

P003 Transfection of Retinal Pigment Epithelium with Pegylated Lipoplexes

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The target cells for ocular gene therapy are often located in the neuroretina or the retinal pigment epithelium (RPE). In this research the transfection of RPE cells with pegylated (polyethylene glycol (PEG) containing) DOTAP DOPE lipoplexes was studied.

RPE cells are easy to transfect with non pegylated DOTAP DOPE lipoplexes. In contrast, even the smallest amount of incorporated PEG decreases the transfection level drastically. Confocal microscopy showed that cellular binding was not a limiting step for transfection with pegylated lipoplexes, as even high pegylated lipoplexes showed good cell binding. However, confocal images showed that the internalization of pegylated lipoplexes is less efficient than for the non pegylated lipoplexes. Moreover, also the long term intercellular processing is different for pegylated and non pegylated lipoplexes. In a next step, the pegylation method of the DOTAP DOPE lipoplexes was altered. Instead of incorporating PEG in the lipoplexes, preformed lipoplexes were coated with PEG containing lipids. The stability of these lipoplexes was comparable with the standard pegylated lipoplexes. However, the post coated variants showed much better transfection efficiency. In conclusion, pegylation of DOTAP DOPE lipoplexes severely affects their transfection capacity for RPE cells. Post coating of DOTAP DOPE lipoplexes can circumvent this problem and forms a good alternative pegylation procedure.