Culture of Rabbit Meibomian Gland Using Collagen Gel

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Rabbit whole meibomian glands were isolated by microsurgical dissection and enzymatic digestion. Both ductal and acinar elements could be separated by subsequent microsurgical manipulation. Epithelial outgrowth could be derived from either the whole gland or the ductal/acinar elements using collagen gel in the serum-containing, growth factor-enriched medium. Two types of outgrowths were observed: a discontinuous spindle-shaped epithelial cell monolayer in which oil-red-O (ORO)-stained meibum containing single cells and cell aggregates were evenly distributed, and a continuous cuboidal epithelial monolayer in which oil-red-O-stained cells formed a gradient with single cells and cell aggregates preferentially distributed in the periphery of the outgrowth. Thus, meibum production and meibomian gland differentiation can be maintained in collagen gel culture and ductal elements may contain progenitor cells for acinar differentiation. This culture system will allow future exploration of the regulatory mechanism of meibomian gland differentiation at the cellular level. Invest Ophthalmol Vis Sci 32:214-223, 1991

The meibomian glands are modified sebaceous glands located within the tarsus of the eyelid. Each gland consists of a main excretory duct that connects with numerous ductules that end as acini. Each acinus is outlined by the basal epithelium that proliferates and differentiates into meibocytes. Through holocrine secretion, meibocytes produce meibum lipids that are secreted onto the lid margin. Meibum, possibly together with lipid secreted by the glands of Zeis, constitutes the superficial layer of the precorneal tear film. Sufficient quantity and quality of this lipid layer stabilizes the tear film by lowering the surface tension at the air-aqueous interface.

Meibomian gland dysfunction is commonly associated with several clinical disorders, including blepharitis, rosacea, seborrhea and hordeolum/chalazion. Patients with these disorders have symptoms of ocular irritation due to an unstable tear film. Specific and effective therapy for meibomian gland dysfunction does not exist. Improved therapy may only be developed through a better understanding of the normal regulatory mechanisms of meibum secretion at a cellular level.

In past research, animal models of meibomian gland dysfunction have been induced by systemic treatment with polychlorinated biphenyls in monkeys or topical epinephrine treatment in rabbits, or by systemic 13 cis-retinoic acid treatment in hamsters and rabbits. These animal models have demonstrated that meibomian gland dysfunction is associated with abnormalities in cellular proliferation and differentiation. However, the intrinsic complexity of the in vivo system precludes direct investigation of the pathogenesis of meibomian gland dysfunction at a cellular level.

We sought to establish a cell culture system for meibomian glands. Previously, only a short-term organ culture has been established for human epidermal sebaceous glands. A culture of avian uropygial glandular epithelium, which has a holocrine mechanism of secretion, has been reported. Several culture systems have been developed for other secretory epithelia derived from mouse mammary glands, mouse submandibular glands, rat cervix, rabbit trachea, porcine thyroid, and rat liver. The success of these culture systems is due to the use of collagen gel, which allows in vitro epithelial growth and differentiation similar to that which occurs in vivo. Based on the above reports detailing culturing of non-sebaceous glandular epithelia, we report herein an in vitro culture system developed for rabbit whole meibomian glandular epithelia, as well as for isolated ductal and acinar glandular elements.
Materials and Methods

Dulbecco’s modified Eagle medium (DMEM), Ham’s F12 supplement, and fetal bovine serum were obtained commercially (GIBCO, Grand Island, NY). Dimethylsulfoxide (DMSO) (95%), mouse epidermal growth factor (mEGF), bovine insulin, cholera toxin and ethylenediamine tetraacetic acid, disodium salt (EDTA), and fluorescein isothiocyanate (FITC)-conjugated F(ab)2 fragment of goat-anti-mouse antibody, were obtained commercially (Sigma Chemical Company, St. Louis, MO). Dispase II and collagenase A (Boehringer-Mannheim, Indianapolis, IN), trypsin (DIFCO, Detroit, MI), and anti-vimentin monoclonal antibody were obtained (DAKO, Santa Barbera, CA). Vascular endothelial cells from a tissue culture of rat thoracic aorta were provided by Dr. Una Ryan, and AE1 anti-keratin antibody was provided by Dr. T-T. Sun.

