Rudolf Guthoff

On the way to in-vivo histology

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in Potsdam

CONFOCAL in-vivo microscopy reveals corneal detail on the cellular level, enabling researchers to discover new features of corneal innervation and learn more about the cells that play a pivotal role in corneal immunity. This offers the potential to help tackle some of the complications plaguing refractive surgery today, report researchers.

"We believe that confocal in-vivo microscopy with the Rostock Cornea Module (RCM) has more clinical potential than was thought up to now. This method provides a magnification of up to 800 times actual size, allowing us to sub-differentiate dry eye and wound healing processes after refractive surgery, examine the different forms of filtration cushions after fistulating glaucoma surgery, as well as understand more about the neoplastic changes of the cornea, conjunctiva, and eye lids. Examinations so far verify ex-vivo histology," said Rudolf Guthoff MD, head of the Rostock University Eye Clinic in Rostock, Germany.

The Rostock Cornea Module's enormous amplification potential allows investigators to break down the corneal layers into further component parts and observe variations in keratocyte cell densities. In this way, a quantitative determination of cells in the superficial, intermediate, and basal strata of the corneal epithelium is possible. Clinical observations, such as 'dry eye' can be investigated on the microscopic level. Similarly, changes in the corneas of contact lens wearers can be explained.

The epithelial layer's topmost stratum (5µm thick), for instance, has around 1000 epithelial cells, while the basal layer has closer to 10,000 epithelial cells, which can each be individually visualised with this technology, Dr Guthoff observed in a presentation of his work at the yearly congress of the DGII (German-Speaking Society for Intraocular Lens Implantation and Refractive Surgery).

Still deeper into the cornea, at roughly 50µm, this technology illuminates the nerve cell layer, as never before seen. While scientists once believed that corneal nerves grew radially inward toward the corneal centre, investigations with the RCM revealed that they grow in a spiral-like fashion. Knowing about the anatomy of corneal nerves and their growth pattern enables us to study the changes seen with innervation deficits, like in herpetic keratitis and after trigeminal nerve damage. It also provides refractive surgeons with better information about the nerve plexus they are disrupting and more clues on how nerves might regenerate after surgery, Dr Guthoff explained.

The RCM makes a three-dimensional image of the corneal strata, representing approximately 250µm cuts of the cornea. In LASIK patients, it shows the precise events

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at the surgical interface, including cell growth, cell differentiation at the interface, and interface fibrosis. Dr Guthoff suggested that femtosecond laser ablation sites be examined with this technology to determine the actual effect of the laser in the cornea.

Dr Guthoff believes that the information won through in-vivo microscopy can help surgeons understand why things happen as they do in the cornea and provide important insights into therapeutic options in refractive surgical patients.

He uses this in-vivo technology to study cell differentiation, as well, amplifying pathologic changes on the cellular level in various strata of the cornea. Leukocytes and dendritic cells are easily visible in the corneal epithelium in eyes with radial keratotomy. Cell movement within capillaries and other bits of functional information can be derived from the live, moving microscopic pictures, he noted.

Dr Guthoff described cell populations hardly known before, such as the corneal, antigen-presenting Langerhans cells. The RCM reveals sub-types of this cell in varying degrees of maturity, such as small, non-dendritic cells, big cells with long dendrites, and a net pattern formed between them. These cells play an important role in the immunologic processes of the cornea, as shown by their increased number in contact lens wearers.

Eyes that had penetrating keratoplasty show a high number of infiltrates within the corneal graft. In vivo microscopy can help show which cells are active in this process, such as white blood cells and Langerhans cells, as well as the genesis of this and other inflammatory processes.

He reported that Langerhans cells increase in number in response to preservatives used in eye drops. Although their exact role is still not clear, he feels that this technology will allow researchers to break through this still-unknown area. Again, in-vivo Langerhans images corresponded to impression cytological images made of these cells from other studies.

The technique relies on a confocal image, which Dr Guthoff compared to the image from the slit lamp, which eye doctors knew from everyday practice. As with slit-lamp imaging, the investigator precisely focuses the confocal microscope on a certain area, blocking out all other light, to concentrate on the illuminated area, only with the advantage of 800-fold image magnification.

The eye comes into direct contact with the machine, using anaesthesia, artificial tears and a small PMMA cap, which covers the objective and helps stabilise the image seen through the microscope. A newer cap design features a thin groove along the contact side, which helps reduce the applanation pressure and allows investigators to examine the corneal epithelium without side effects.

Dr Guthoff believes that researchers could easily take this technology a step further to perform in-vivo biopsies, to check for corneal pathologies. Also, the 'activated' keratocytes that are thought to cause corneal net formation in keratoconus patients could be amplified using the RCM 3-D imaging technology to reveal and possibly explain the complex network of interacting cells.

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