Synthesis and application of magnetic nanoparticles (MNPs) in siRNA delivery to the retina

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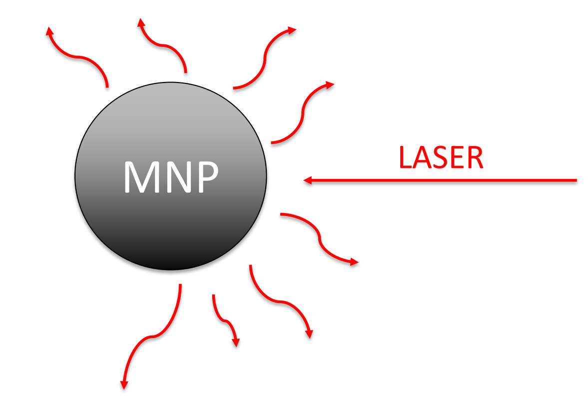
INTRODUCTION

Small interfering RNA (siRNA) is an important RNA interference (RNAi) tool that has found significant applications in ocular therapy[1]. Despite its therapeutic potential, clinical trials in humans showed intravitreous injections (IVT) of siRNA have unfavorable intraocular pharmacokinetics and pharmacodynamics[2]. For this reason, there is a considerable demand of siRNA delivery systems (DS) able to effectively transfect cells within the retina. Up to now several DS based on nanocarriers such as lipids, nanoparticles and polymers have been tested for this application but in all the cases invasive injections are the only administration routes explored[2]. Moreover, a major limitation shared by all these delivery systems is the lack of active targeting toward the posterior segment of the eye that hampers high transfection efficiencies.

Therefore, **we aim to develop a new magnetic nanoparticles-mediated delivery technology that shall allow safe and efficient siRNA delivery to the retina assisted by magnetic targeting.**

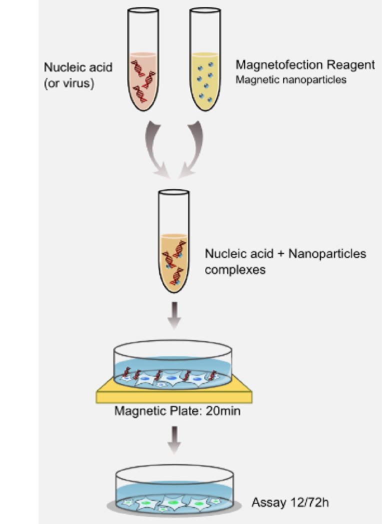
MATERIALS AND METHODS

1- MNPs synthesis & characterization



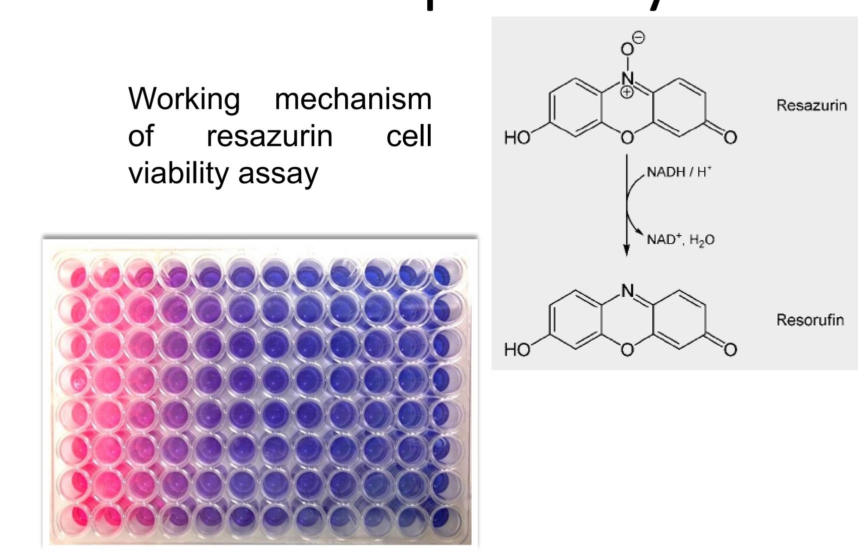
Schematic representation of Dynamic Light Scattering (DLS) technique

