Application of In Vivo Confocal Microscopy in Dry Eye Disease

Yukihiro Matsumoto and Osama M. A. Ibrahim

Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan

Confocal microscopy is a new, emerging, noninvasive technology that can aid in the in vivo assessment of structural changes in several ocular surface diseases at the cellular level. In the dry eye field, in vivo confocal microscopy has been applied to the examination of the cornea, bulbar and palpebral conjunctiva, Meibomian gland, and lacrimal gland. The device can assess the morphology, including superficial/wing/basal epithelial cell density, stromal keratocyte density, endothelial cell density, nerve fiber density, the number of beadings, nerve tortuosity, nerve reflectivity, and inflammatory cell density in the cornea. Furthermore, the device can not only assess epithelial cell density and area, goblet cell, microcyst, and inflammatory cell density but also the cellular architecture, including nucleocytoplasmatic ratio in conjunctiva. The device also can disclose acinar unit density, acinar unit longest diameter, acinar unit shortest diameter, and inflammatory cell density in the Meibomian gland and lacrimal gland by other potential applications. Relevant research in Europe and the United States focused on the morphologic changes in the cornea in the dry eye field, while Japanese research focused on the conjunctival, Meibomian gland, and lacrimal gland alterations. The application of in vivo confocal microscopy in dry eye disease will be a powerful method to evaluate the morphologic change of the ocular surface around the world in the future.

Keywords: confocal microscopy, dry eye, cornea, conjunctiva, Meibomian gland, lacrimal gland

IN VIVO CONFOCAL MICROSCOPY

Confocal microscopy is a noninvasive technology that is useful as a supplementary diagnostic tool for the in vivo assessment of the histopathology of many ocular surface diseases and anterior-segment disorders at cellular level.1-6 Notably, the application of in vivo confocal microscopy in the real-time diagnosis of infectious keratitis, such as Acanthamoeba and fungal keratitis has been immensely useful.3,4 The first generation confocal microscopy has been developed from an in vivo white-light through-focusing confocal microscope (Tandem Scanning Corp., Reston, VA, USA), and advanced to an in vivo white-light slit-scanning confocal microscope (ConfoScan, Nidek Technologies, Vigonza, Veneto, Italy) (second generation). Recently, examination of the cornea by a new generation of in vivo laser-scanning confocal microscope (Heidelberg Retina Tomograph, Rostock Corneal Module [HRT-RCM]; Heidelberg Engineering GmBH, Heidelberg, Baden-Württemberg, Germany) has been reported to yield impressive, high-quality images in many corneal pathologies. In the dry eye field, in vivo confocal microscopy has been applied for the examination of the cornea, bulbar and palpebral conjunctiva, meibomian gland, and lacrimal gland.7-15

CORNEA: DRY EYE DISEASE ASSOCIATED WITH SjÖGREN’S SYNDROME

Epithelium

Many corneal in vivo confocal microscopic findings have been reported from Europe and the United States in dry eye disease associated with Sjögren’s syndrome (SS) and with other aqueous tear deficiency dry eye diseases. In the previous reports by Benitez-del-Castillo et al.7,8 from Spain during 2004 through 2007 and Villani et al.9,10 from Italy during 2007 through 2008, the corneal superficial epithelial cell density was shown to be significantly decreased in SS patients (741 ± 306 to 971 ± 262 cells/mm² and 965.4 ± 96.0 to 993.1 ± 104.8 cells/mm², respectively) compared with the healthy control subjects (1431 ± 283 to 1528 ± 341 cells/mm²; 1485.6 ± 133.7 to 1511.6 ± 136.0 cells/mm², respectively) by using ConfoScan. Villani et al.9,10 reported a significant increase in the corneal basal epithelial cell density in SS patients (83, 5980.3 ± 192.6 to 6261.1 ± 168.4 cells/mm²; control, 5602.2 ± 234.9 to 5861.7 ± 260.4 cells/mm²); however, Benitez-del-Castillo et al.7,8 reported no significant differences between the SS patients and the healthy control subjects (83, 5744 ± 627 to 6173 ± 634 cells/mm²; control, 5783 ± 841 to 5858 ± 702 cells/mm²). Erdélyi et al.16 reported that the superficial and wing epithelial cell densities in the cornea were significantly smaller in the patients with aqueous tear deficiency dry eye (843 ± 198 cells/mm² and 5028 ± 582 cells/mm², respectively) than in the normal subjects (1212 ± 242 cells/mm² and 5804 ± 513 cells/mm², respectively) with HTRCM from Hungary in 2007. There was no significant difference in the corneal basal epithelial cell density between the aqueous tear deficiency dry eyes (9131 ± 1701 cells/mm²) and the controls (9858 ± 990 cells/mm²). In light of the previous literature, the corneal superficial epithelial cell density is thought to decrease in dry eye diseases (Fig. 1); however, it is still not clear whether the wing and basal cell densities change. The superficial epithelial cell loss is considered to be caused by apoptotic cell death in severe aqueous-deficient dry eye diseases.
Stroma

