Journal of Clinical & Experimental Ophthalmology

Yuan, J Clin Exp Ophthalmol 2012, 3:6
DOI: 10.4172/2155-9570.1000230

Research Article Open Access

In vivo Laser Scanning Confocal Microscopy Used in Herpes Simplex Keratitis Research

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Abstract

In vivo Laser Scanning Confocal Microscopy (LSCM) is a new technology that can be used to observe morphologic changes of the cornea on a cellular level. Non-invasive, high-contrast *in vivo* images of the cornea at different depths can be reliably and rapidly captured by LSCM. The high resolution of corneal structures, close to 1 μm, demonstrates the sensitivity of this technique, and the images can be stored for further research. The amount of magnification is high enough to visualize individual cells. *In vivo* images of the cornea at different depths from the epithelium to the endothelium are obtained immediately, directly and reproducibly. The *in vivo* laser scanning confocal microscopy is useful in HSK (Herpes Simplex Keratitis) research because it provides a new way to observe HSK and provides a new path for treatment.

Keywords: *In vivo* laser scanning confocal microscopy; Herpes simplex keratitis; Herpes simplex virus type 1; Epithelial keratitis; Stromal keratitis

Introduction

Herpes Simplex Keratitis (HSK) is a recurrent disease and a major cause of visual morbidity. It impacts vision through corneal scarring, thinning and neovascularisation. Although predominantly unilateral, the disease occurs bilaterally in 1.3%–12% of cases, and it now occurs in a younger age group and tends to be more severe [1]. HSK results from Herpes Simplex Virus Type 1 (HSV-1) infection. Primary infection may involve an ocular or non-ocular site, after which latency may be established principally in the trigeminal ganglion, but also in the cornea. Herpetic stromal disease is due to the immune response to the virus within the cornea, and the ability of the virus to cause corneal stromal disease is correlated with its ability to induce corneal vascularisation.

HSK research has primarily depended on laboratory tests and animal experiments. Current laboratory assay methods comprise virus isolation and culture, immunofluorescence assay, polymerase chain reaction, Western blotting and enzyme-linked immunosorbent assay, while primary and secondary HSK animal models have been developed for *in vivo* research. HSK research still lacks simple, real-time methods for direct observation of the disease.

In vivo Laser Scanning Confocal Microscopy (LSCM) is a new technology that can be used to observe morphologic changes of the cornea on a cellular level. Non-invasive, high-contrast *in vivo* images of the cornea at different depths can be reliably and rapidly captured by LSCM. It is now widely used to observe the corneal structure *in vivo* to help in diagnosing diseases and thus direct treatment.

In this study, LSCM was used to observe two patients (three eyes) with HSK. One patient had unilateral primary HSK, and the other had bilateral recurrent HSK.

Patients and Methods

One patient, a 58-year-old woman, had a primary HSV infection in her right eye. The second patient, a 28-year-old man, had recurrent HSV infection in both eyes; one eye had an active infection, while infection in the other eye was quiescent. Two patients (three eyes) were examined by using an *in vivo* confocal microscopy, the Heidelberg Retina Tomography/Rostock Cornea Module (HRTII-RCM) (Heidelberg Engineering GmbH, Dossenheim, Germany). Before examination, one drop of 0.4% oxybuprocaine hydrochloride topical anaesthetic (Benoxil; Santen Pharmaceutical Co., Ltd., Osaka, Japan) and one drop of 0.2% carbomer gel tear substitute (WeiDiXi; Boslum & Furuida Pharmaceutical Co., Ltd., Shandong, China) were instilled in the lower conjunctiva fornix. A 60× water-immersion objective lens (Olympus Europa GmbH, Hamburg, Germany) was used with a 670-nm diode laser as a light source to scan an area of 384 by 384 m, with lateral and vertical resolutions of 1 m and up to 800× magnification. The x–y positions of the image and section depth were controlled manually.

Since HRTII-RCM confocal microscopy is routinely used for examination of ocular surface disorders, is non-invasive and painless, and does not present any risk of complication [2], its use did not require specific approval. Informed consent for the purpose of the examination was obtained from all patients.

Results

Epithelial keratitis in the patient with primary infection

The epithelial cells were found to be damaged, and oedema and vacuole degeneration were present. Nerves were decreased, and broken dendritic cells (DCs) were found near the nerves. No neovascularisation or inflammatory cells were found in the stroma, and there was no damage to the endothelial cells (Figure 1).

