Corneal damage revealed by endogenous cellular fluorescence and second harmonic signals from collagen.

Julia G. Lyubovitsky[1], Tatiana B. Krasieva[1], Joel A. Spencer[2], Bogi Andersen[2], Bruce J. Tromberg[1]

1. Laser Microbeam and Medical Program (LAMMP), Beckman Laser Institute, University of California, Irvine, USA
2. Department of Medicine, University of California, Irvine, Irvine, CA 92697

Abstract

An intrinsic cellular emission allied with second harmonic signals are promising *in-vivo* clinical diagnostic tools for corneal abnormalities, cancer and wound healing. The extent of corneal damage in K14-DN-Clim mice will be addressed.

INTRODUCTION

We explored the capability of multiphoton microscopy (MPM) to detect structural changes in the stromal collagen and cellular layers of the mice corneas in the backscattering geometry. The extent of corneal damage in K14-DN-Clim mice model was addressed. The dominant negative (DN) Clim (Co-factor of LIM - a diverse group of transcription factors) was expressed in the epithelial tissues in mice using keratin 14 (K14) promoter, which resulted in a disrupted epithelial transcriptional complex. The K14-DN-Clim mice are patch-bald and have corneas that undergo an ultimate transformation into epidermis-like structures. The corneas become highly neovascularized, inflamed, have downregulation of the corneal specific protein K12, concomitant with upregulation of epidermal markers K10 and filaggrin (data not shown). Eighty percent of mice are blind due to corneal opacities. In severe cases, the effected mice exhibit abnormal overgrowths of the stratified squamous epithelium, which are visually detectable and confirmed by histology. In moderately effected individuals, endogenous cellular fluorescence and second harmonic signals from collagen showed displasia in the squamous epithelium, irregular collagen arrays in the stroma, and a compromised posterior endothelium. K14-DN-Clim mice is an excellent model for MPM to explore corneal abnormalities ranging from cellular displasia to stromal neovascularization.

MATERIALS AND METHODS

Sample preparation

Animals were euthanized by asphyxiation with CO_2. The eyes were removed, placed into saline solution and imaged immediately following euthanasia. The corneas were not dissected out of the eyes and were amendable to imaging for a few hours. About twenty normal and fifteen K14-DN-Clim mice eyes were imaged.

SHG/TPF imaging and spectra

Multiphoton Microscopy in the Biomedical Sciences V, edited by Ammasi Periasamy, Peter T. C. So, Proceedings of SPIE Vol. 5700 (SPIE, Bellingham, WA, 2005)
1605-7422/05/$15 · doi: 10.1117/12.590006

The images and spectra were obtained using combined second harmonic(SHG)/two-photon fluorescence(TPF) microscope described earlier[1-11]. The excitation wavelength was 766 nm. Focusing objective (Zeiss; 63X water immersion; N.A., 1.2) was used. The time for a typical x-y scan was one to three seconds.

Histological evaluations

Both eyes were enucleated and placed into 0.9% saline solution. The eyes remained in the saline for approximately 3-5 hours during which the NLOM images were recorded. Specimens were then fixed in 10% buffered formalin (Sigma-Aldrich) overnight, dehydrated by an ethanol gradient, and embedded in paraffin wax (Fisher). The 6 micron-sections closest to the center of the eye were stained with hematoxylin and eosin.

RESULTS

SHG/TPF signals and spectra

Healthy mice cornea

All the topographic features seen in MPM x-y scans (Figure 1B, C, D) have excellent coregistration with (H&E) stained sections (Figure 1A), however structural organization of collagen stroma evident in SHG/TPF images is not apparent in H&E processed tissues. Regardless of animals ages the size of the cornea was estimated to be 150$\pm$10 μm from MPM data, with epithelial layer making up the first 50 μm, stroma with parallel arrangement of collagen layers (at ~60μm, Figure 1C) and endothelial layer at 150$\pm$10 μm (Figure 1D).