In the previous reports, both Benitez-del-Castillo et al.7,8 and Villani et al.9,10 showed a significant increase in corneal anterior keratocyte density in SS patients (1322 ± 156 to 1348 ± 220 cells/mm² and 1226.5 ± 69.8 to 1274.9 ± 32.7 cells/mm², respectively) compared with the healthy control subjects (1062 ± 183 to 1107 ± 210 cells/mm² and 970.3 ± 104.5 to 1098.4 ± 80.4 cells/mm², respectively). Villani et al.9,10 reported a significant increase in the corneal posterior keratocyte density in SS patients (SS, 835.5 ± 64.7 to 854.5 ± 44.7 cells/mm²; control, 702.2 ± 79.2 to 797.9 ± 42.0 cells/mm²). Nevertheless, Benitez-del-Castillo et al.7,8 reported no significant changes between SS patients (808 ± 117 to 815 ± 131 cells/mm²) and the healthy control subjects (722 ± 99 to 741 ± 142 cells/mm²). In short, it is still unknown whether the keratocyte density increases or decreases in dry eye diseases.

Endothelium

A thorough PubMed search revealed that no confocal microscopy studies have been reported in regard to the corneal endothelial cell density in patients with SS. Erdélyi et al.16 reported no changes in endothelial cell density in the patients with aqueous tear deficiency dry eye (2572 ± 656 cells/mm²) compared with control subjects (3193 ± 1042 cells/mm²). In another study using HRT-RCM, Kheirkhah et al.17 showed a significant decrease in corneal endothelial cell density in dry eye patients (2595.8 ± 356.1 cells/mm²) compared with control subjects (2812 ± 395.2 cells/mm²). The change in corneal endothelial cell density in dry eye is not yet clear. Corneal endothelial changes can occur in association with corneal epithelial disease, but this hypothesis is worthy of investigation in the future.

Inflammatory Cells

Lin et al.18 showed that the inflammatory dendritic cell density increased dramatically in the corneal epithelium in dry eye patients with non-SS and SS from China in 2010 (non-SS, 89.8 ± 10.8 cells/mm²; SS, 127.9 ± 23.7 cells/mm²; control, 34.9 ± 5.7 cells/mm²). Machetta et al.19 showed that the inflammatory dendritic cell density was significantly different in the subbasal epithelium between non-SS and SS patients (non-SS, 22 cells/mm²; SS, 79 cells/mm²). Kheirkhah et al.20 also reported that inflammatory dendritic cell density at the subbasal epithelial area significantly increased in the aqueous-deficient immunologic dry eye patients (239.6 ± 52.9 cells/mm²), such as SS and graft versus host disease (GVHD), compared with the aqueous-deficient nonimmunologic dry eye patients (78.6 ± 17.6 cells/mm²), evaporative dry eye patients (58.9 ± 9.4 cells/mm²), and controls (19.6 ± 2.8 cells/mm²).

The dendritic antigen-presenting cells play a critical role in corneal immunology and have been observed by in vivo confocal microscopy in both healthy and affected corneas. Dry eye can induce the upregulation and secretion of proinflammatory mediators such as TNF-α, IL-1β, and matrix metalloproteinase 9 into the tear film by the ocular surface epithelium.21,22 The migration and maturation of dendritic cells are activated in response to proinflammatory stimulation. The dramatic increases of dendritic cells in the corneal epithelium are thought to have a role in dry eye pathophysiology (Fig. 2).