Stromal keratitis in the patient with recurrent infection

Epithelial cells were damaged, and oedema and vacuole degeneration were present. A quantity of DCs were seen in the epithelial layer and near neovascularisation, which was found at every depth of the stroma. Inflammatory cells flew quickly through the vessels and migrated from

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Received May 29, 2012; **Accepted** June 30, 2012; **Published** July 05, 2012

Citation: Yuan X, Ping HZ, Hua SY, Ping YL (2012) *In vivo* Laser Scanning Confocal Microscopy Used in Herpes Simplex Keratitis Research. J Clin Exp Ophthalmol 3:230. doi:10.4172/2155-9570.1000230

the vessels to the stroma. Endothelial cells and nerve structure could not be observed (Figure 2).

Quiescence state in the patient with recurrent infection

Epithelial cells, epithelial nerves and endothelial cells were found to be normal. Many highly reflective dots occurred near the nerve and may have been immature DCs. Empty blood vessels were found at every depth of the stroma; some had a few blood cells in them and their flow was sluggish. No inflammatory cells were found in the stroma (Figure 3).

Discussion

Epithelial keratitis

The earliest epithelial keratitis lesions had a characteristic branching linear shape with large terminal bulbs and swollen epithelial borders. From the LSCM images, swollen epithelial cells and broken nerves could be observed in the lesion, DCs were found near the nerves and limbus, no neovascularisation or inflammatory cells were found, and the endothelial cells were normal. It has been suggested that the epithelial layer is the primary site of damage by the virus. The professional antigen-presenting cells, such as Langerhans cells or monocyte macrophages, acquire viral antigens in the epithelium, before promoting inflammation in the stroma [3]. The prevalence of HSV-1 antigen in the stroma compared to the epithelium or endothelium indicates that the stroma is the site directly affected by the immune response [4].

Stromal keratitis

Stromal keratitis may be chronic and recrudescent, leading to stromal scarring, thinning, neovascularisation and lipid deposition. Stromal neovascularisation can be sectoral or diffuse and frequently occurs in several layers of the cornea [5]. HSV could potentially leave the cornea with trafficking immune cells and then return at a later time with these cells [6-10]. The LSCM images showed neovascularisation in every layer of the stroma, with numerous inflammation cells