2- *In vitro* RNAi using MNPs



Magnetofection® procedure

3- MNPs biocompatibility *in vitro*



Example cell viability experiment with resazurin

RESULTS

MNPs physico-chemical characterization

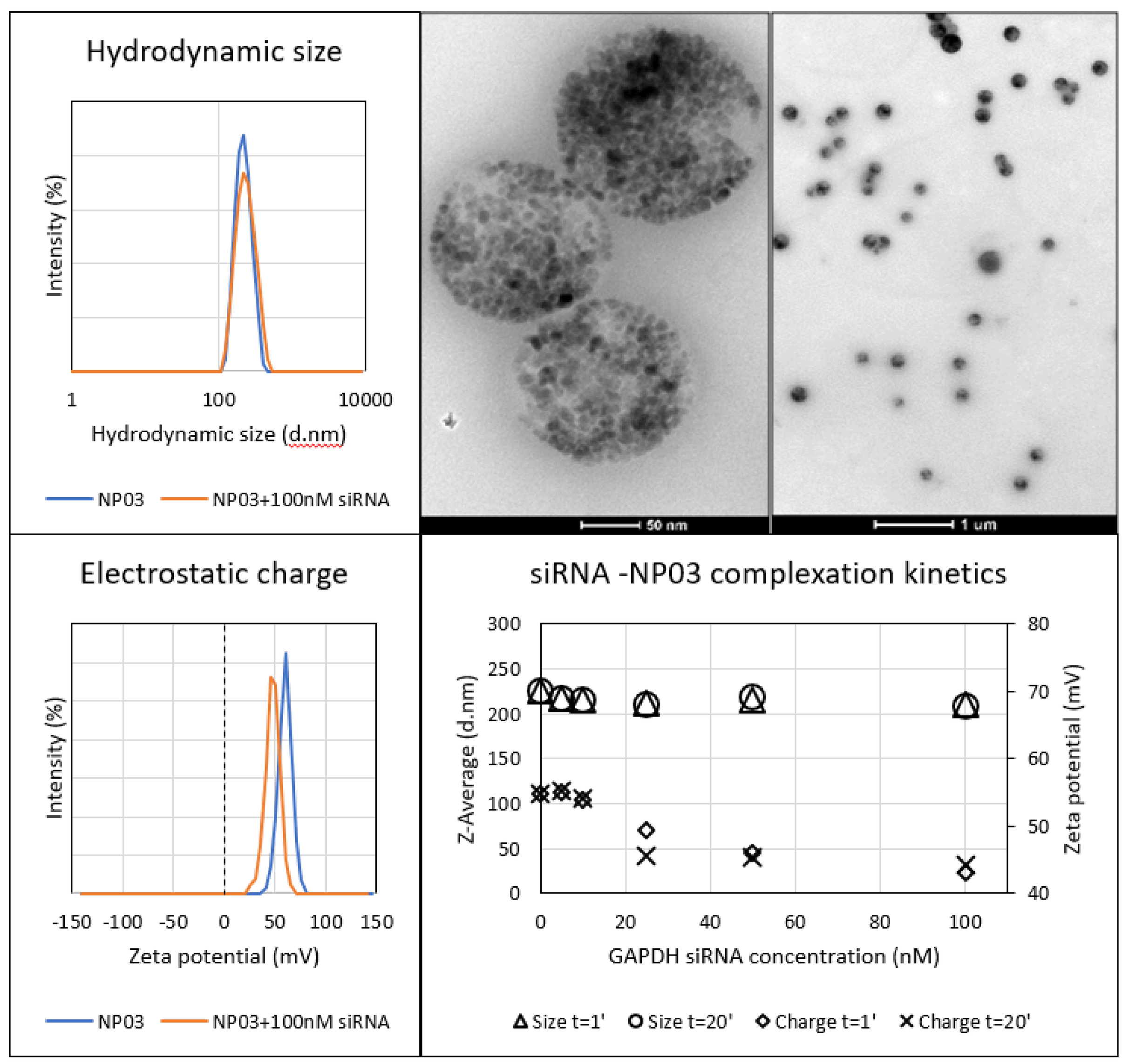


Figure 1: hydrodynamic size (A) and zeta potential (B) of NP03 before and after siRNA complexation; TEM images of NP03 (C,D); siRNA complexation kinetics by NP03 with fixed NP03 concentration (E).