Corneal Nerve Density

A significant decrease of the subbasal nerve numbers in the SS patients was reported by Benitez-del-Castillo et al.7,8 (SS, 2.7 ± 0.8 to 2.8 ± 1.2 nerves/frame; control, 4.6 ± 0.8 to 4.6 ± 0.8 nerves/frame) and Villani et al.9,10 (SS: 3.2 ± 0.7 to 3.3 ± 0.81 nerves/frame; control: 5.0 ± 0.8 to 5.1 ± 0.8 nerves/frame). However, both Tuominen et al.24 using Tandem Scanning in 2003, and Tuisku et al.25 using ConfoScan in 2008 from Finland, reported no significant differences between the SS patients (5.4 ± 1.8 nerves/frame, 5.9 ± 2.2 nerves/frame, respectively) and control subjects (5.0 ± 1.4 nerves/frame, 6.1 ± 2.5 nerves/frame, respectively). Benitez-del-Castillo et al.7,8 also reported a significant decrease of the subbasal nerve density in the SS patients (SS, 508 ± 128 to 511 ± 106 µm/frame; control, 769 ± 88 to 787 ± 105 µm/frame). Xu et al.26 showed that the corneal sensitivity decreased in dry eyes with SS. Benitez-del-Castillo et al.9 Villani et al.10 and Labbé et al.27 reported that there was a statistically significant correlation between the corneal sensitivity and the corneal subbasal nerve number or density. However, Tuisku et al.25 reported no correlation between the corneal sensitivity and the corneal subbasal nerve density. The nerve fibers have an important influence in corneal tropism and contribute to the maintenance of healthy corneas. It was postulated that the inflammatory cells might induce a diminution of nerve fibers in the subbasal nerve plexus in dry eyes with SS.

Benitez-del-Castillo et al.7,8 reported a significant decrease of the sub-basal nerve numbers (3.0 ± 0.6 to 3.3 ± 0.7 nerves/frame) and density in non-SS patients (591 ± 90 to 593 ± 127 µm/frame). On the other hand, Hoşal et al.28 reported no...
significant decrease of sub-basal nerve numbers and thickness in the dry eye patient (2.0 ± 2.1 nerves/frame, 4.6 ± 0.9 μm, respectively) with SS and non-SS compared with that of control subjects (2.3 ± 1.3 nerves/frame, 4.7 ± 0.7 μm), and no significant correlation between the corneal sensitivity and the corneal subbasal nerve morphologic changes by using Confoscan from Turkey in 2005. Erdelyi et al.16 also reported that the number of subbasal nerves was similar in the patients with aqueous tear deficiency dry eye (data not shown) compared with the normal subjects (data not shown). In summary, there are many reports suggesting that the corneal subbasal nerve density may decrease in dry eyes with SS. However, the kind of alterations in dry eyes with non-SS is still unclear.

**Corneal Nerve Morphology**

Benitez-del-Castillo et al.7,8 reported a significant increase in the number of beadings and nerve tortuosity (non-SS, 307 ± 75 to 323 ± 65 beadings/100 μm, 2.3 ± 0.5 grade; SS, 364 ± 64 to 387 ± 62 beadings/100 μm, 3.2 ± 0.8 grade; control, 192 ± 61 to 198 ± 65 beadings/100 μm, 1.1 ± 0.5 grade) but no significant change in the nerve reflectivity (non-SS, 2.8 ± 0.6 grade; SS, 2.6 ± 0.8 grade; control, 2.6 ± 0.8 grade) in both non-SS and SS patients. In addition, Villani et al.9,10 also reported a significant increase in the number of beadings and nerve tortuosity (SS, 332.9 ± 64.2 beadings/100 μm, 2.7 ± 0.5 to 2.7 ± 0.7 grade; control, 196.2 ± 0.7 beadings/100 μm, 1.2 ± 0.7 to 1.3 ± 0.6 grade) but no significant change in the nerve reflectivity (SS, 2.0 ± 0.7 to 2.1 ± 0.8 grade; control, 1.9 ± 0.9 to 2.0 ± 1.0 grade) in SS patients. Morphologic changes such as beadlike formation, tortuosity, and reflectivity are considered to be indices of metabolic activity of the nerve plexus. In summary, there is a significant increase in the number of beadings and nerve tortuosity in dry eye diseases; however, the changes in nerve reflectivity are still unclear.