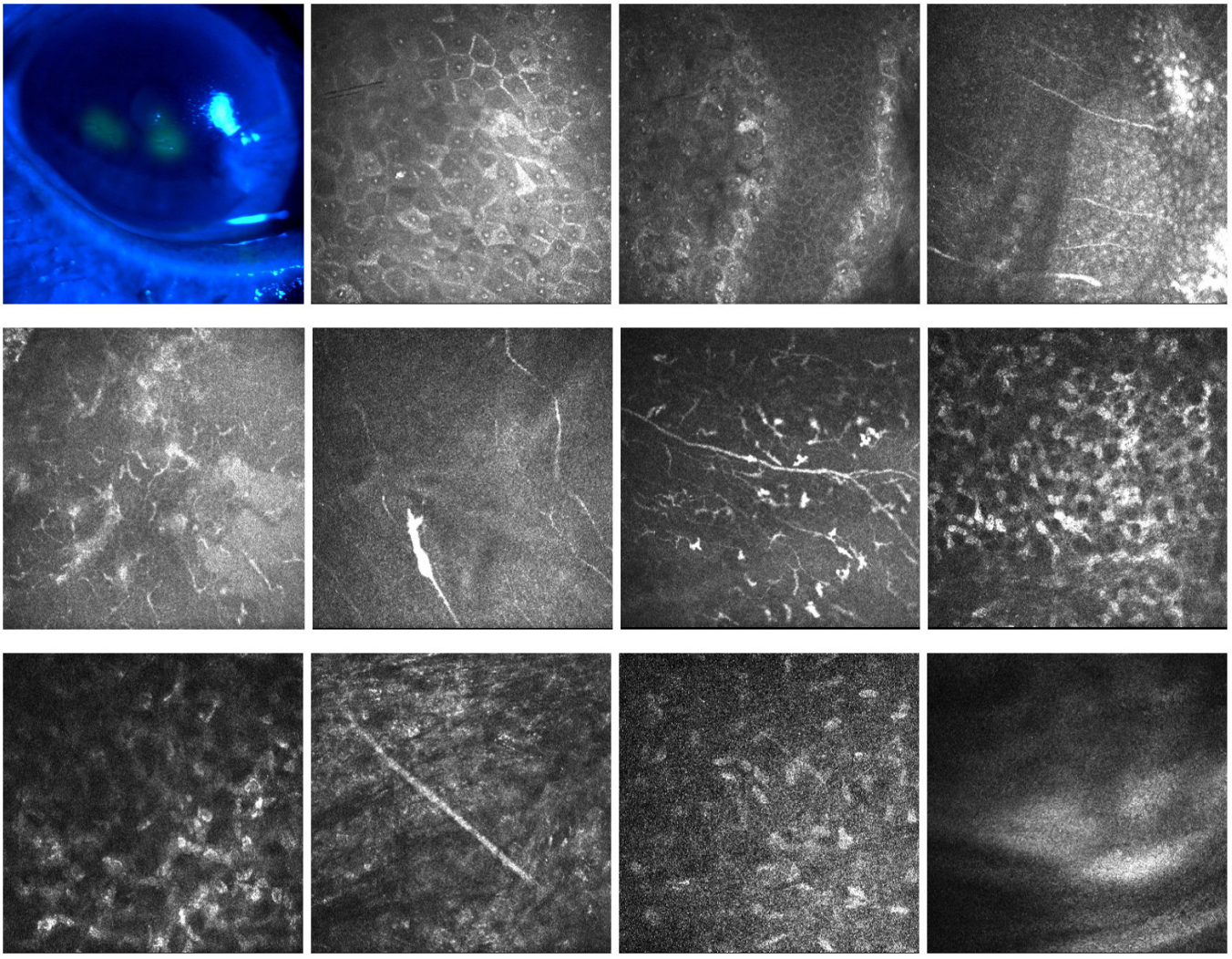

Figure 1: HRTII-RCM images (300 μm by 300 μm). (A) The primary HSK cornea under the slit lamp microscope. (B) Epithelial cells loosing and oedema. (C) Normal epithelial cells and degenerating cells. (D,E) The broken subepithelial nerves in the lesion. (F) The broken nerve and expanding stump. (G) Quantity of DCs near the nerve. (H,I,J,K) The stromal cell oedema, no inflammatory cells or neovascularisation in the stroma, and stromal nerve thickening. (L) No endothelial cell damage.