K14-DN-Clim mice cornea

The high resolution MPM images (Figure 2) are highly effective in highlighting the displastic areas within the epithelial layer and irregular organization of collagen arrays within the stroma. In several severe cases, partial absence of endothelial layer was also noted and confirmed by histology. Where present, the cells in the endothelial layer, were found to be irregularly shaped.

DISCUSSION AND CONCLUSION

Reflection multiphoton microscopy has potential use as non-invasive visualization technology for high-resolution structures of cornea. This work describes the normal and pathological corneas in mice using MPM (TPF and SHG combined). The high-resolution imaging highlights the structural abnormalities in the K14-DN-Clim mice corneas, which are validated with a conventional histology. Most K14-DN-Clim mice develop first evidence of corneal abnormality around six weeks of age, however, partial loss of Clim function leads to a complex phenotype with a severity of the condition varying greatly among individuals of the same age. To overcome the challenge of detecting early stages in K14-DN-Clim mice corneal pathology, we are currently implementing single, live animal imaging.

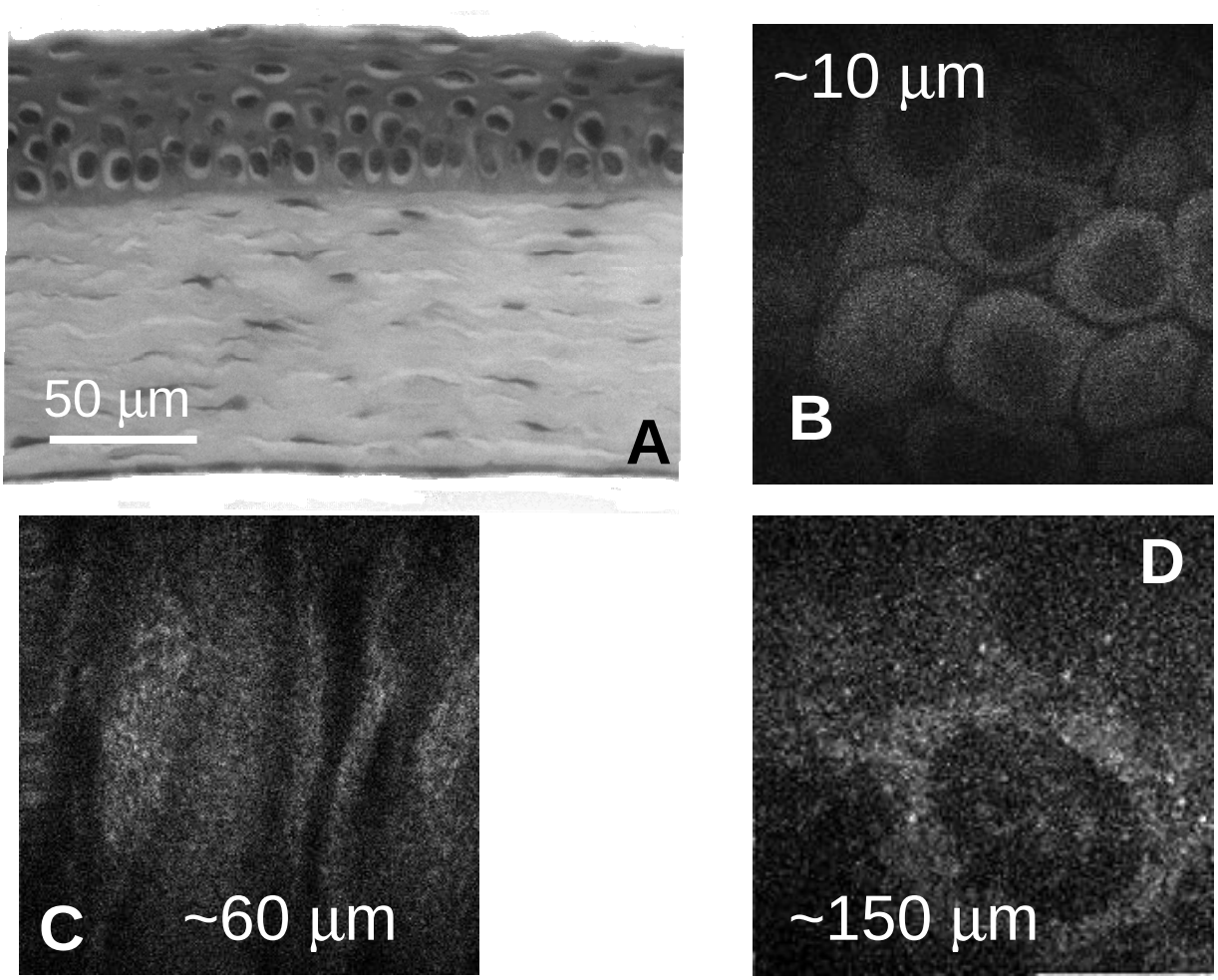

Figure 1. (A) H&E stained section of the healthy mouse cornea; (B), (C), (D) TPF/SHG signals from healthy mouse cornea, x-y scans.