**Assessment of Treatment Effectiveness**

In vivo confocal microscopy is a useful tool to evaluate the efficacy of treatment for dry eye disease. Kheirkhah et al.35 reported that the subbasal nerve fiber length did not significantly change after 4 weeks of treatment with topical corticosteroids (0.5% loteprednol etabonate ophthalmic suspension) for dry eye disease associated with Melboman gland dysfunction (MGD) by using HRT-RCM from the United States in 2015. However, Villani et al.36 reported that the subbasal dendritic cell density and activated keratocyte density significantly decreased after 4 weeks of treatment with topical corticosteroids (0.5% loteprednol etabonate ophthalmic suspension) in dry eye patients using HRT-RCM. Iaccheri et al.37 reported that in vivo confocal microscopy showed an increase in cell density of the corneal intermediate epithelium; a decrease in hyperreflective keratocytes; and a decrease in density, tortuosity, and reflectivity of corneal nerve fibers after 6 months of treatment with 0.05% cyclopentolate eye drops in patients with dry eye disease. On the other hand, Levy et al.38 reported an increase in corneal subbasal nerves, with a decrease in dendritic cell density after 6 months of treatment with topical 0.05% cyclopentolate in patients with SS by using HRT-RCM. Mahelkova et al.55 reported that the corneal basal epithelial cell density decreased significantly in patients with dry eye disease after 3 months of treatment with autologous serum eye drops, while the superficial epithelial cell density did not change. Semeraro et al.54 reported that the numbers of nerve branches and beadings were lower in the autologous serum eye drop treatment group compared with the artificial tear treatment group in SS-related dry eyes by using in vivo confocal microscope. In the future, in vivo confocal microscopy will be able to evaluate the efficacy of new treatments, such as topical 3% diquafosol sodium and 2% rebamipide for dry eye disease.

**Other Dry Eye Diseases**

In vivo confocal microscopy was applied to evaluate the corneal changes for other severe dry eye diseases, such as Stevens-Johnson syndrome and toxic epidermal necrosis. Vera et al.35 reported superficial epithelial cell changes, frequent pathologic nerve damage, and prevalent presence of inflammatory dendritic cells from France in 2009. Steger et al.56 described corneal findings, including a reduced number of subbasal nerve branches, increased tortuosity, and reduced reflectivity in subbasal nerve morphology, in patients with ocular chronic GVHD after allogeneic stem cell transplantation in Austria in 2015. On the other hand, Kheirkhah et al.57 also described no significant differences in patients with dry eyes due to GVHD and non-GVHD in relation to corneal epithelial dendritic cell density, corneal subbasal nerve density, and conjunctival epithelial immune cell density. Tepelus et al.38 evaluated the morphologic changes of the corneal epithelial layers, subbasal nerve plexus, and anterior stroma in dry eye patients with ocular GVHD and non-GVHD. The in vivo confocal microscopic images were analyzed in the GVHD patients, non-GVHD patients, and healthy controls as follows, respectively: superficial epithelial cell density, 656.1 ± 101.1 cells/mm², 827.0 ± 99.6 cells/mm², and 1277.2 ± 121.4 cells/mm²; wing cell density, 4499.8 ± 976.4 cells/mm², 4662.9 ± 319.7 cells/mm², and 6556.4 ± 504.0 cells/mm²; basal cell density, 7850.9 ± 723.5 cells/mm², 8570.0 ± 913.3 cells/mm², and 9759.8 ± 252.0 cells/mm²; nerve fibers density, 11.2 ± 5.5 mm/mm², 14.5 ± 4.3 mm/mm², and 19.6 ± 4.8 mm/mm²; and subbasal dendritic cell density, 67.9 ± 71.8 cells/mm², 40.1 ± 32.0 cells/mm², and 29.5 ± 8.1 cells/mm² (all parameters had significant differences). He et al.59 also evaluated the subbasal nerves and inflammatory cells in the cornea of Japanese GVHD patients and non-GVHD patients. The in vivo confocal microscopic images produced using HRT-RCM and analyzed in GVHD patients and non-GVHD patients were as follows, respectively: subbasal dendritic cell density, 119.3 ± 79.8 cells/mm² and 37.6 ± 21.7 cells/mm²; subbasal globular immune cell density, 41.6 ± 10.1 cells/mm² and 24.7 ± 25.6 cells/mm²; subbasal nerve density, 17.8 ± 6.2 mm/mm² and 13.7 ± 5.1 mm/mm²; and subbasal nerve tortuosity, 3.1 ± 0.7 grade and 2.2 ± 0.5 grade (each parameter except for subbasal nerve density had a significant difference).

