SINGLE PARTICLE TRACKING TO MEASURE THE MOBILITY OF RETINAL GENE THERAPEUTICS AFTER INJECTION IN THE VITREOUS HUMOR OF THE EYE

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Thanks to recent advances in genomic screening, several disorders affecting vision have been linked to genetic defects of the retina. Instead of treating the symptoms, gene therapy offers the possibility to cure these dystrophies, by either blocking a dysfunctional gene or by delivering a new functional one to the target cells. However, the biggest hurdle remains the delivery of the therapeutic nucleic acids to the appropriate target site, without compromising the nucleic acid integrity and functionality by the harsh extracellular environment. For that reason, the negatively charged nucleic acids are usually complexed with e.g. cationic polymers, forming non-viral gene delivery vehicles of about 100–200 nm in size. In retinal gene therapy, the preferred method of administration is injection in the central part of the eye, the vitreous humor (Fig 1). However, if delivery to the retina is intended, efficient mobility of the gene nanocarriers in the vitreous humor towards the posterior part of the eye is of critical importance.

In this work, we have optimized an ex vivo assay based on excised bovine eyes in which single particle tracking (SPT) experiments can be performed to investigate the diffusive properties of fluorescent nanoparticles in an intact model of bovine vitreous humor [1]. From analyzing many hundreds of single particle trajectories (Fig 2) of model polystyrene nanospheres, we found that both electrostatic and hydrophobic interactions are key determinants for the diffusion rate of nanoparticles in vitreous humor. By analyzing the mode of motion of the fluorescent polystyrene beads, we also found that particles with a size up to 1 µm are able to move freely in the vitreal network. Finally, we employed this methodology to determine whether a promising class of non-viral gene delivery vectors are suitable for intravitreal administration. Complexes of anionic plasmid DNA (pDNA) and cationic CBA-ABOL polymers [2] were evaluated with our assay, confirming the findings that cationic charge and hydrophobicity should be avoided when developing nanomedicines for retinal delivery.

1. Martens, T., et al., Nanomedicine UK, accepted for publication