



Lipid nanoparticles for the sustained release of miRNA: new perspectives into intervertebral disc regenerative medicine.

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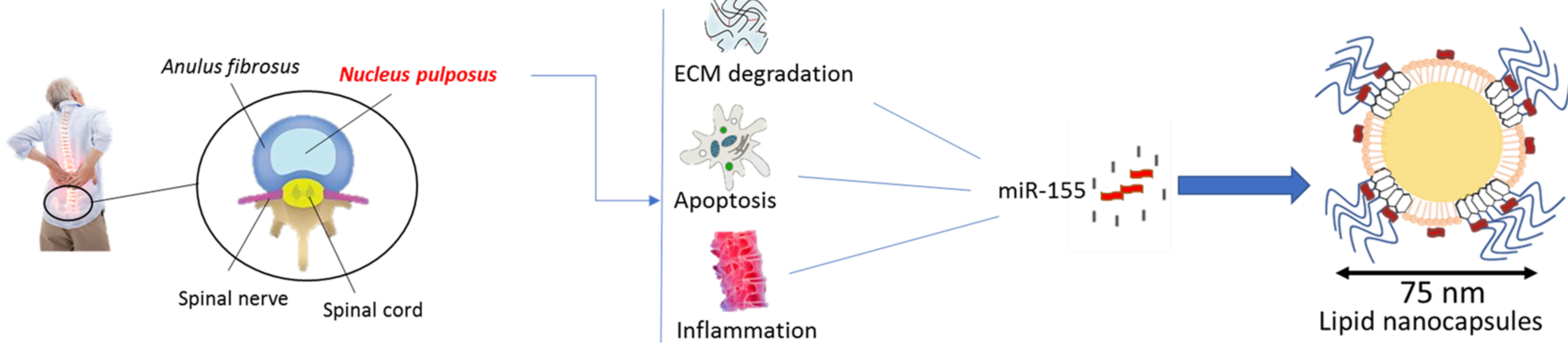
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Introduction

Disregulation of miRNAs, notably the miRNA-155, and their targets have been associated with several diseases including intervertebral disc degeneration (IDD). The relevance of unprotected miRNAs for therapeutic applications suffers from their fast *in vivo* degradation, requiring iterative deleterious injections. Thus, the development of nanocarriers is a prerequisite for a miRNA therapeutic use. Therefore, miRNAs protection from nucleases is considered as a prior condition for the development of sustainable systems. Among relevant options, nanoparticles, such as lipid nanocapsules (LNCs), exhibit a suitable strategy thanks to their ability to encapsulate DNA and siRNA (Catherine P.et al. Patent FR3026009. 2014).

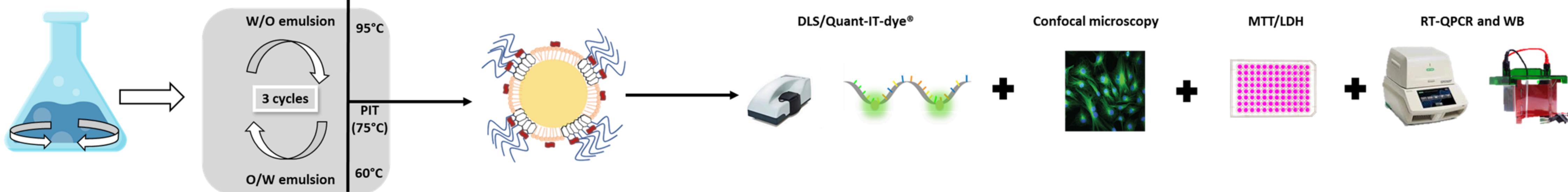
Objectives

For the present study, the objectives were to develop and fully characterized miRNA loaded LNCs (encapsulation efficacy, size, PDI and zeta potential, miRNA release, protection), to evaluate the potential cytotoxicity of the formulation on *Nucleus pulposus* (NP) cells and to study the cell internalization and the *in vitro* bioactivity.



