Biomedical imaging, the cornea, and regeneration

Optical imaging provides a means for non-invasive and in-vivo examination and diagnosis with sub-cellular resolution and without the use of extrinsic contrast agents. In the Marie Curie International Incoming Fellowships program 'MICROCORNEA' optical coherence tomography, in vivo confocal microscopy, and fluorescence microscopy have been used to examine corneas in clinical and experimental studies, to elucidate the mechanisms of tissue regeneration, wound healing, and pathologic changes in the injured, diseased or surgically-treated cornea. The work involved live-cell imaging and the interpretation of observed phenomena for scientific and diagnostic purposes.

The objectives of this research program were divided along clinical and experimental lines. In the clinical part of the research, the main objectives were to: i) present a detailed microscopic picture of how a biomaterial interacts with a patient's cornea after implantation; ii) to examine the impact of laser-tissue interaction in the cornea in terms of microscopic-level clinical outcomes in a cross-sectional group of patients; and iii) to examine microscopic-level corneal pathology in inherited corneal diseases in Swedish families. Objectives of the experimental part of the research were to: i) develop a means for non-invasive imaging to track cell behavior during wound healing in biomaterials in vitro; and ii) to attempt the first in-vivo detection of invisible corneal lymph vessels in a murine model of corneal inflammation.

Since the start of the MICROCORNEA program, the specific program objectives have been addressed by performing clinical corneal patient examinations across transplant and hereditary disease patient groups, and analyzing corneal microstructure by image analysis and statistical methods, reporting novel findings and their implications. In experimental work, new models have been developed to enable corneal vessels and cells to be studied in vitro and in-vivo by non-invasive means.

In the biosynthetic cornea study, the first group of trial patients to receive biosynthetic tissue to replace human donor corneas was followed for two years. In MICROCORNEA, the regeneration of host nerves and cells to cover and invade the implanted material was envisaged directly, and the slow but gradual conversion of the biomaterial into a human corneal architecture over time was found. The results were reported in translational medicine journals in 2009 and 2010. A third study was submitted in 2011, describing biointegration of implants within hosts, comparing microscopic corneal architecture with a parallel group of transplant patients with human donor corneas over the same three-year period. Numerous data was collected, detailing the structural and functional similarity of biosynthetic and human donor material in vivo, giving further evidence that laboratory-made biosynthetic materials could one day be used in place of scarce human donor tissue for the treatment of corneal blindness.

In the laser phototherapeutic keratectomy cross-sectional study, 39 patients treated for a corneal epithelial dystrophy over an 8-year period were examined for microscopic and clinical signs of recurrence of the disorder. After the laser treatment, the cornea is thought to regenerate to resemble a normal, healthy cornea. Microscopically, however, signs of

the dystrophy were found in 40% of patients, with a greater number of treated patients developing these signs over time. The micro-structure of the dystrophy was found to be milder than in untreated eyes in the same patients. A complex relationship between the micro-morphology and the clinical symptomatology of the dystrophy was uncovered. The study lays the groundwork for a deeper investigation into mechanisms of recurrence of the dystrophy after treatment, which is painful for patients and costly to re-treat.

Several Swedish families have been examined, to document for the first time, the genetic and microsopic changes in the cornea in several hereditary diseases. In one family, a new type of dystrophy that erodes the corneal epithelium was documented. In affected corneas of this family, multiple focal opacities developed in the cornea under the epithelium. In another group of families, a genetic disorder, aniridia, causing underdevelopment and malformation of the iris and other eye strcutures was examined. In affected eyes, but with milder pathology and clear corneas, a high density of inflammatory cells and nerves was quantified, the presence of opaque lesions in the epithelium was documented, and an absence of the epithelial stem cell niche was detected microscopically for the first time. These findings form the basis of further investigations that may help in identifying new treatment options for these patients in the future.

An experimental study of corneal cell migration and wound healing in cell-free biomaterials was undertaken in a newly-developed culture model using bovine corneas. The central section of the cornea was replaced by a biomaterial and an in-vivo confocal microscope was used to image the movement of cells into the biomaterial from the host. The development of scar tissue and fibroblast accumulation at the host-material border was imaged, and a slow movement of cells into the material paralleled clinical findings in patient corneas implanted with a similar material. This validation of the model and technique will form the basis of future studies aimed at influencing the rate and nature of cell migration during wound healing, for the purpose of improving the speed of healing while avoiding vision-limiting scar tissue formation after surgery.

In another experimental study, a model using in-vivo confocal microscopy of murine corneas undergoing inflammatory corneal angiogenesis was developed to enable the first in-vivo, label-free detection of lymph vessels in the cornea. Thought to have been invisible previously, lymphatics and the cells carried therein can now be viewed and quantified non-invasively. Conclusive proof of the identity of the in-vivo structures as lymphatics was presented by locating the same vessels in-vivo and ex-vivo after immunostaining. The morphological characteristics of the unstained in-vivo vessels were subsequently used to detect corneal lymphatics in a live human subject. This technique could be used to screen patients to determine the risk of corneal transplant rejection both prior to and after surgery, thereby avoiding costly regimens of medication or re-transplantation.

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