Microsurgical and Enzymatic Separation of Whole Meibomian Gland or Isolated Ductal or Acinar Elements

New Zealand white rabbits of either sex, weighing 2-3 kg, were anesthetized with an intramuscular injection of 30 mg xylazine hydrochloride and 30 mg ketamine hydrochloride. One drop of gentamicin sulfate solution was applied in the conjunctival cul-de-sac, which was then rinsed with betadine*-balanced salt (1:1) solution. Using a sterile technique, the anterior lid skin and muscle lamella were removed using Wescott® scissors and tissue forceps (Storz Instruments, St. Louis, MO), leaving intact the posterior lid lamella including the meibomian gland-embedded tarsal connective tissue and conjunctival mucosa. This posterior lamella was then excised and rinsed in a balanced salt - gentamicin (1:1) solution. The animal was killed with an intravenous overdose of sodium pentobarbital.

Under a dissecting microscope, the posterior lamella was cut with a razor blade into several segments, each containing approximately three meibomian glands. To isolate individual whole meibomian glands, various enzyme solutions were surveyed for their capability of completely digesting the dense tarsal connective tissue. This endpoint would be signaled by gland flotation due to low specific gravity of meibum lipid. Each three-gland segment was placed in an autoclaved eppendorf tube containing 1 ml of various filtered enzyme solutions in DMEM/F12 (1:1). The first set of tested enzyme solutions included: (1) collagenase A, 0.1%; (2) collagenase A, 0.01%; (3) collagenase A, 0.025%, trypsin, 0.025%, and EDTA, 0.25 mM; (4) collagenase A, 0.05%, trypsin, 0.05%, and EDTA, 0.5 mM; (5) collagenase A, 0.05%, and Dispase II, 0.6 u/ml; and (6) control containing DMEM/F12 1:1 only. The tubes were placed in a 37°C shaker water bath for 24 hr.

To reduce incubation time and achieve a more complete digestion, additional enzyme systems were evaluated. These included: (7) collagenase A 0.5% and Dispase II 0.6 u/ml, and (8) collagenase A, 0.25% and Dispase II, 0.6 u/ml. After incubation for a desired period of time, the partially digested segments were removed and rinsed in enzyme-free culture media consisting of an equal volume of Hapes buffered DMEM with bicarbonate and Ham’s F12, supplemented with 0.5% DMSO, 2 ng/ml mEGF, 1 ug/ml bovine insulin, 0.1 ug/ml cholera toxin, and 5% fetal bovine serum. The three glands of each segment were separated with a siliconized razor blade.

To study separated ductal and acinar elements of meibomian glands, the meibomian gland was obtained in the same manner as described above, and also evaluated using collagenase A 0.25% alone. The isolated whole gland was transferred into a sterile petri dish. Under a phase contrast microscope, the acinar elements were separated from the ductal elements by cutting at the ductule-acini junction with a razor blade.

Preparation of Collagen Gel

Collagen gel was prepared according to the method of Elsdale and Bard,24 with minor modifications. Specifically, rat tail tendons were obtained and sterilized in 70% ethanol at 4°C for 24 hr. Collagen was solubilized by stirring the tendons for 48 hr at 4°C in a sterile 0.1% acetic acid solution (150 ml/1 gram of tendon). The solution was filtered through sterile gauze to remove the unsolubilized tissue debris. After centrifugation at 16,000 x g for 1 hour at 4°C, the supernatant was collected as stock solution and stored at −20°C before use.

Culture of Whole Meibomian Gland or Isolated Ductal or Acinar Elements

Whole meibomian glands or isolated acinar and ductal elements were transferred to different culture dishes. The ice-cold collagen solution, which had just been thawed, was mixed simultaneously at 4°C to raise the pH to 7.2 with 0.34 M NaOH and 5X concentrated DMEM in a volume ratio of 8:1:2. This cold mixture was then dispensed into the culture dishes containing preplaced whole glands or isolated elements, and the dishes were incubated at 37°C for 1 hour to allow collagen gelation. Some isolated ducts were placed on the collagen gel after gelation. After overlaying with fresh media, the cultures were incubated in humidified 5% CO2/95% air at 37°C. The media were changed every 2-3 days.

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Morphologic Studies

To evaluate which cellular components were preserved after enzymatic/microsurgical manipulation, isolated whole meibomian glands or separated ducts were fixed in 2.5% glutaraldehyde, embedded in epon, cut into 1-2 μm thick sections and stained with 1% toluidine blue for 1 ½ min.

To assess which whole meibomian glands or isolated elements after various enzymatic treatments showed cellular outgrowth after culture, the post-cultured glands, together with the surrounding collagen gel, were also fixed in 2.5% glutaraldehyde, processed, and stained as above. For flat-mount preparation, the culture was terminated by fixing in formalin, followed by rinsing with distilled water. The culture explants, including the surrounding collagen gel, were excised with a razor blade and transferred onto a gelatin-coated glass slide.

