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CONFOCAL *IN VIVO* PROBING OF PROTEINS AND WATER IN THE RABBIT CORNEA

JONGSMA F.H.M.[1], ERCKENS R.J.[2], WICKSTED J.P.[3], MOTAMEDI M.[2], HENDRIKSE F.[1], MARCH W.F.[2]
University of Limburg[1], Maastricht, The Netherlands; University of Texas Medical Branch[2], Galveston, TX; Oklahoma State University[3], Stillwater, OK.

Purpose: To develop an instrument with high spatial resolution probing chemical composition of corneal tissue in live animal model without interference of signals originating from adjacent tissues.
Methods: A Raman probe for *in vivo* studies was developed that has the required resolving power in space, obtained by a confocally placed fiber for transportation of the Raman signal. Resolving power in time, to suppres movement artifacts, was obtained by a microcope objective with a high light gathering power and a long working distance.
Results: *In vivo* Raman spectra of protein and water collected along the optical axis of the excitation beam were obtained from rabbit corneas at exposure times of 1 s at spatial intervals of 70 µm with a resolution in depth of 30 or 150 µm.
Conclusions: The shallow probing depth of 30 µm proved to have enough spatial and temporal resolution to perform reliable measurements of the protein and water gradients in the corneal tissue in an animal model. However, for measurements where gradients are not relevant, the 150 µm probing depth, that requires less radiation energy, is adequate for intra corneal measurements.

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MONITORING THE WATER GRADIENT IN THE *IN VIVO* RABBIT CORNEA USING CONFOCAL RAMAN SPECTROSCOPY.

ERCKENS R.J.[1], JONGSMA F.H.M.[2], WICKSTED J.P.[3], MOTAMEDI M.[1], HENDRIKSE F.[2], MARCH W.F.[1]
University of Texas Medical Branch[1], Galveston, TX; University of Limburg[2], Maastricht, The Netherlands; Oklahoma State University[3], Stillwater, OK.

Purpose: Information of the corneal hydration status could be a helpful tool in the follow up on the healing process of the cornea after surgery or disease, and in the response of the cornea after the use of drugs. The aim of this study was to use Raman spectroscopy for monitoring the hydration status in the live rabbit cornea with a noninvasive confocal probe.
Methods: A noninvasive confocal probe was designed to collect Raman signals from the cornea with an integration depth of 30 µm. Argon laser light of 514.5 nm at 25 mW was used for excitation. The Raman scattered light was dispersed with a single grating spectrometer and detected by means of a CCD detector at a 1 s integration time. Three NZW rabbits were anesthetized. Normal Raman spectra were obtained while scanning through the cornea. Next, a hypertonic ophthalmic ointment (Muro 128) was added to dehydrate the cornea and a new set of Raman spectra were made.
Results: The CH stretching mode at 2945 cm^{-1} and the OH mode at 3390 cm^{-1} was used to determine the hydration status of the cornea. After instillation of the Muro 128 it was clearly visible that the the water concentration of the cornea decreased.
Conclusions: Changes in the water concentration of the cornea could be detected after topical instillation of a dehydrating drug. The dynamic changes in the cornea could be followed with subsequent scans at an energy level of 25 mJ for each scan.

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LOCALIZATION AND CHARACTERIZATION OF A NOVEL EXTRACELLULAR MATRIX PROTEIN βIG-H3

Rawe I[1], Zhan Q, Komai-Hori Y[1], Burrows R[1], Kublin CL[1], Hirano K[1], Bennett K[2], Cintron C[1]. [1]Schepens Eye Research Institute. [2]Bristol-Myers Squibb.

Purpose. In preparations of type VI collagen from rabbit corneas in the presence of N-ethylmaleimide an additional 66 kD protein was observed in coomasie blue stained gels only after reduction. We sought to characterize and localize the 66 kD protein.
Methods and Results. Co-elution of type VI collagen and the additional protein fractions from gel filtration chromatography support the contention that these proteins are associated. Sequencing of a full length cDNA clone from a corneal fibroblast library showed 88% homology with the published sequence of human βig-h3.Immunohistology with βig-h3 antibody was positive in many organs often associated, but not exclusively, with basement membrane. Positive fluorescence was also seen in guttata and abnormally thick epithelial basement membrane in Fuch's dystrophic corneas. Immunocytochemical analysis co-localized βig-h3 with type VI collagen filaments. In situ hybridization indicated that βig-h3 message is located in corneal epithelium, foetal stromal cells, and healing corneal wounds.
Conclusion. The novel protein βig-h3 is present in the extracellular matrix of corneal stroma where it is associated with filaments of type VI collagen. The highly conserved sequence homology of βig-h3 and the temporal expression of βig-h3 message suggest this protein plays a role in normal and pathological morphogenesis of extracellular matrix.