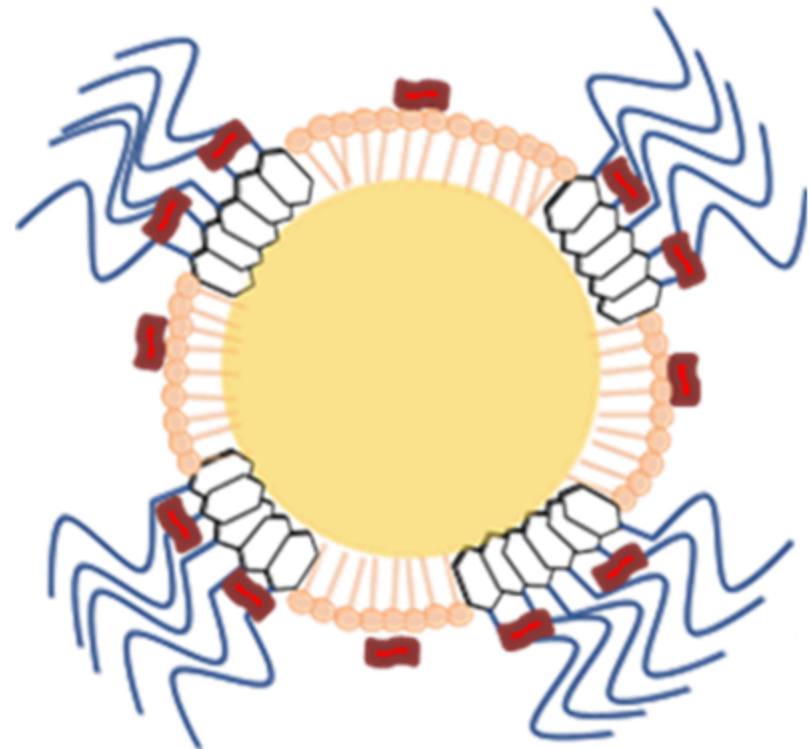
Materials and Methods

- Formulation** of miR-155-LNCs was performed by phase inversion process.
- Characterization** of miR-155-LNCs was done by dynamic light scattering measurement and fluorescent quantification of miRNA, after purification.
- Protection and release** of miR-155 were investigated by gel electrophoresis before and after human serum exposition and by dialysis in PBS at 37°C respectively.
- Internalization and cytocompatibility** study were assessed by confocal microscopy and LDH/MTT tests on human adipose stromal cells (hASC).
- Bioactivity** was investigated by RT-QPCR and western blot on ERK1/2, one of the main targets of miR-155.



