XPMag Results.

1. Description

XPMag, is a novel magnetic nanoparticles formulation dedicated to gene transfection in organotypic cultures of explant by "**Reverse Magnetofection**". "Reverse Magnetofection" provides a novel and non-toxic strategy for nucleic acids (DNA & siRNA) based gene therapy in the retina that can be transferred to a wide variety of organ explants.

XPMag, associated to Reverse Magnetofection allows the delivery of nucleic acids (NA) up to the deepest explant layers. The magnetic force delivered by a specific magnetic plate guides and concentrates complexes of NA and XPMag magnetic nanoparticles from the culture media to the tissue. This novel method of transfection associating XPMag and Reverse Magnetofection is fully biocompatible and does induce neither apoptosis nor inflammatory reactions.

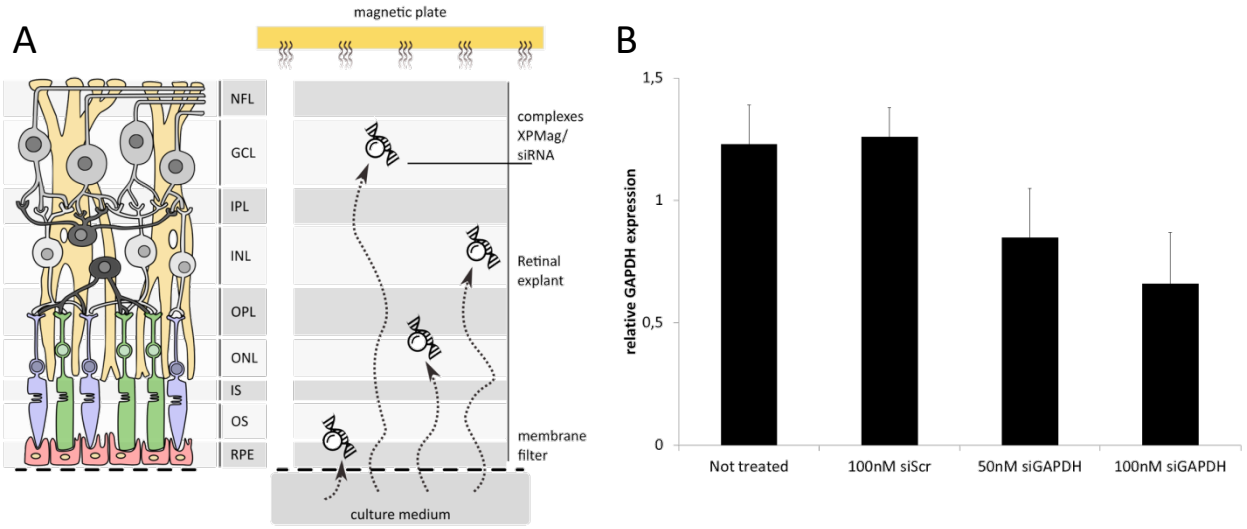
2. Storage and shipping condition

Storage: Upon reception and for long-term use, store XPMag at -20°C; stability: 1 year
Shipping condition: The reagent is shipped at RT

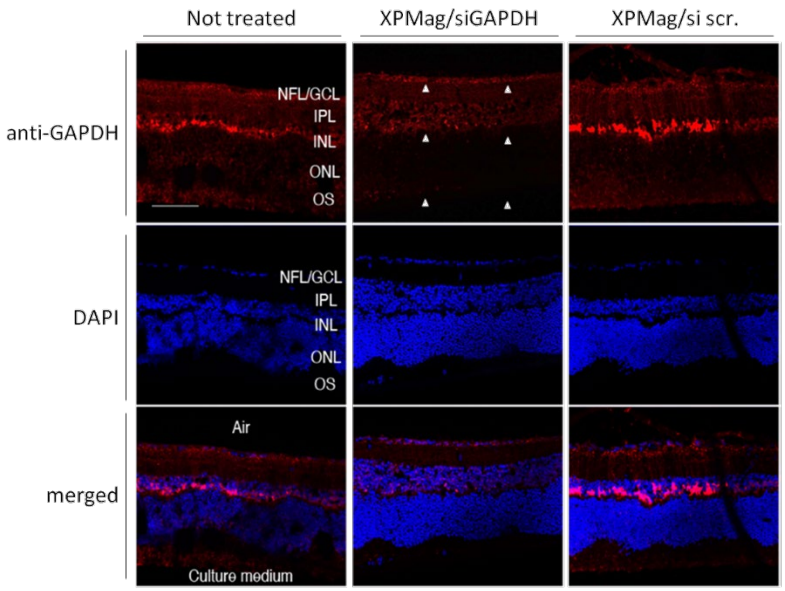
XPMag and Reverse Magnetofection allow gene silencing through the whole thickness of the retina.