Oil-Red-O (ORO) Staining

To detect meibum production, isolated glands or their elements as well as flat-mount preparation of their culture outgrowths in collagen gel, were submitted to ORO staining, which gives an orange-red color for lipid-laden cells. The staining procedure began with immersion in 100% propylene glycol for 2 min, and then 0.5% ORO in propylene glycol for 30 min. Then, 85% propylene glycol was applied for 1 min to eliminate non specific staining.

To verify the specificity of the ORO staining for lipid-laden cultured cells, we compared the staining of the meibomian gland culture with the staining of a positive control specimen of human sebaceous cell carcinoma and three negative control specimens, including rabbit conjunctival fibroblasts, rabbit conjunctival epithelial cells, and rat aorta endothelial cells. After surgical excision, a specimen of human sebaceous cell carcinoma was cut into approximately 3 × 3 × 3 mm pieces and placed into culture dishes. DMEM/F-12 medium with 20% fetal bovine serum was added in a low volume to prevent explant flotation. After the outgrowth of fibroblasts and epithelial cells became evident, the media was switched to serum-free. Neoplastic epithelial cells could be identified by their atypical nuclei and sebaceous differentiation. The outgrowth of the explant onto the plastic dish was used for ORO staining. Rabbit conjunctival fibroblasts were obtained from conjunctival explant outgrowth onto collagen gel in culture media of DMEM/F-12 with 5% fetal bovine serum. Rabbit conjunctival epithelial cells grown on collagen gel were obtained in the same way as previously described. Vascular endothelial cells at the passage of 33 to 36 were derived from a tissue culture of rat thoracic aorta. These cells were plated on collagen gel, and cultured in DMEM/F-12 with 5% fetal bovine serum. All culture media were changed every 2–3 days.

Fluorescent Studies

To verify that the outgrowing cultured cells were indeed of an epithelial origin, nonfixed frozen sections of meibomian gland and acinar elements cultures on gelatin-coated slides were rinsed with PBS pH 7.2 and then overlaid for 1 hr with undiluted hybridoma supernatant containing AE1 anti-keratin or 1:5 anti-vimentin monoclonal antibody. No blocking agent was used. After a PBS rinse, the slides were incubated with 1:200 fluorescein isothiocyanate (FITC) conjugated F(ab)2 fragment of goat-anti-mouse antibody for 1 hr, and then visualized with a Zeiss axiophot microscope equipped with epi-fluorescence capacity. For control, the same specimens were also stained with the supernatant of the myeloma parental secretor. Positive control for vimentin antibody was rabbit skin fibroblasts.

Results

Epithelial Components Preserved After Enzyme Digestion

After a 24 hr incubation in the first set of enzyme solutions (1–5), no glands were floating. However, partial digestion of tarsal connective tissue occurred in solutions (1) and (5), which contained at least 0.05% collagenase without the inhibition by EDTA. The control solution (6) without any enzyme did not show any connective tissue digestion. Attempts at surgical separation were unsuccessful without injuring the glands.

Additional enzyme systems (7 and 8) were tested to determine the incubation time could be reduced with a more complete digestion. By 20 hr of incubation, both solutions showed gland flotation. Therefore, the milder solution (h), 0.25% collagenase A and 0.6 u/ml Dispase II, was chosen for subsequent experiments. A 20 hr digestion produced glandular remnants consisting of the main duct, branching ductules, and numerous sac-like termini (Fig. 1A). ORO staining of this isolate indicated that meibum was well retained (Figure 1B). To evaluate the extent of preservation of epithelial cell components, thick-section histologic study of the above isolate showed preservation of primarily intraductal meibum (indicated by *) and ductal lamellar sheet lining the external aspect of the duct (indicated by arrow) (Figure 1C). The term “lamellar sheet” was used previously in the detailed description of the meibomian gland. No dis-
crete acini were identified, although rare detached epithelial cells of unknown source were noted (Figure 1C). To determine whether more epithelial components could be preserved, the incubation time was reduced. Six hour incubation preserved intact basal and suprabasal acinar epithelium; however, intercellular adhesion of the ductular epithelia was lost and ductal epithelia were completely detached from the ductal lamellar sheet. Four hour incubation preserved intact ductular and ductal epithelium with only mild intercellular separations at the duct–ductule junction (Figure 1D, E). Further shortening of incubation to 2 hr preserved intact acinar and ductal epithelium and also a large amount of undigested surrounding connective tissue containing fibroblasts. These results indicate that incubation for 4 hr is optimal for the isolation of whole meibomian glands.