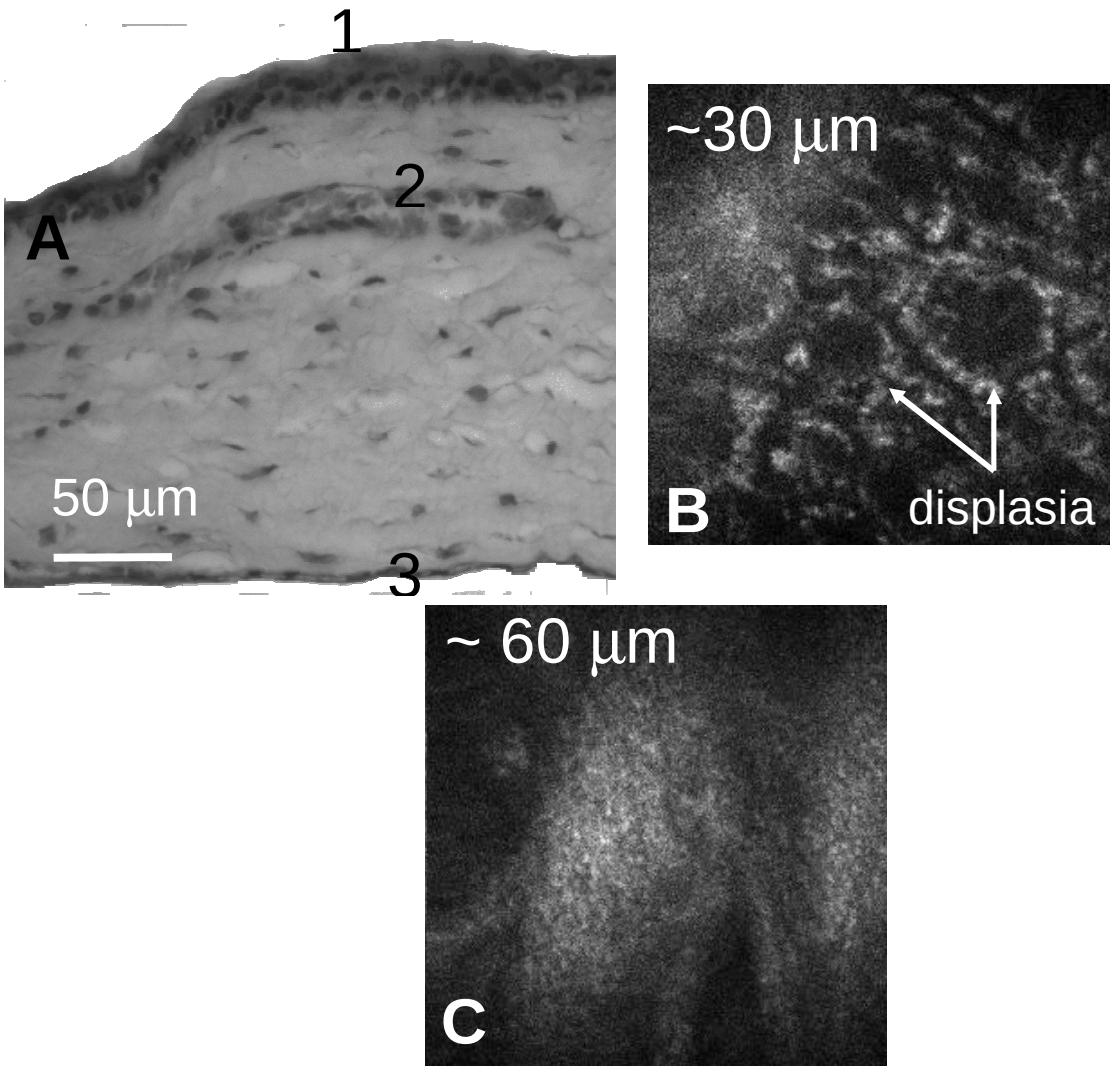

Figure 2. (A) H&E stained section of the K14-DN-Clim mouse cornea. 1. Epithelial cell displasia in the strat. squam. epithelium; 2. Cell invasion and neovascularization in stroma; 3. Compromised posterior endothelium (B), (C), (D) TPF and SHG signals from K14-DN-Clim mouse cornea, x-y scans.

REFERENCES

(1) Agarwal, A.; Coleno, M. L.; Wallace, V. P.; Wu, W. Y.; Sun, C. H.; Tromberg, B. J.; George, S. C. *Tissue Engineering* **2001**, *7*, 191-202.

(2) Dunn, A. K.; Wallace, V. P.; Coleno, M.; Berns, M. W.; Tromberg, B. J. *Applied Optics* **2000**, *39*, 1194-1201.

(3) Kelly, T. F.; Sutton, F. M.; Wallace, V. P.; Wong, B. J. F. *Otolaryngology-head and neck surgery* **2002**, *127*, 256-270.

(4) LaMorte, V. J.; Zoumi, A.; Tromberg, B. J. *J. Biomed. Opt.* **2003**, *8*, 357-361.

(5) Wong, B. J. F.; Wallace, V.; Coleno, M.; Benton, H. P.; Tromberg, B. J. *Tissue Engineering* **2002**, *7*, 599-606.