XPMag silences gene expression of GAPDH through the whole thickness of retina explants.

The magnetic plate placed above the explant attracts complexes upward, from the medium to the inner layer of the explant. In retina, Ganglion cell layer (GCL) can be reached with magnetic complexes using Reverse Magnetofection (A). 100 nM siRNA against GAPDH (siGAPDH) or scrambled siRNA (siScr) were transfected by Reverse Magnetofection in sections of the central retina (B). Gene silencing was evaluated by western blot using antibodies against GAPDH. Bands intensities were normalized and expressed as relative GAPDH expression.



72H after transfection as described before, gene silencing was evaluated by immunostaining using antibodies against GAPDH. Bands intensities were normalized and expressed as relative GAPDH expression



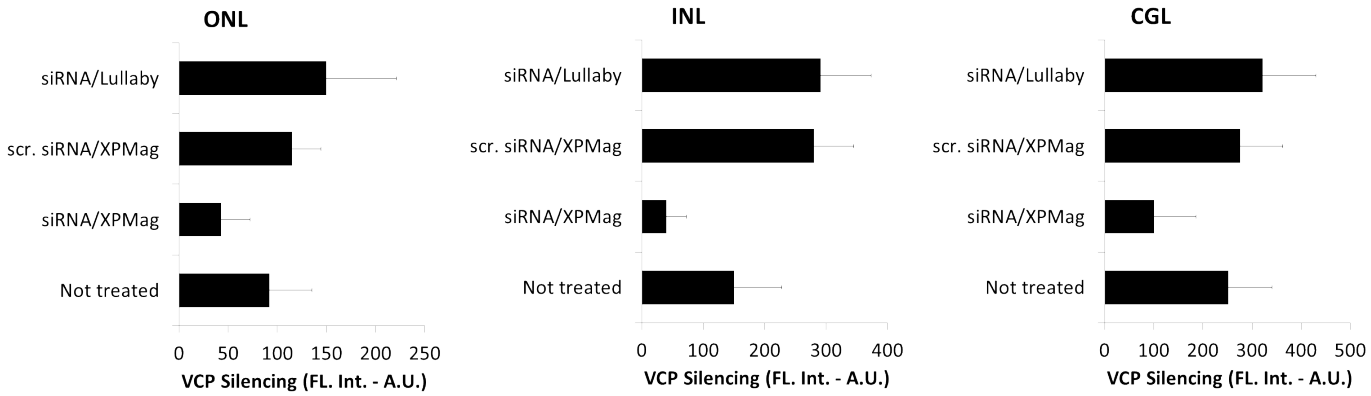
Results adapted from Bassetto M., *et al*, 2021, in press.

These results demonstrated the capacity of XPMag to silence gene expression of endogenous GAPDH gene through the whole retina explant.

Functional gene silencing in retina explant with XPMag and Reverse Magnetofection.

XPMag efficiently silences gene expression of Valosin-Containing-Proteins (VCP) in Rat retinal organotypic culture by reverse Magnetofection through the entire layers of the retinal explant 72 h after treatment.

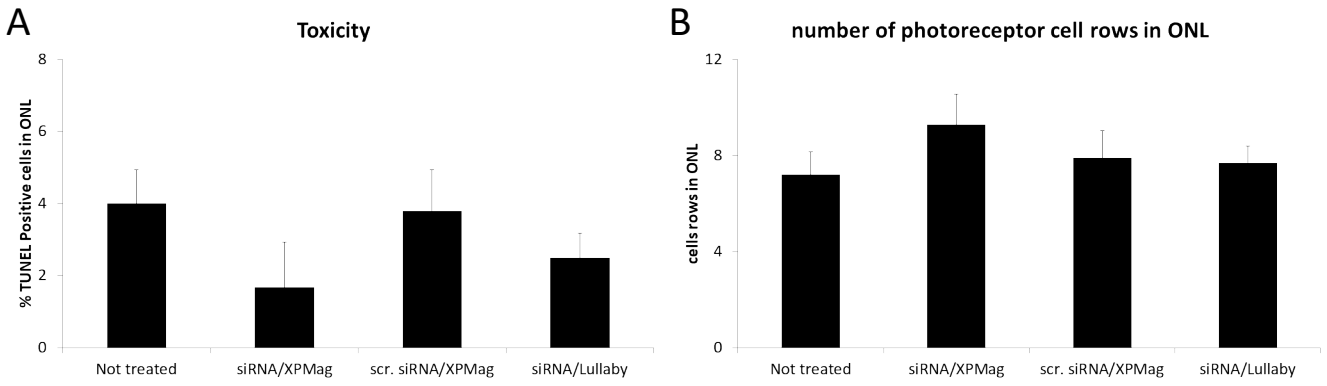
Retinae of postnatal day 12 rats were explanted, cultured for 3 days and treated or not with 50 nM VCP siRNA complexed to XPMag or Lullaby transfection reagent. Quantification of VCP immunofluorescence intensity was performed in ONL, INL and GCL.