To isolate ductal and acinar elements, the 4 hr whole-gland incubation in 0.25% collagenase A plus 0.6 u/ml Dispase II was followed by surgical separa-

tion at the ductule–acinar junction. This method preserved the intraductal meibum and ductal lamellar sheet, but the most of the epithelial cells were detached (Fig. 2A). For this reason, Dispase II was eliminated from the enzyme solution. Four-hour incubation in 0.25% collagenase A alone preserved nearly all suprabasal epithelium (Fig. 2B). (For ductal epithelium, basal cells lie external to suprabasal 2.) Incubation for 3 hr preserved a poorly adherent multilayer epithelium and possible rare acinar epithelium (Fig. 2C). The 2 hr incubation preserved a cohesive, intact multilayered epithelium, a trace amount of acinar epithelium, and surrounding connective tissue (Fig. 2D). These results indicate that the epithelial components were vulnerable after the enzymatic digestion used for whole meibomian gland isolation and could not withstand additional microsurgical manipulation during the isolation of ductal or acinar elements. By eliminating Dispase II and reducing the incubation time, it became feasible to isolate glandular elements.
Culture of Whole Meibomian Glands and Isolated Elements

To assess the growth potential of isolated whole glands, a collagen gel culture was performed. After a 16–20 hr digestion in 0.25% collagenase A and 0.6 u/ml Dispase II, the isolated glands did not show any outgrowth after 2 wk in serum-containing culture media enriched with growth factors (Fig. 3A, B). In fact, the glands underwent gradual degeneration with loss of some ductule-acini units (c.f. Fig. 3A, B, arrows). ORO staining of the post-cultured gland verified the retention of meibum lipid. In a 2 wk culture of 18 explants, we observed one (5.5%) outgrowth. These results indicate that the 16–20 hr digestion did not preserve those epithelial cells with proliferative capacity, a finding consistent with the above histologic study (Fig. 1). The sensitivity and specificity of ORO staining were confirmed by the negative staining findings in cultures of rat thoracic aorta endothelium, rabbit conjunctival epithelium, and rabbit conjunctival fibroblasts (Fig. 3C) and by the positive staining for human sebaceous carcinoma cells (Fig. 3D).

When the enzyme incubation time was reduced to 2–6 hr, we found that the outgrowth frequency during a 2-wk culture increased to 10 of 15 (67%) explants, a significant increase compared with the 5.5% outgrowth frequency after 16–20 hr of incubation ($P = .001$). Two patterns of explant outgrowth were seen. Most of the outgrowth morphologic characteristics seen in all 10 growing whole gland cultures was characterized by a discontinuous monolayer of spindle-shaped epithelial cells interspersed with multiple
Fig. 3. The gland remnant from a 16–20 hour digestion in 0.25% collagenase A and 0.6 U/ml Dispase II before (a) and two weeks (b) after collagen gel culture showing the gradual degeneration with loss of some ductule-acinar units (arrows). The negative control for ORO staining was from rat thoracic aorta endothelium (c). The positive control is human sebaceous carcinoma cells (d) (phase contrast, original magnification ×32 (a–c), ×64 (d)). The bar in (a) measures 50 μm and may be used for (b, c). The same bar used for (d) measures 25 μm.

cell aggregates (Figs. 4A, B). Throughout the monolayer were individual large cellular inclusions that gave a dark density under phase contrast microscopy (Figs. 4A, B). These inclusions were of a higher concentration, forming clusters in the cell aggregates. On ORO staining, the cell aggregates yielded a pinkish-orange coloration, and the cellular inclusions stained red (Figs. 4C, D). A second growth pattern was of a continuous cuboidal epithelial monolayer sheet seen in one of ten (10%) growing whole gland cultures. This type of sheet growth also contained individual dark cellular inclusions at a higher concentration in the sheet periphery (Fig. 4E). Epithelial cell aggregates, although few in number, were also identified in the sheet periphery (Fig. 4E). The cell aggregates were stained pinkish-orange by ORO, and the central sparse dark inclusions as well as the peripheral denser dark particles stained orange-red (Fig. 4F, G). This distribution of a centrifugal gradient of ORO-stained single cells and cell aggregates toward the periphery of the sheet growth suggests that cellular differentiation takes place along with cell proliferation during the explant outgrowth.