**Conjunctiva**

De Nicola et al.40 observed and reported the images of conjunctival epithelial cell morphology produced using HRT-RCM in both tear evaporative and tear deficient dry eye diseases in 2005 in France. They found squamous metaplasia, inflammatory cell infiltration, and goblet cell depletion in the conjunctival epithelium with dry eye. There were no reports of conjunctival in vivo confocal microscopy in dry eye disease since then until Wakamatsu et al.11 reported it in Japan in 2010. The conjunctival alterations in Japanese patients with SS and non-SS were demonstrated by Wakamatsu et al.11 using HRT-RCM. In their report, temporal bulbar conjunctival epithelial cell densities were significantly lower in SS and non-SS patients compared with control subjects, and the density of conjunctival epithelial microcysts was significantly higher in SS than in healthy control eyes (data not shown) (Fig. 5). The conjunctival inflammatory cell densities consisting of
polymorphs, dendritic cells, and lymphocytes were significantly higher in SS (435.0 ± 435.8 cells/mm²) and non-SS (134.8 ± 124.2 cells/mm²) patients compared with control subjects (10.0 ± 17.9 cells/mm²). In vivo confocal microscopic images showed a considerable reduction of conjunctival inflammatory infiltrates after 1 month of dry eye treatment (artificial tears, topical 0.1% fluorometholone and 0.3% sodium hyaluronate, and lacrimal punctal plugs insertion) in the patient with SS. These results suggested that chronic inflammation might lead to both corneal and conjunctival epithelial cell loss in the ocular surface of dry eyes with SS.

On the other hand, Villani et al.41 reported that the densities of inferior tarsal conjunctival epithelial, goblet, and inflammatory cells were significantly higher in SS patients (4331 ± 1143 cells/mm², 688 ± 318 cells/mm², and 1662 ± 818 cells/mm², respectively) compared with control subjects (2515 ± 303 cells/mm², 36 ± 41 cells/mm², and 717 ± 390 cells/mm², respectively; older subjects, 1819 ± 502 cells/mm², 325 ± 431 cells/mm², 905 ± 382 cells/mm², respectively) by using HRT-RCM in 2011. The goblet cell density measured by impression cytology on the temporal bulbar conjunctiva in SS patients was lower compared with controls in a previous report by Pfliugfelder et al.42 This conflict of goblet cell densities was probably due to the peculiar problems in confocal microscopic identification and analysis. Superior bulbar conjunctival goblet cell density in SS patients measured by HRT-RCM was 332 ± 137 cells/mm² in a report by Hong et al.43 They showed a significant positive correlation in conjunctival goblet cell density between the confocal microscopic images and impression cytology.

Kojima et al.44 investigated the cytologic findings of nasal bulbar conjunctiva in Japanese dry eye patients with SS by using HRT-RCM in 2010. The study reported that the conjunctival epithelial cell areas in superior limbic keratoconjunctivitis and controls were 786.5 ± 463.9 μm² and 311.5 ± 78.3 μm², respectively, and the nucleocytoplasmic ratios were 0.4 ± 0.1 and 0.7 ± 0.0, respectively. It was also reported that the inflammatory cell densities in superior limbic keratoconjunctivitis and controls were 439.5 ± 240.3 cells/mm² and 75.3 ± 37.8 cells/mm², respectively. There was a significant difference in relation to inflammatory cell densities between the control subjects and the patients with superior limbic keratoconjunctivitis.

Meibomian Gland

Messmer et al.46 observed and reported the images of Meibomian glands by using HRT-RCM in both blepharitis and normal subjects in Germany in 2005. The device showed that the dilation and obstruction of Meibomian gland ducts could be visible in patients with blepharitis. There were no reports on Meibomian glands observed by in vivo confocal microscopy after their report. However, Matsumoto et al.47 evaluated the morphologic changes of Meibomian glands in Japanese patients with MGD compared with normal subjects by using new confocal microscopic parameters measured by HRT-RCM for the first time in the literature in 2008.12 In their report, the acinar unit density of Meibomian glands was significantly lower in MGD patients (47.6 ± 26.6 glands/mm²) than in control subjects (101.3 ± 33.8 glands/mm²). The acinar unit diameter was significantly larger in MGD patients (98.2 ± 53.3 μm) than in controls (41.6 ± 11.9 μm). The acinar unit density as measured by HRT-RCM was significantly related with the severity of both Meibomian gland dropout and expressibility grades. The acinar unit diameter was also significantly related with the severity of both Meibomian gland dropout and expressibility grades. They also showed the atrophy in the Meibomian glands with extensive periglandular fibrosis in the severe MGD patients (Fig. 4).