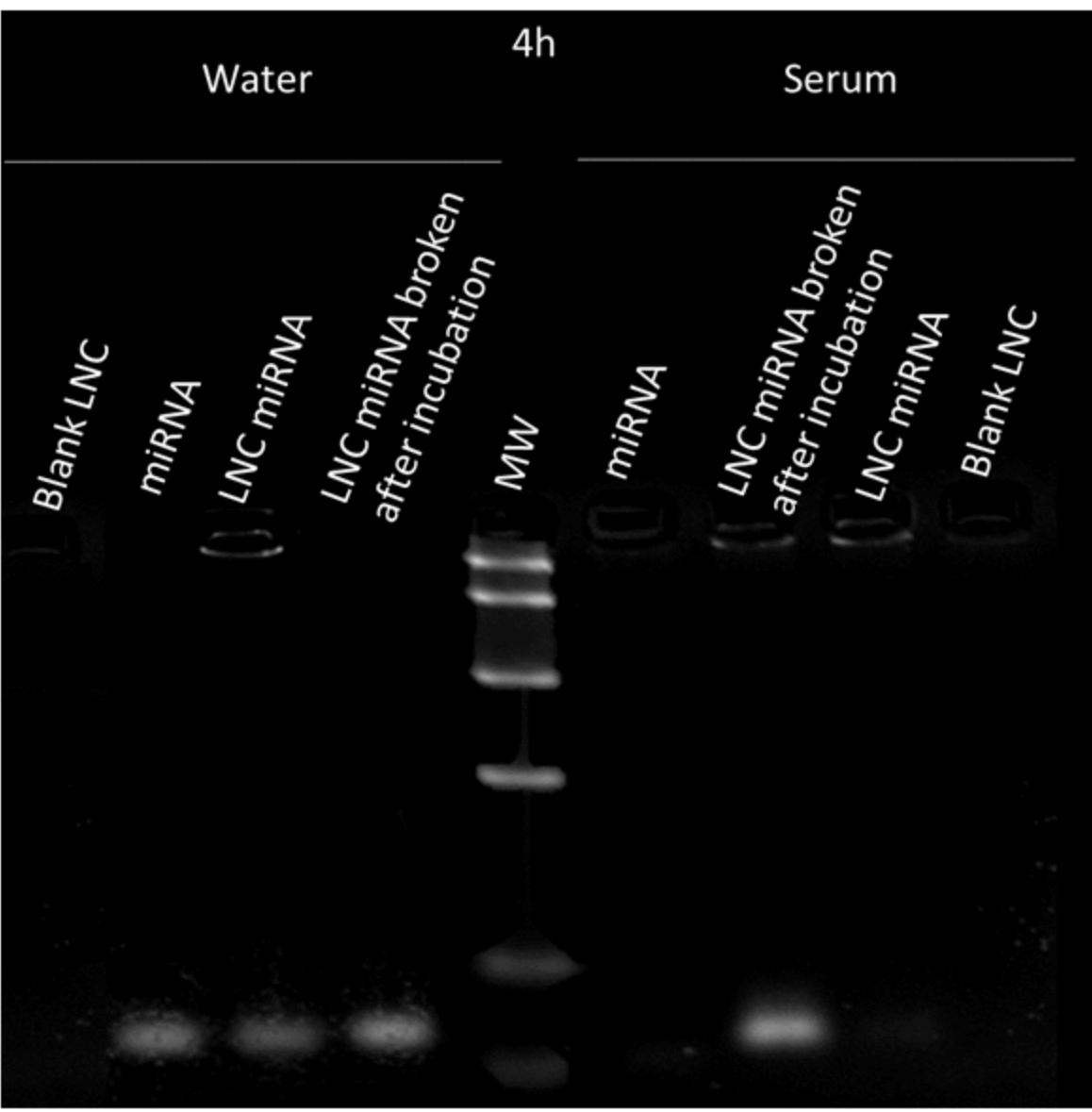
Results

Characterization



Diameter: 72.1 +/- 3.2 nm
Polydispersity index: 0.06 +/- 0.03
Encapsulation efficiency: 75.6% +/-1.6
Drug loading: 590.2 +/- 2.5 µg/g LNC
Zeta potential: +10 +/- 3.5 mV