Results adapted from Sen M., Bassetto M., *et al*, Pharmaceutics 2021, 13(2), 225: "Efficient Ocular Delivery of VCP siRNA via Reverse Magnetofection in RHO P23H Rodent Retina Explants".

Viability is not affected by XPMag driven by Reverse Magnetofection and VCP silencing is neuroprotective.

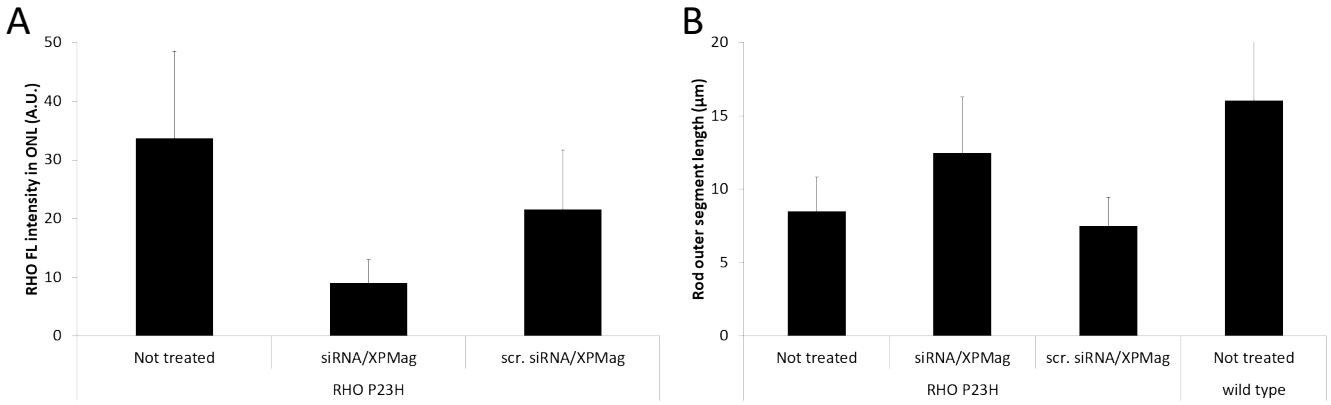
Retinae of postnatal day 12 rats were explanted, cultured for 3 days and treated or not with 50 nM VCP siRNA complexed to XPMag or Lullaby transfection reagent. Toxicity was determined by TUNEL assay (A) and photoreceptor cell rows number was counted in ONL (B).



Results adapted from Sen M., Bassetto M., *et al*, Pharmaceutics 2021, 13(2), 225:"Efficient Ocular Delivery of VCP siRNA via Reverse Magnetofection in RHO P23H Rodent Retina Explants".

VCP silencing using XPMag and Reverse Magnetofection corrects sublocalization of rhodopsin and increases length of Rod outer segment in rhodopsine-defective rats (RHO P23H).

Retinae of postnatal day 12 RHO P23H transgenic and wild type rats were explanted, cultured for 3 days and treated or not with 50 nM VCP siRNA complexed to XPMag. Quantification of RHO mean intensity (A) and measurement of length of the OS in rod cells were performed in the ONL (B).