Furthermore, Ibrahim et al.13 evaluated the sensitivity and specificity of confocal microscopic parameters of acinar unit density, acinar unit longest diameter, acinar unit shortest diameter, and inflammatory cell density of Meibomian glands in the diagnosis of MGD in Japanese patients. In that study, the acinar unit density of Meibomian glands was significantly lower in MGD patients (67.8 ± 15.1 glands/mm²) than in normal controls (113.7 ± 36.6 glands/mm²). The acinar unit longest diameter was significantly larger in MGD patients (86.3 ± 18.9 μm) than in controls (56.3 ± 10.4 μm). The acinar unit shortest diameter was significantly larger in MGD patients (34.8 ± 9.2 μm) than in controls (17.4 ± 4.2 μm). The inflammatory cell density was significantly greater in MGD patients (1026.1 ± 557.3 cells/mm²) than in controls (56.6 ± 32.1 cells/mm²). The confocal microscopic images with HRT-RCM disclosed morphologic alterations in patients with MGD, including extensive periglandular inflammatory cell infiltration and enlargement of glandular acinar units. The cutoff values for acinar unit density, acinar unit longest diameter, acinar unit shortest diameter, and inflammatory cell density of Meibomian glands in the diagnosis of MGD were 70 glands/mm², 65 μm, 25 μm, and 300 cells/mm², respectively. The sensitivity and specificity values of these parameters under these cutoff values were 81% and 81% for acinar unit density, 90% and 81% for acinar unit longest diameter, 86% and 96% for acinar unit shortest diameter, 100% and 100% for inflammatory cell density of Meibomian glands, respectively.

Matsumoto et al.17 also evaluated the alterations of Meibomian glands in Japanese MGD patients before and after anti-inflammatory treatment (lid hygiene, topical 0.5% levofloxacin and 0.1% fluorometholone, and oral 100mg minocycline) by using HRT-RCM in 2009. The study showed that the
inflammatory cell density of Meibomian glands before the initiation of trial was $1216 \pm 328$ cells/mm$^2$, but at the final follow-up (after 12 weeks) was significantly reduced to $700 \pm 436$ cells/mm$^2$ by the anti-inflammatory treatment. In their follow-up (after 12 weeks) was significantly reduced to $700 \pm 436$ cells/mm$^2$ by the anti-inflammatory treatment. In their study, they demonstrated morphologic changes of the Meibomian glands based on new diagnostic parameters, such as acinar unit diameter, density, and inflammatory cell density by using the in vivo confocal microscope.

Villani et al$^{48}$ evaluated the morphologic changes of Meibomian glands in SS patients in 2011. The acinar unit diameter and density of Meibomian glands were measured by HRT-RCM in SS patients, MGD patients, and controls. The acinar unit density was significantly higher, whereas the diameter was significantly shorter in SS patients ($138 \pm 69$ glands/mm$^2$, $53 \pm 31$ μm, respectively) compared with MGD patients ($57 \pm 21$ glands/mm$^2$, $106 \pm 41$ μm, respectively), but did not differ significantly from controls ($110 \pm 31$ glands/mm$^2$, $53 \pm 14$ μm, respectively).

Ban et al$^{49}$ evaluated the morphologic changes of Meibomian glands in Japanese patients with GVHD compared with non-GVHD patients by using HRT-RCM in 2011. In that study, the acinar unit diameter and density of Meibomian glands was significantly lower in GVHD ($57.8 \pm 38.3$ glands/mm$^3$) than in non-GVHD patients ($88.8 \pm 26.6$ glands/mm$^3$). The acinar unit longest diameter was significantly shorter in GVHD ($37.5 \pm 24.4$ μm) than in non-GVHD patients ($60.4 \pm 11.8$ μm). The acinar unit shortest diameter was significantly shorter in GVHD patients ($17.7 \pm 11.8$ μm) than in non-GVHD (26.6 \pm 6.05 μm). The fibrosis grade was significantly higher in GVHD (1.4 ± 0.7 grade) than in non-GVHD patients (0.1 ± 0.3 grade). A confocal microscopy assessment showed atrophic Meibomian glands and excessive fibrosis in dry eye patients with GVHD.

