of docosahexaenoic and eicosapentaenoic acid on human meibomian gland epithelial cells. Exp Eye Res. 2015;140:139–148.
12. Liu Y, Kam WR, Ding J, Sullivan DA. Effect of azithromycin on lipid accumulation in immortalized human meibomian gland epithelial cells. JAMA Ophthalmol. 2014;132:226–228.
13. Han X, Liu Y, Kam WR, Sullivan DA. Effect of brimonidine, an alpha2 adrenergic agonist, on human meibomian gland epithelial cells. Exp Eye Res. 2018;170:20–28.
14. Richards SM, Schirra F, Sullivan DA. Development of a defined, serum-free culture system for the maintenance of epithelial cells from the mouse meibomian gland. Invest Ophthalmol Vis Sci. 2002;43:3150–3150.
15. Naik S, Larsen SB, Gomez NC, et al. Inflammatory memory sensitizes skin epithelial stem cells to tissue damage. Nature. 2017;550:475–480.
16. Gaucher S, Jarraya M. Technical note: comparison of the PrestoBlue and LDH release assays with the MTT assay for skin viability assessment. Cell Tissue Bank. 2015;16:325–329.
17. Sullivan DA, Liu Y, Kam WR, et al. Serum-induced differentiation of human meibomian gland epithelial cells. Invest Ophthalmol Vis Sci. 2014;55:3866–3877.
18. Jester JV, Potma E, Brown DJ. PPARgamma regulates mouse meibocyte differentiation and lipid synthesis. Ocul Surf. 2016;14:484–494.
19. Parfitt GJ, Xie Y, Reid KM, Dervillez X, Brown DJ, Jester JV. A novel immunofluorescent computed tomography (ICT) method to localise and quantify multiple antigens in large tissue volumes at high resolution. PLoS One. 2012;7:e53245.
20. Olivares-Trigo P, Penaf CF, Cavierres MF. A simple short term method to study thyroid disruption using a fetal rat thyroid culture. J Pharmacol Toxicol Methods. 2017;88:19–24.
21. Doussau F, Dupont JL, Neel D, Schneider A, Poulain B, Bossu JL. Organotypic cultures of cerebellar slices as a model to investigate demyelinating disorders. Expert Opin Drug Discov. 2017;12:1011–1022.
22. Munnamalai V, Fekete DM. Organotypic culture of the mouse cochlea from embryonic day 12 to the neonate. Methods Mol Biol. 2016;1427:293–303.
23. Ju Q, Fimmel S, Hinz N, Stahlmann R, Xia L, Zouboulis CC. 2,3,7,8-Tetrachlorodibenzo-p-dioxin alters sebaceous gland cell differentiation in vitro. Exp Dermatol. 2011;20:320–325.
24. Maskin SL, Tseng SC. Culture of rabbit meibomian gland using collagen gel. Invest Ophthalmol Vis Sci. 1991;32:214–223.
25. Rotzer V, Melega F, Garreis F, Paulsen F, Waschke J. E-Cadherin is important for meibomian gland function as revealed by a new human ex vivo slice culture model. Am J Pathol. 2019;189:1559–1568.
26. Xie HT, Sullivan DA, Chen D, Hatton MP, Kam WR, Liu Y. Biomarkers for progenitor and differentiated epithelial cells in the human meibomian gland. Stem Cells Transl Med. 2018;7:887–892.
27. Rosen ED, Spiegelman BM. PPARgamma: a nuclear regulator of metabolism, differentiation, and cell growth. J Biol Chem. 2001;276:37731–37734.
28. Kim SW, Xie Y, Nguyen PQ, et al. PPARgamma regulates meibocyte differentiation and lipid synthesis of cultured human meibomian gland epithelial cells (hMGEC). Ocul Surf. 2018;16:463–469.
29. Solomon A, Dursun D, Liu Z, Xie Y, Macri A, Pflugfelder SC. Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. Invest Ophthalmol Vis Sci. 2001;42:2283–2292.
30. Zhang L, Su Z, Zhang Z, Lin J, Li DQ, Pflugfelder SC. Effects of azithromycin on gene expression profiles of proinflammatory and anti-inflammatory mediators in the eyelid margin and conjunctiva of patients with meibomian gland disease. JAMA Ophthalmol. 2015;133:1117–1123.
31. Landsend ECS, Utheim OA, Pedersen HR, et al. The level of inflammatory tear cytokines is elevated in congenital aniridia and associated with meibomian gland dysfunction. Invest Ophthalmol Vis Sci. 2018;59:2197–2204.