Cationic MNPs complex siRNA efficiently

In vitro RNAi of GFP reporter gene in retinal cell line

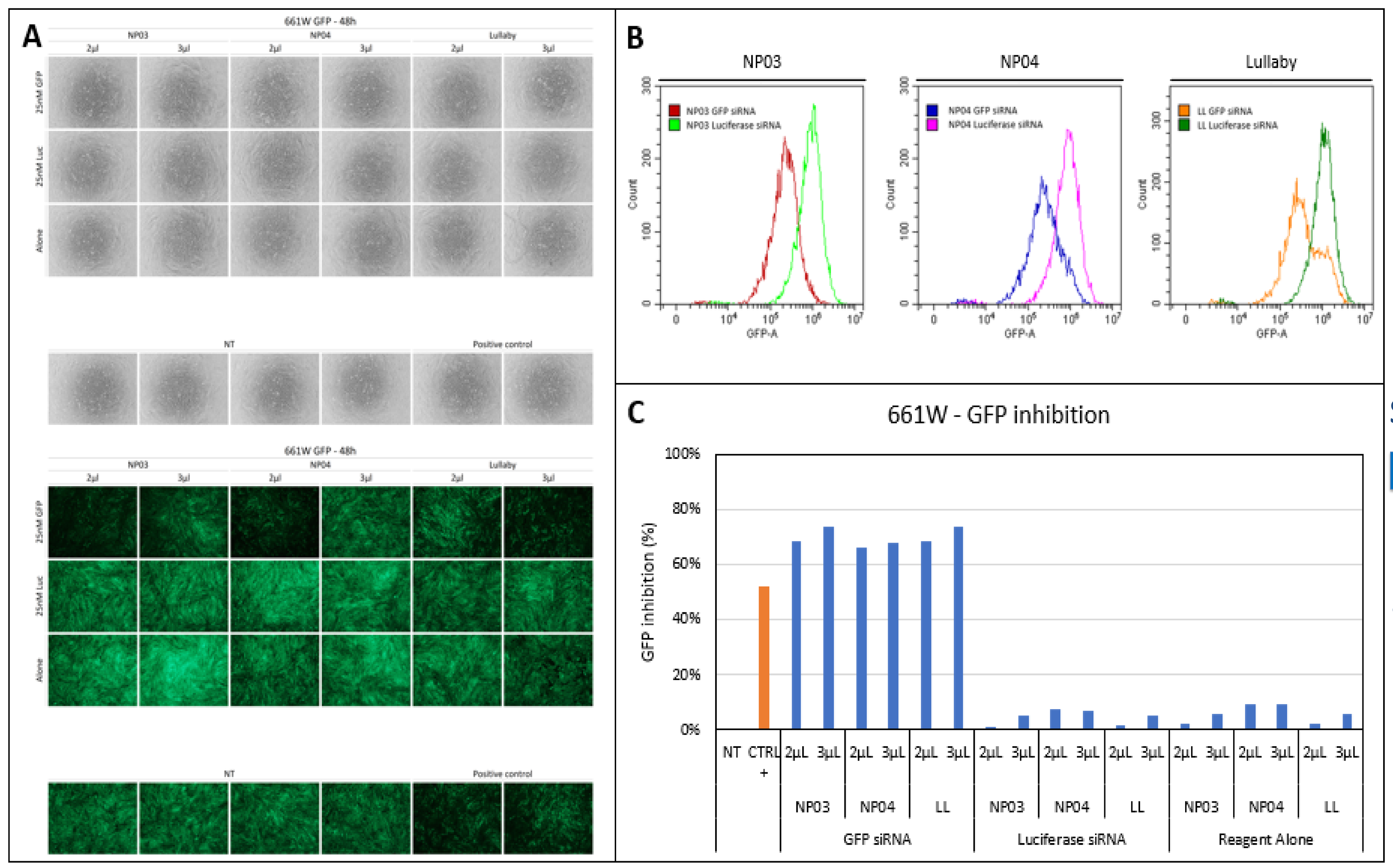


Figure 2: example of *in vitro* GFP gene silencing with MNPs in 661W GFP (photoreceptors) cell line. Fluorescence microscopy after GFP siRNA delivery (A); GFP gene silencing by flow cytometry at 48h from the treatment (B); GFP inhibition expressed as % compared to the not treated cells (C).