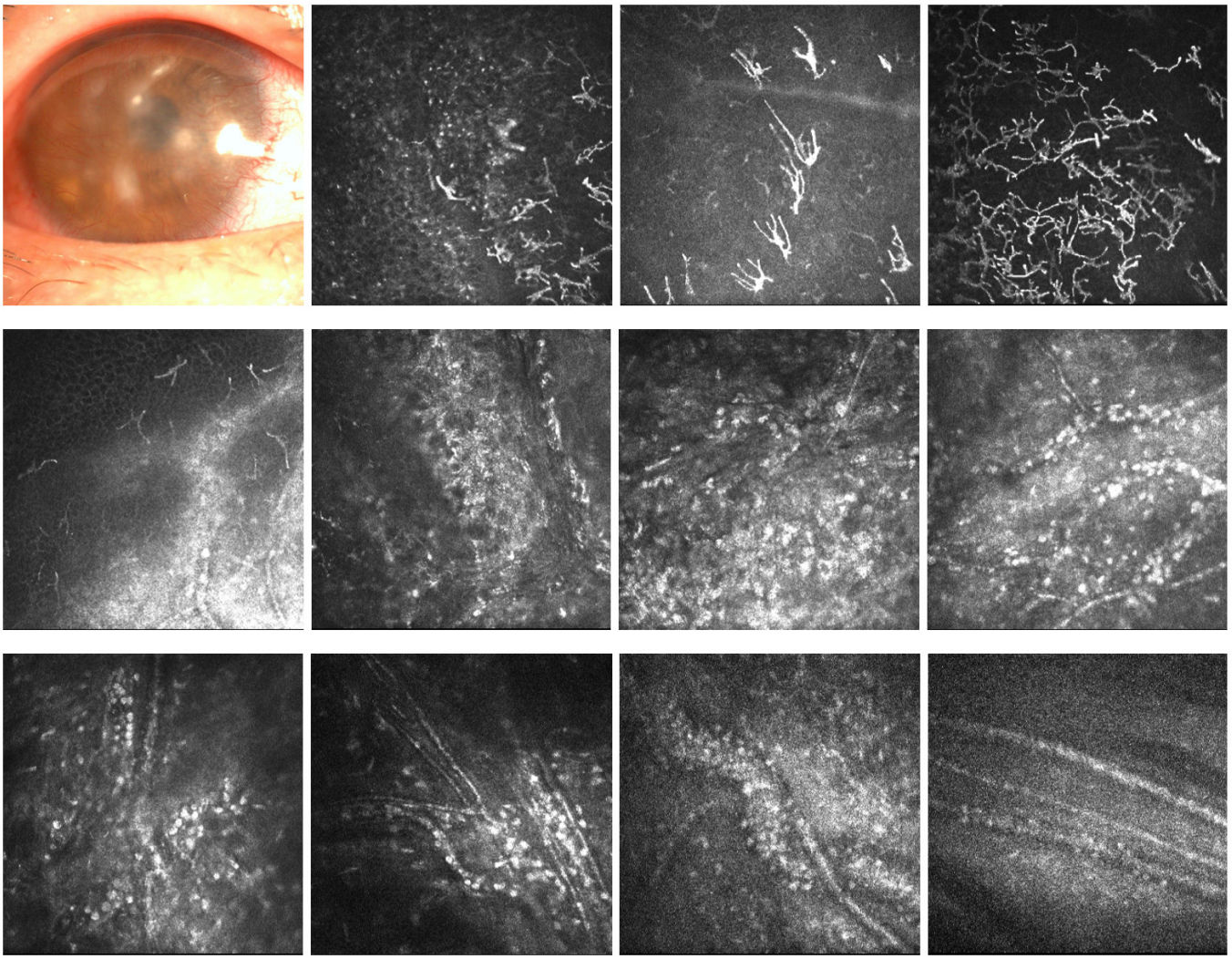

Figure 2: HRTII-RCM images (300 μm by 300 m). (A) The cornea with recurrent HSK under the slit lamp microscopy. (B,C) Quantity of mature DCs in the epithelial layer. (D) The degeneration of the epithelial cell vacuoles and the DCs infiltration. (E) Cross section of the cornea, neovascularisation in the stroma, and the DC migration from the limbus to the epithelial layer. (F) The proliferation of stroma fibrosis. (G,H,I,J,K,L) Reduced stromal nerves, neovascularisation at every depth of the stroma, rapid blood cell flow, and migration of the inflammatory cells to the stroma.

transported by the vessels to every depth of the stroma. Nerve damage was found to be more serious, endothelial cells were affected, a lot of neovascularisation and immune cells could be found, and DCs were much more numerous in the recurrent infection than in the primary infection. DCs were observed near the limbus and found to migrate to the epithelial layer. They were likely released from the conjunctiva in response to the infection, and they might present the HSV-1 antigens to T cells in the draining lymph nodes [11]. From the LSCM images, we found a quantity of inflammatory cells in every layer of stroma near neovascularisation. The polymorphonuclear leucocytes contribute to virus removal in response to chemokines [4,12], and they are also a source of vascular endothelial growth factor and matrix metalloproteinase-9, which play crucial roles in the development of corneal vascularisation [13,14]. These cells acted both to kill the virus and to damage the cornea.

Quiescence state in recurrent infection patient

HSV-1 can establish both productive and latent infections. Although HSV-1 could conceivably enter via immune cells through the epithelium or in new blood vessels formed with blood–ocular barrier breakdown, it is apparent that HSV-1 resident in the cornea can initiate stromal disease. Many highly reflective dots near the nerve were found in the LSCM images and may have been immature immune cells. Empty new blood vessels in every layer of the stroma provide the chance for the virus latency and reactivation. Compared with the actively infected eye of the recurrent patient, almost every cornea structure was normal in the quiescence state. In the epithelial layer, some immature immune cells could be found, but in the stroma, the new blood vessels were empty, with no more than a few blood cells, and no immune cells could be found.