To assess the growth potential of the isolated ducts and acini, the isolated elements were also cultured for 2 wk using collagen gel. After 4 hr incubation in 0.25% collagenase A with 0.6 U/ml Dispase II or 0.25% collagenase A alone followed by microsurgical separation, the isolated ducts yielded no growth in a total of 30 and 4 explants, respectively. When the incubation in 0.25% collagenase A alone was reduced to 3 or 2 hr, the outgrowth frequency was significantly increased to 5 of 9 (56%) and 5 of 14 (36%) explants, respectively (p < .005). After 4 hr incubation in 0.25% collagenase A with 0.6 U/ml Dispase II or 0.25% collagenase A alone followed by microsurgical separation, the isolated acini yielded 1 growth in 13 (8%) explants and no growth in 5 explants, respectively. Reduction of incubation time to 3 or 2 hr increased the outgrowth frequency to 6 of 18 (33%) and 8 of 12 (67%) explants, respectively (P < .05). The outgrowth morphologic features of both ductal and acinar elements (Figure 4H, I) were similar to those of whole gland cultures (Figs. 4B, G). The pattern of a discontinuous monolayer of spindle epithelioid cells interspersed with multiple cell aggregates was seen in all 10 growing ductal explants and in 12 of 14 growing acinar explants. The second outgrowth pattern of a continuous epithelial sheet was also seen in 4 of 10 growing ductal explants and in 8 of 14 growing acinar explants. ORO staining of these cultures confirmed the presence of meibum in single
cells, with clustering at sites of cell aggregates, the same as those in whole gland cultures.

To verify that the outgrowth was of an epithelial origin, frozen sections of one whole gland culture and one acinar culture were stained for immunofluorescence with AE1 anti-keratin monoclonal anti-

Fig. 4. Culture outgrowth of rabbit meibomian gland in collagen gel. Type 1 morphology showing a discontinuous monolayer of spindle-shaped epithelial cells with interspersed cell aggregates (a, b). Note the numerous dark cellular inclusions throughout the monolayer, but most concentrated at the cell aggregates (a, b arrow). ORO stained the cell aggregates pinkish-orange, and the dark cellular inclusions red (c, d). The explant was at the upper right hand corner in a, c. Type 2 morphology showing a continuous monolayer of epithelial cells (e–g). The explant was inferior in the micrograph; the arrow directs to the extension of the outgrowth. There was a gradient of dark cellular inclusions, sparse centrally and dense peripherally. Few cell aggregates are seen in the periphery of the outgrowth. In (f), the ORO staining of (e) demonstrates the same type of staining as seen in (c). There is a greater density of meibum in the periphery than the central part of the outgrowth (f, g). In the isolated acinar or duct element culture (h), there was also Type 1 morphology of a discontinuous monolayer of spindle shaped epithelial cells and cell aggregates. Note the scattered differentiated cells (arrow) as well as the cluster of differentiated cells over the cell aggregates (arrowhead). Type 2 morphology of the continuous epithelial sheet could be identified containing scattered single differentiated cells (i, arrow), as well as a cluster of differentiated cells over a cell aggregate (i, arrowhead) (original magnification ×12.8 (a, c, e, f), ×32 (b, d, g, h) and ×64 (i)). The bar in (a) measures 0.2 mm and may be used for (c), (e) and (f). The bar in (b) measures 50 μm and may be used for (d), (g), and (h). The bar in (i) measures 25 μm.
body. Positive staining was noted throughout the section decorating the epithelial cells (Figs. 5A,B,E) confirmed with phase contrast micrographs (Figs. F,G), as compared with the control (Figs. 5C,D). To verify the lack of fibroblast contamination, acinar outgrowth frozen sections and in situ whole gland cultures on plastic (unpublished results) were stained for immunofluorescence with anti-vimentin monoclonal antibody. No staining was noted compared with positive control using rabbit skin fibroblasts.