Efficient siRNA delivery and gene silencing in retina cells

In vitro RNAi of endogenous gene & biocompatibility

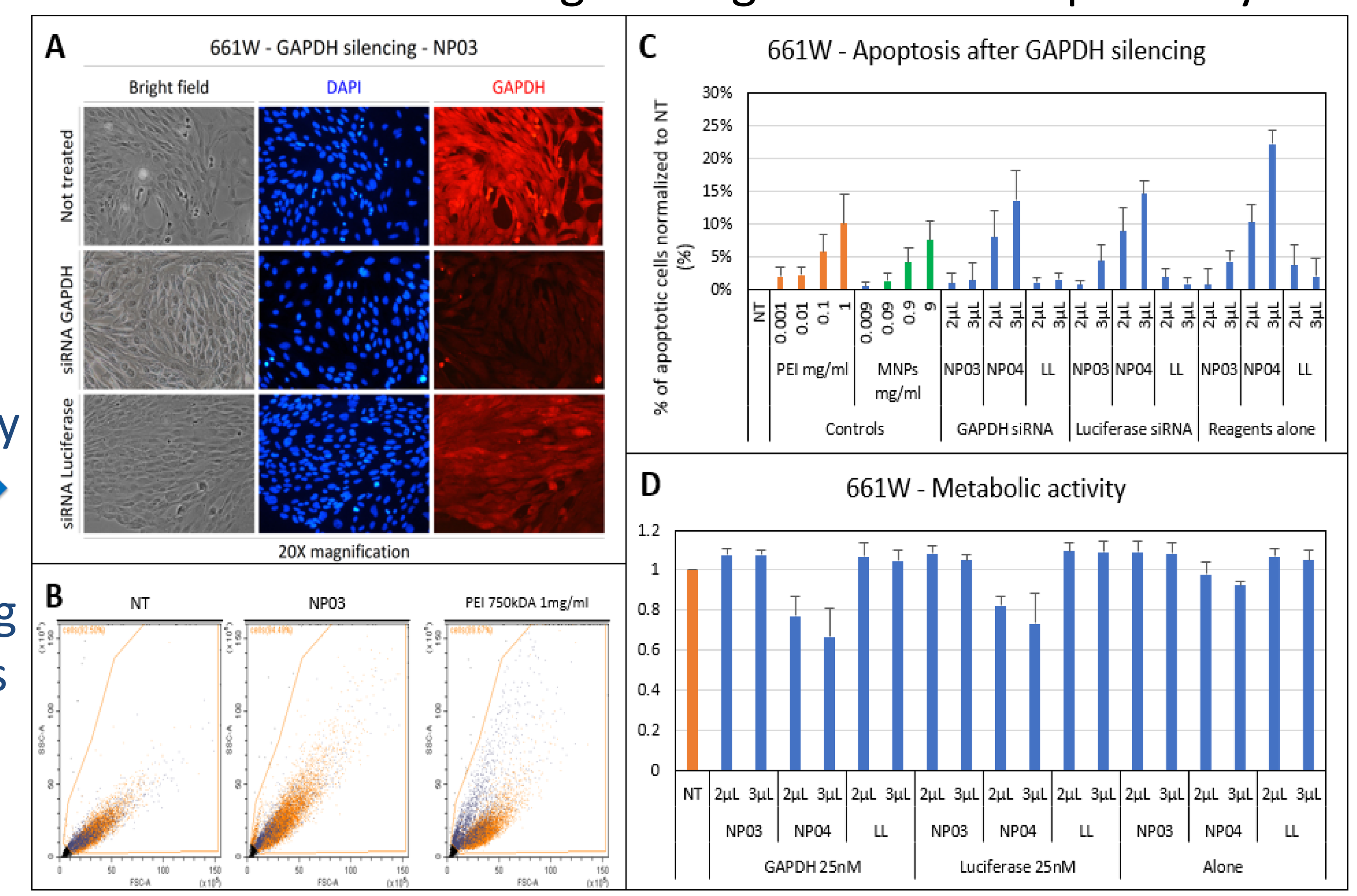


Figure 3: Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene silencing in 661W cells with NP03 by immunofluorescence (A); effect of NP03 on 661W cell population (B); cell apoptosis in 661W cells 48h after transfection, n=3 (C); resazurin assay 48h after gene silencing experiment in 661W cells, n=3 (D).

CONCLUSIONS

- Stable MNPs formulations have been established loading and deliver siRNA to retinal cells
- Efficient gene silencing has been achieved using MNPs and magnetic targeting *in vitro*
- The formulations are biocompatible and tolerated by retinal cells *in vitro*

REFERENCES

1. Kaiser PK et al; RNAi-based treatment for neovascular age-related macular degeneration by Sirna-027. (2010). Am J Ophthalmol 150: 33–39.e2
2. Guzman-Aranguez A. et al; Small-interfering RNAs (siRNAs) as a promising tool for ocular therapy. (2013). British Journal of Pharmacology (2013) 170 730–747

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