In addition, it was reported by Wei et al$^{50}$ in 2011 that the acinar unit density of Meibomian glands in healthy subjects significantly decreased with age by using HRT-RCM ($71 \pm 27$ glands/mm$^2$; mean age, $43 \pm 23$ years). Villani et al$^{51}$ reported similar age-related findings in 2013 (under 50 years old, $118 \pm 29$ glands/mm$^2$; over 50 years old, $97 \pm 26$ glands/mm$^2$, respectively). Villani et al$^{51}$ also reported that the acinar unit diameter of Meibomian glands in healthy subjects significantly decreased with age (under 50 years old, $47 \pm 8$ μm; over 50 years old, $36 \pm 8$ μm, respectively).

In summary, there are significant decreases in the acinar unit density and increases in the acinar unit longest diameter, acinar unit shortest diameter, and inflammatory cell density of Meibomian glands in patients with MGD. The activation of inflammatory cells may induce the morphologic changes of Meibomian glands; however, the mechanism of enlargement of glandular acinar units in MGD patients is also not yet fully understood. The device may be useful as a supplementary diagnostic tool for the in vivo assessment of Meibomian gland in patients with MGD and/or other Meibomian gland diseases in the future.

**Lacrimal Gland**

There were no reports on in vivo confocal microscopic images of lacrimal glands until Sato et al$^{14}$ reported them from Keio University in Japan in 2010. The alterations of lacrimal glands in Japanese patients with SS were demonstrated by Sato et al$^{14}$ using HRT-RCM for the first time. In their report, the confocal microscopic parameters of acinar unit density, acinar unit diameter, and inflammatory cell density of lacrimal glands were significantly worse in SS patients ($19.0 \pm 6.8$ glands/mm$^2$, $75.7 \pm 17.4$ μm, and $1146.0 \pm 692.0$ cells/mm$^2$, respectively) compared with controls ($66.1 \pm 49.7$ glands/mm$^2$, $61.5 \pm 15.2$ μm, and $377.0 \pm 152.0$ cells/mm$^2$, respectively) (Fig. 5). In vivo confocal microscopy examination could aid in the visualization of the acinar units, glandular ducts, and interstitial matrix of lacrimal glands in healthy individuals and patients. In the future, the device may be an efficient tool in the noninvasive diagnostics of lacrimal gland in patients with SS or other lacrimal gland diseases.

**CONCLUSIONS**

Confocal microscopy is a new, emerging, noninvasive technology that can aid in the in vivo assessment of structural changes in several ocular surface diseases at the cellular level. To delineate the status of the ocular surface epithelium and any coexisting inflammation in dry eye
diseases, cytomorphologic findings of conventional ocular surface examination techniques, such as vital staining, impression, and brush cytology, have been reported to be important components of clinical evaluation and diagnosis.22 However, in vivo confocal microscopy can assess not only the morphology, including corneal/conjunctival cell density, nerve fiber density and inflammatory cell density, but also the cellular architecture, including nucleocytoplasmic ratio, by observation of the nucleus, cytoplasm, cellular borders in patients with dry eye. The device also can disclose acinar unit density, acinar unit longest diameter, acinar unit shortest diameter, and inflammatory cell density in the Meibomian gland and lacrimal gland by other potential applications. As mentioned above, many studies using in vivo confocal microscope focused on the corneal morphologic changes in aqueous-deficient dry eye diseases with SS and non-SS for a long time in Europe and the United States. Recently, several dry eye researchers have focused on the morphologic changes in other dry eye diseases, such as GVHD, and assessment of treatment effectiveness in dry eye disease. In Japan, the morphologic alterations of conjunctiva in dry eyes with SS or in superior limbic keratoconjunctivitis have been mainly studied, while in vivo confocal microscopy was applied to imaging the Meibomian glands in MGD or GVHD and lacrimal glands in dry eyes with SS. In the future, an evaluation of the sensitivity and specificity of confocal microscopic parameters and determination of the cutoff values in relation to dry eye will be necessary. In summary, the application of in vivo confocal microscopy in dry eye disease can be a powerful method to evaluate the morphologic change of the ocular surface, including the cornea, conjunctiva, Meibomian gland, and lacrimal gland.

Acknowledgments

Funding of the publication fee and administration was provided by the Dry Eye Society, Tokyo, Japan. The Dry Eye Society had no role in the contents or writing of the manuscript.

Disclosure: Y. Matsumoto, None; O.M.A. Ibrahim, None

References