Protection and release

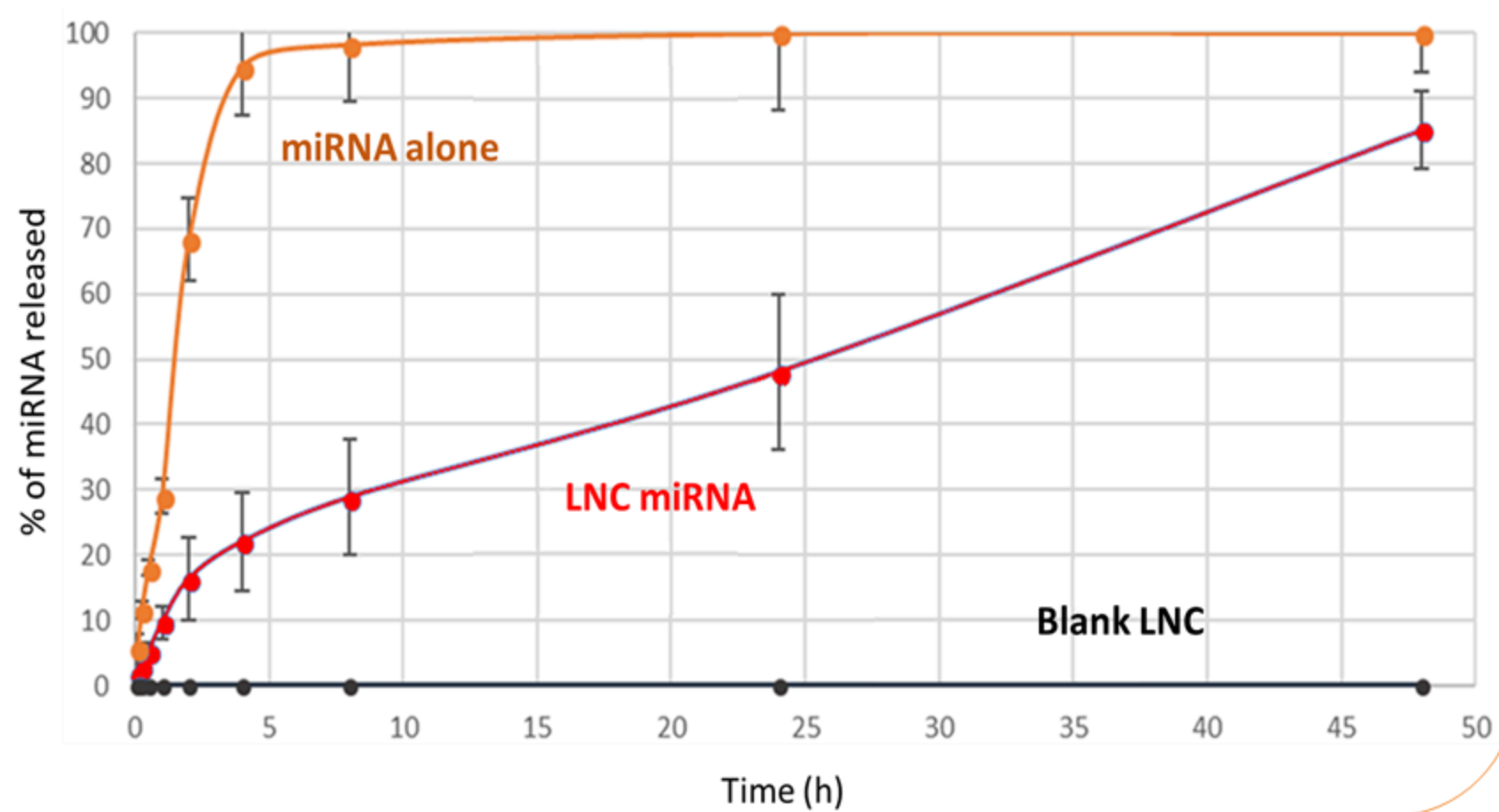


Gel electrophoresis

The miRNA alone was totally degraded after 4h, whereas the miRNA inside the LNCs was undegraded. Compared to the profile in water, gel electrophoresis demonstrated that LNCs protected the miRNA from nuclease degradation, after serum exposition.

Release study

The release profile showed that LNCs delayed the miRNA release compared to free miRNA: at 4 h, 23.3 % ± 7.1 was released compared to 94.8 % ± 5.4 for free miRNA.