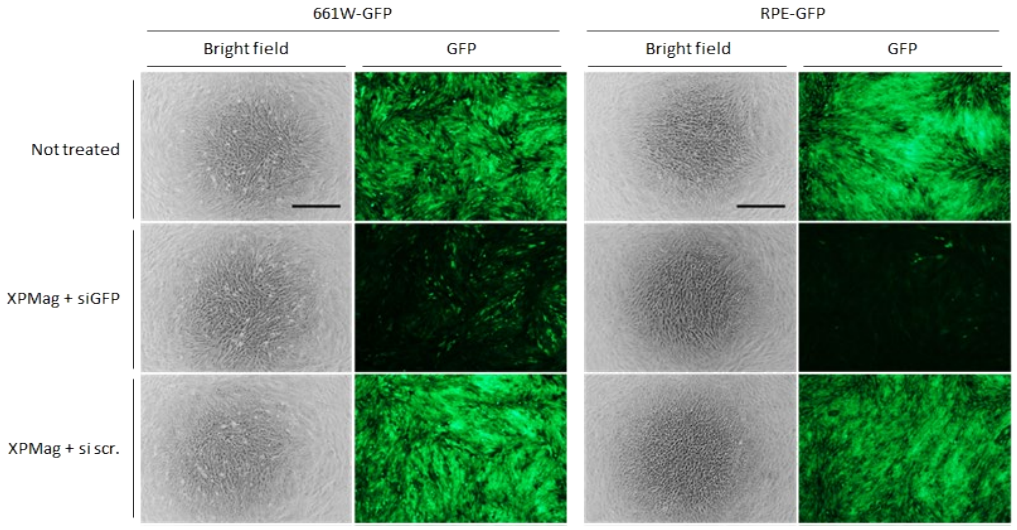
Results adapted from Sen M., Bassetto M., *et al*, Pharmaceutics 2021, 13(2), 225:"Efficient Ocular Delivery of VCP siRNA via Reverse Magnetofection in RHO P23H Rodent Retina Explants".

Altogether these results demonstrated the capacity of XPMag to silence gene expression of functional gene and to restore a phenotype in the whole retina explant.

Gene Silencing in retinal cell line 661W & RPE.

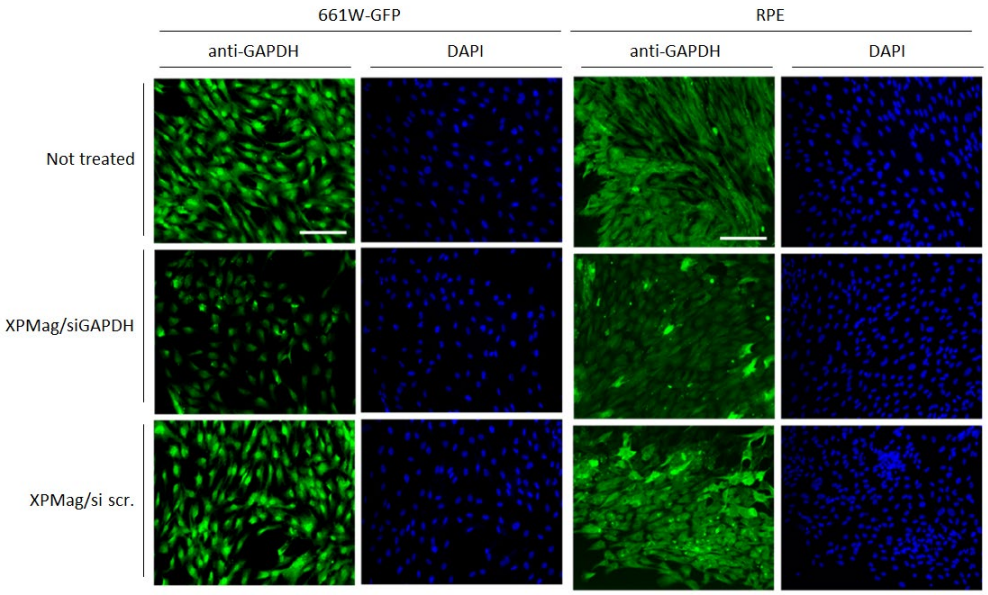
XPMag efficiently silences gene expression in retinal cell lines 661W and RPE.

GFP stably transduced photoreceptors cell line (661W) and retinal pigment epithelial cell line (RPE) were transfected with 2 µL of XPMag complexed with 50 nM GFP (siGFP) or scrambled siRNA (si scr.). Gene silencing was monitored 72 h after transfection by fluorescence microscopy.



Results adapted from Bassetto M., *et al*, 2021, in press.

661W and RPE cell lines were transfected with 2 µL of XPMag complexed with 50 nM siRNA targeting GAPDH (siGAPDH) or scramble siRNA (si scr.). 72 h after transfection, GAPDH expression was monitored by immunostaining.



Results adapted from Bassetto M., *et al*, 2021, in press.

These results demonstrated the capacity of XPMag to silence gene expression in retinal cell lines.