(6) Yeh, A. T.; Nassif, N.; Zoumi, A.; Tromberg, B. J. *OPTICS LETTERS* **2002**, *27*, 2082-2084.

(7) Yeh, A. T.; Choi, B.; Nelson, J. S.; Tromberg, B. J. *J. Invest. Derm.* **2003**, *121*, 1332-1335.

(8) Yeh, A. T.; Kao, B. S.; Jung, W. G.; Chen, Z. P.; Nelson, J. S.; Tromberg, B. J. *J. Biomed. Opt.* **2004**, *9*, 248-253.

(9) Zoumi, A.; Yeh, A.; Tromberg, B. J. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11014-11019.

(10) Zoumi, A. In *Biomedical Engineering*; UCI: Irvine, 2003; p 202.

(11) Zoumi, A.; Lu, X.; Kassab, G. S.; Tromberg, B. J. *Biophys. J.* **2003**, *87*, 2778-2786.

Acknowledgment

Julia G. Lyubovitsky thanks George E. Hewitt and George E. Hewitt Foundation for Medical Research for the fellowship. This work was made possible, in part, through access to the Laser Microbeam and Medical Program (LAMMP) at the University of California, Irvine. LAMMP facility is supported by the National Institutes of Health under grant from National Center for Research Resources, NIH #P41RR01192