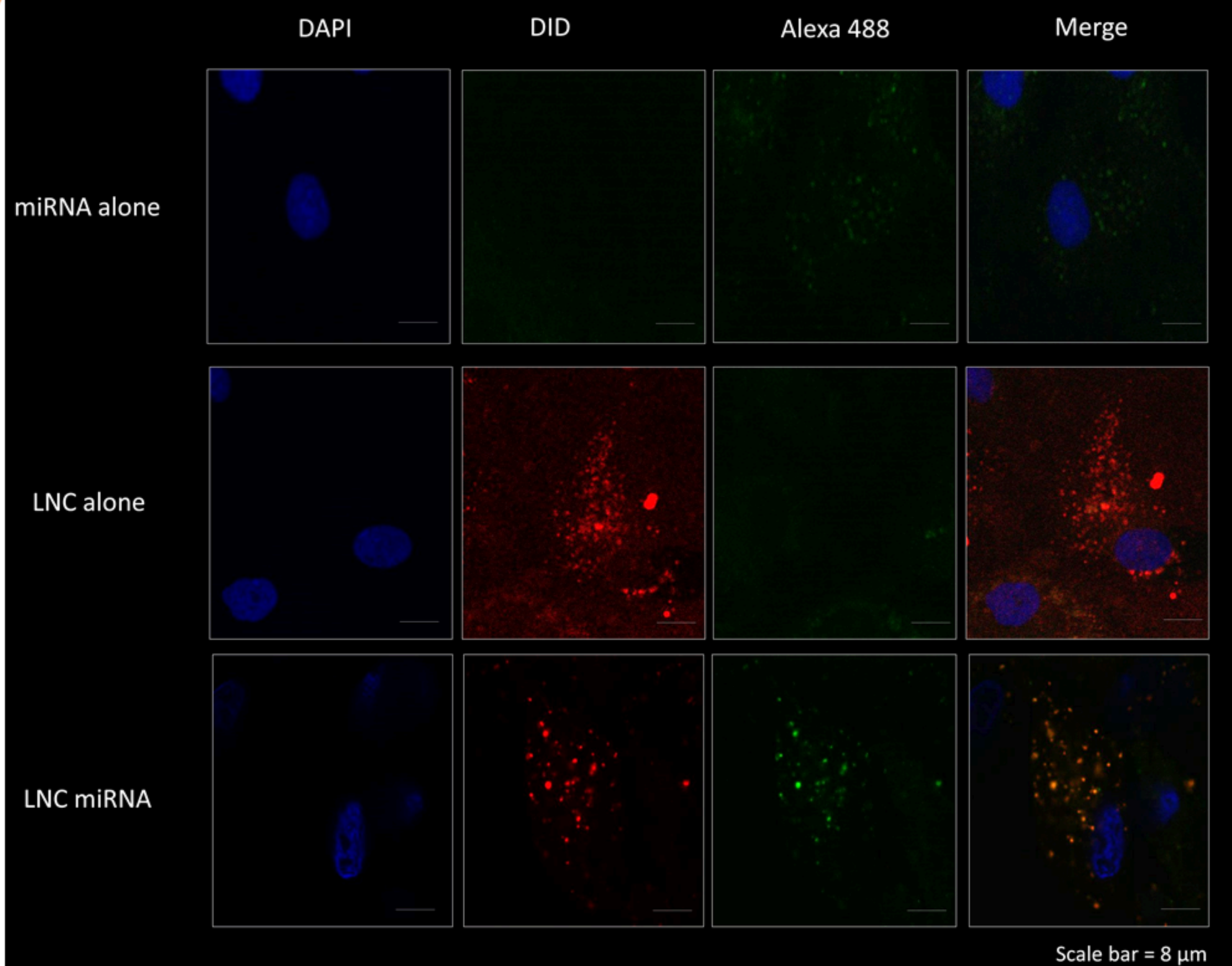


Bioactivity

RT-qPCR and Western blot

RT-qPCR and Western blot showed that LNC miR-155 reduced drastically the ERK1/2 genic and protein expressions and proved the necessity of LNC to protect miR-155, in allowing its bioactivity inside the cells. The different control, LNC without miRNA (blank LNC), LNC with unspecific miRNA (LNC miRNA ctrl) and LNC with anti-miRNA blocking the miR-155, confirmed the results