Corneal nerve damaged in HSK

Corneal nerves penetrate the corneal periphery in a radial distribution and form the subbasal nerve plexus between the Bowman's layer and the basal epithelium. Corneal innervation provides

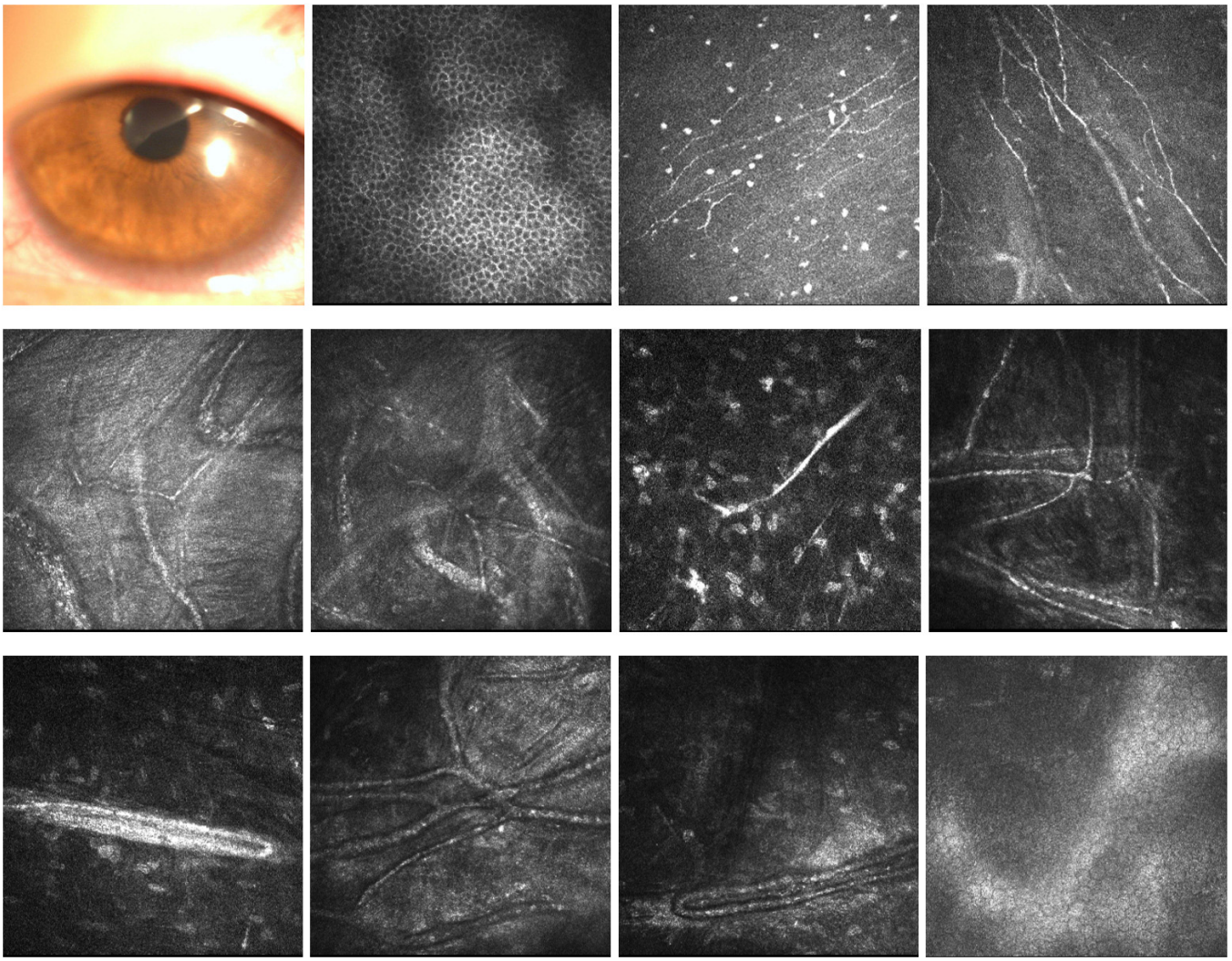

Figure 3: HRTII-RCM images (300 μm × 300 μm). (A) The cornea with recurrent HSK in the quiescence state under the slit lamp microscopy. (B) The epithelial layer is not plant. (C) Quantity of highly reflective dots near the subepithelial nerves. (D) The subepithelial nerve plexus. (E,F,H,I,J,K) The empty blood vessels in the anterior stroma, the cells in the vessels flowed slowly, and no inflammatory cells were in the stroma. (G) The stromal nerve was thickened. (L) The endothelial cells were normal.

protective and trophic functions and regulates epithelial integrity, proliferation and wound healing [2,15]. The complex stromal and epithelial branching of nerves is not visible by conventional slit-lamp biomicroscopy but can be visualized by LSCM. In the LSCM images, the nerves were broken or absent, and terminal bulbs were large and swollen in the active infection state, while in the quiescence state, the nerves were observable, but decreased in number compared with a normal eye.

