Nanopores in Track-Etched Polymer Membranes as Substitutes for the Tight Junctions in a Novel Concept of an Artificial Corneal Endothelium *

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This collaborative project is aimed at creating an artificial substitute for the eye's corneal endothelium. The availability of an artificial endothelium able to duplicate the transendothelial ion-and-fluid transport function of the natural endothelium will remove the need for donor tissue or cell-based constructs in the treatment of corneal blindness. To date, no attempts were successful to substitute a dysfunctional corneal endothelium with an artificial membrane.

The natural endothelium consists of a monolayer of specialized cells, and has an essential role in maintaining the transparency of our cornea by performing a process called "pump-barrier function", which assures a constant hydration of the corneal stroma. The loss of this function leads eventually to blindness.

The mechanism of the pump-barrier function is still disputed. Based on Fischbarg's hypothesis [1] that the fluid transport through the endothelium is achieved by electroosmosis through the leaky tight junctions between cells, we proposed [2] that the nanopores in track-etched membranes can mimic the natural tight junctions. An essential condition for the electoosmosis is fulfilled by the fact that the internal pore walls are electrically charged.

Poly(ethylene terephthalate) (PET) membranes (12-µm thick) were irradiated at the UNILAC using Au ions (11.1 MeV/u) and a fluence of either 10^6 or 10^7 ions/cm^2. Subsequent track etching was performed at 50 ºC in 2M NaOH for various times (5, 25, 125 and 250 min) yielding cylindrical, parallel oriented pores of diameter 20, 100, 500 and 1000 nm, respectively.

Preliminary experiments have been performed to quantify the electroosmotic flow (EOF) through track-etched polymer membranes. Wang and co-workers did perform similar experiments earlier, where they recorded a mass flow by direct displacement of the electrolyte [3, 4]. In our work, we used a fluorophore to monitor the mass transport through the membrane, with the view that the fluorophore will also allow to account for other effects such as diffusion and electrophoresis.

In our experiment, two compartments were separated by a track-etched polymer membrane where the average pore diameter was 100 nm. One compartment was loaded with an electrolyte containing 100 mM KCl and 1 mg/ml Eosin Y, and the other was filled with exactly 1 ml of electrolyte without the dye. Then, gold electrodes were placed in each compartment and a potential of 500 mV was applied for two hours.

Figure 1 shows photoluminescence spectra of a diluted aliquot of the electrolyte-only compartment after two hours of application of the potential. The red spectrum shows the results when the anode was placed in the electrolyte-only compartment, the black spectrum corresponds to the reverse polarisation. The arrows indicate the direction of the respective flow components. By comparing these two spectra, it is evident that EOF is the dominating contribution, however, diffusion plays a significant role as well. Further studies will be necessary to quantify all transport mechanisms systematically.

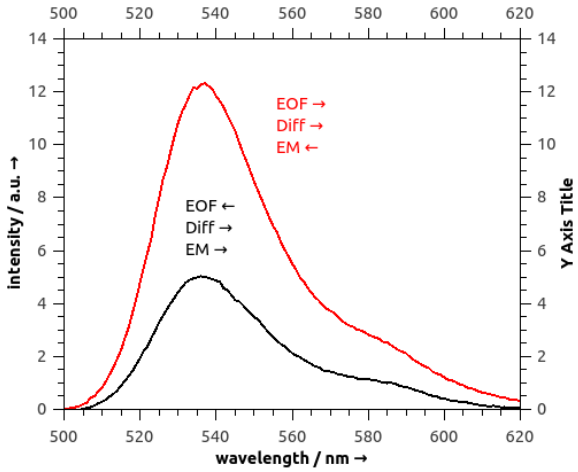

Figure 1: Photoluminescence spectra from electrolyte-only compartment after two hours of voltage application indicating the electroosmotic transport of Eosin Y. For the red (black) spectrum the anode (cathode) was placed in the electrolyte-only compartment. EOF is the direction of the electroosmotic flow, Diff denotes diffusion, and EM means electrophoretic flow.

References

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