**Discussion**

We developed a method for establishing an in vitro culture system for rabbit meibomian gland. Through the course of experiments, we noted that it was essential to use collagenase-containing enzymatic digestion to isolate intact whole glands. The incubation time was critical. Prolonged incubation up to 20 hr in collagenase A 0.25% and Dispase II 0.6 u/ml preserved only meibum without epithelial cells. This finding was confirmed by histologic findings and poor culture growth. This preparation, although not useful for subculture, may be valuable for obtaining a large quantity of uncontaminated meibum lipids. This new method of meibum lipid preparation could be used for biochemical analysis of its lipid composition, and the data could then be compared with earlier published results. Reducing the incubation time from 20 to 2-6 hr preserved more epithelial components and yielded a significantly higher outgrowth rate. For the isolation of viable ductal and acinar elements for subculture, we further confirmed that preservation of basal epithelial was crucial as the results of various enzyme incubation times were compared (Figs. 1 and 2). These data indicate that the basal epithelia possess proliferative capacity. In contrast to the isolation of whole glands, for ductal and acinar elements, elimination of Dispase II and reduction of incubation time were important. We attribute this further loss of basal epithelial cells to the additional microsurgical manipulation that was required to obtain the isolated elements. This is probably because the initial enzyme treatment had rendered the whole gland isolates vulnerable to subsequent microsurgical manipulation.

For ductal element isolation, reduction of enzyme incubation to 2 hr had not further improved, but on the contrary, decreased the growth rate compared with that of the 3 hr incubation. Under the former condition, we noted poor surgical visualization of the ductal structures due to the residual connective tissue; surgically induced damage was inevitable. However, the experience was not the same for isolating acinar elements because, as the acini are termin structures, surgical visualization was not significantly impaired at 2 hr, as compared with that at 3 hr. This may explain why the outgrowth rate of ductal culture was best after 3 hr of incubation but that of acinar culture was best after 2 hr of incubation.

Several reports of in vitro culturing of secretory epithelia have shown that their successes can be attributed to the use of a collagen gel extracellular matrix as growth substrate. By doing so, epithelial growth and differentiation in vitro can mimic their in vivo counterparts. In this report, we also found that collagen gel was useful in establishing the culture growth for the whole rabbit meibomian gland as well as for isolated ductal and acinar elements. In the case of meibomian glands, the use of collagen gel to embed the isolates offers the additional advantage of keeping the glands from floating.

Two morphologic patterns of outgrowths were observed in this culture system. Type I was a discontinuous spindle-shaped epithelial cell monolayer growth that had interspersed cell aggregates with ORO-positive inclusions. This pattern was noted in 32 of 34 (94%) cumulative growing cultures (whole gland plus duct and acini). Type II was a continuous epithelial monolayer growth with ORO positivity predominantly in the periphery. This pattern was noted in 13 of 34 (38%) cumulative growing cultures (whole gland plus duct and acini). These results suggest that there was intracellular de novo meibum production in the explant outgrowth. We speculate that the Type I growth pattern may represent the acinar cell differentiation due to its scattered and even distribution of meibum-containing cells in the monolayer. The finding that more than over 90% of culture growths showed there morphologic features suggests a predominance of acinar cell differentiation. Type II growth pattern of the continuous epithelial monolayer may represent a predominant ductal cell differentiation. Because numerous meibum-containing cell aggregates developed from isolated duct as well as in the periphery of the whole gland growth, we speculate that the isolated ductal elements may give rise to acinar cell differentiation early on, or after a stage of predominant ductal epithelial monolayer growth. This interpretation implies that there may be a common stem cell for ductal and acinar epithelium, a concept previously reviewed by Montagna for epidermal sebaceous glands. To resolve this issue definitively, it is important to develop specific monoclonal antibody markers for these two types of cell differentiation. Although we were impressed with the growth potential and meibum differentiation of the whole gland, duct, and acinar explants, we did not show direct evidence of cellular proliferation or de novo meibum synthesis. One approach to verify its prolif-

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Fig. 5. Immunofluorescent staining of the whole meibomian gland (a, e at high magnification) and acinar (b) culture using AE1 antikeratin monoclonal antibody showing the diffuse positive staining of Type I (b) and Type II (a) culture morphology. The same specimen stained with the supernatant of the myeloma parental secretor was negative (c, d). Phase contrast micrographs (f, g) confirm cells throughout the sections immunoreacted (original magnification X32 (a, f), X128 (b-e, g). The bar in (a) measures 50 um and may be used for (c). It may also be used for (b, d, e, f, g) where it measures 12.5 um.
erative and differentiative capacity is to establish a single cell clonal growth culture derived from these isolated glands. Our preliminary results supported this observation. In conclusion, we believe that establishing this in vitro culture system for meibomian glands will provide a model to explore the regulatory mechanisms of meibum production and meibomian gland epithelial proliferation and differentiation at the cellular level. Through such explorations, we hope to better understand the pathogenesis of various models of meibomian gland dysfunction and to develop effective treatments for such clinical disorders.

Key words: collagen gel, epithelium, meibomian gland, meibium, organ culture, sebaceous gland

References