Conclusion

HRTII-RCM *in vivo* LSCM is a new technique that is non-invasive and painless and does not carry any risk of complication [16]. The high resolution of corneal structures, close to 1 μm, demonstrates the sensitivity of this technique, and the images can be stored for further research. The amount of magnification is high enough to visualize individual cells. *In vivo* images of the cornea at different depths from the epithelium to the endothelium are obtained immediately, directly and reproducibly. The *in vivo* laser scanning confocal microscopy is useful in HSK research because it provides a new way to observe HSK and provides a new path for treatment. It may allow objective evaluation of treatment response through quantification of cellular and nerve changes in the cornea.

References

1. Liesegang TJ (2001) Herpes simplex virus epidemiology and ocular importance. Cornea 20: 1–13.
2. Guthoff RF, Wienss H, Hahnel C, Wree A (2005) Epithelial innervation of human cornea: a three-dimensional study using confocal laser scanning fluorescence microscopy. Cornea 24: 608 –613.
3. Polcicova K, Biswas PS, Banerjee K, Wisner TW, Rouse BT, et al. (2005) Herpes keratitis in the absence of anterograde transport of virus from sensory ganglia to the cornea. Proc Natl Acad Sci USA 102: 11462-11467.
4. Kaye SB, Baker K, Bonshek, Maseruka H, Grinfeld E, et al. (2000) Human herpes viruses in the cornea. Br J Ophthalmol 84: 563-571.
5. Liesegang TJ (1999) Classification of herpes simplex virus keratitis and anterior uveitis. Cornea 18: 127-143.

6. Fukuda M, Deai T, Hibino T, Higaki S, Hayashi K, et al. (2003) Quantitative analysis of herpes simplex virus genome in tears from patients with herpetic keratitis. Cornea 22: S55-S60.

7. Neufeld MV, Steinemann TL, Merin LM, Stroop WG, Brown MF (1999) Identification of a herpes simplex virus-induced dendrite in an eye-bank donor cornea. Cornea 18: 489-492.

8. Openshaw H, McNeill JI, Lin XH, Niland J, Cantin EM (1995) Herpes simplex virus DNA in normal corneas: persistence without viral shedding from ganglia. J Med Virol 46: 75-80.

9. Kaye SB, Shimeld C, Grinfeld E, Maitland NJ, Hill TJ, et al. (1992) Non-traumatic acquisition of herpes simplex virus infection through the eye. Br J Ophthalmol 76: 412-418.

10. Thomas J, Gangappa S, Kanangat S, Rouse BT (1997) On the essential involvement of neutrophils in the immunopathologic disease: herpetic stromal keratitis. J Immunol 158: 1383-1391.

11. Jager MJ, Bradley D, Atherton S, Streilein JW (1992) Presence of Langerhans cells in the central cornea linked to the development of ocular herpes in mice. Exp Eye Res 54: 835-841.

12. Tumpey TM, Chen SH, Oakes JE, Lausch RN (1996) Neutrophil mediated suppression of virus replication after herpes simplex virus type 1 infection of the murine cornea. J Virol 70: 898-904.

13. Zheng M, Deshpande S, Lee S, Ferrara N, Rouse BT (2001) Contribution of vascular endothelial growth factor in the neovascularization process during the pathogenesis of herpetic stromal keratitis. J Virol 75: 9828-9835.

14. Lee S, Zheng M, Kim B, Rouse BT (2002) Role of matrix metalloproteinase-9 in angiogenesis caused by ocular infection with herpes simplex virus. J Clin Invest 110: 1105-1111.

15. Oliveira-Soto L, Efron N (2001) Morphology of corneal nerves using confocal microscopy. Cornea 20: 374–384.

16. Labbe A, Dupas B, Hamard P, Baudouin C (2005) In vivo confocal microscopy study of blebs after filtering surgery. Ophthalmology 112: 1979.