Internalization and cytocompatibility

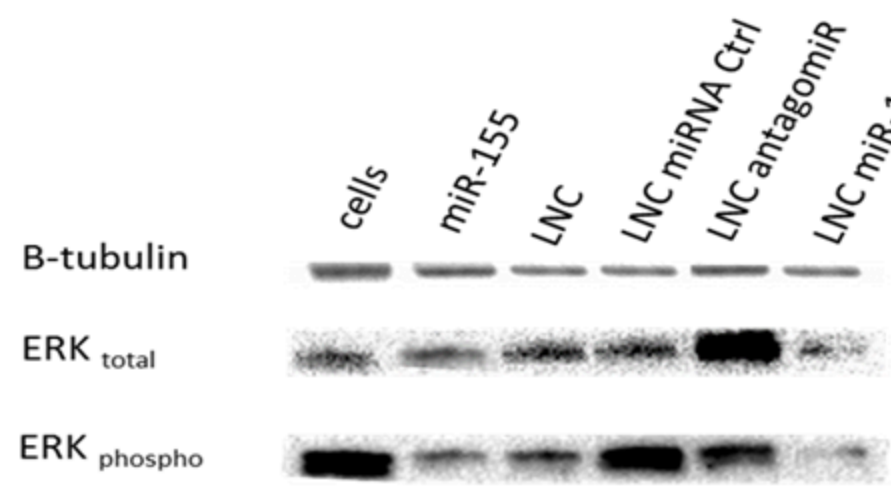
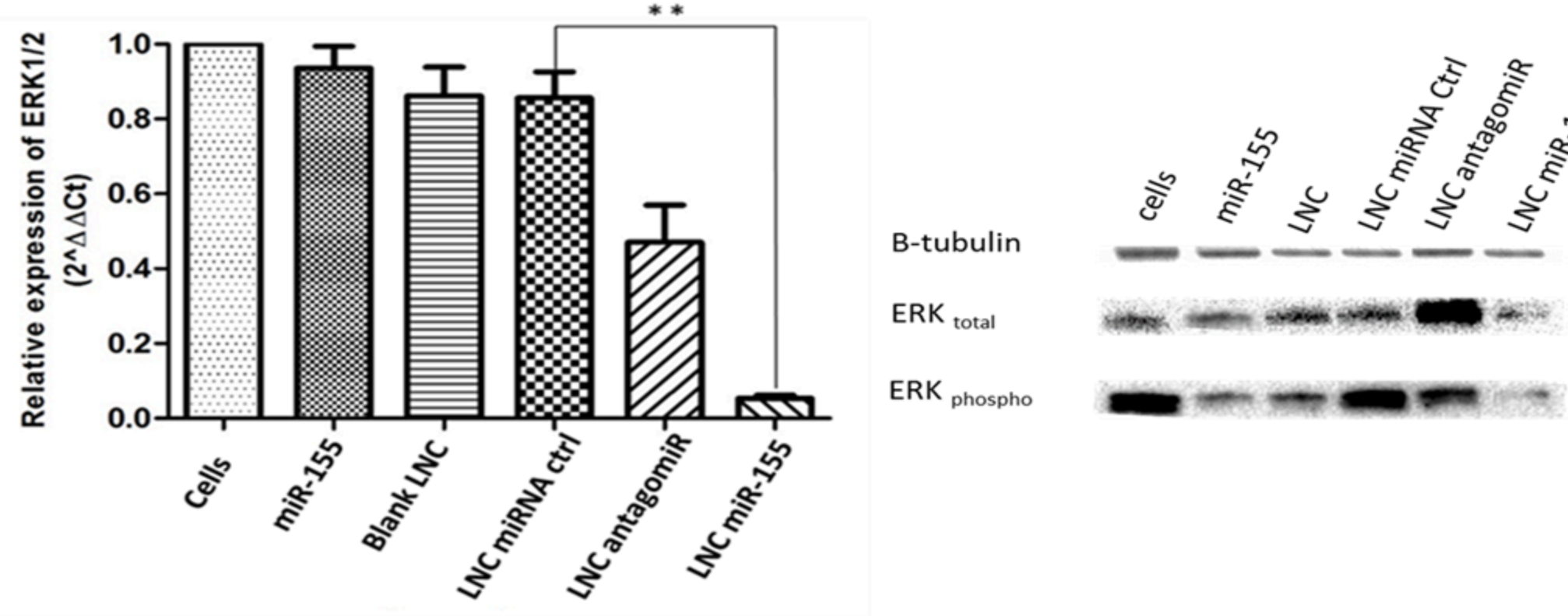
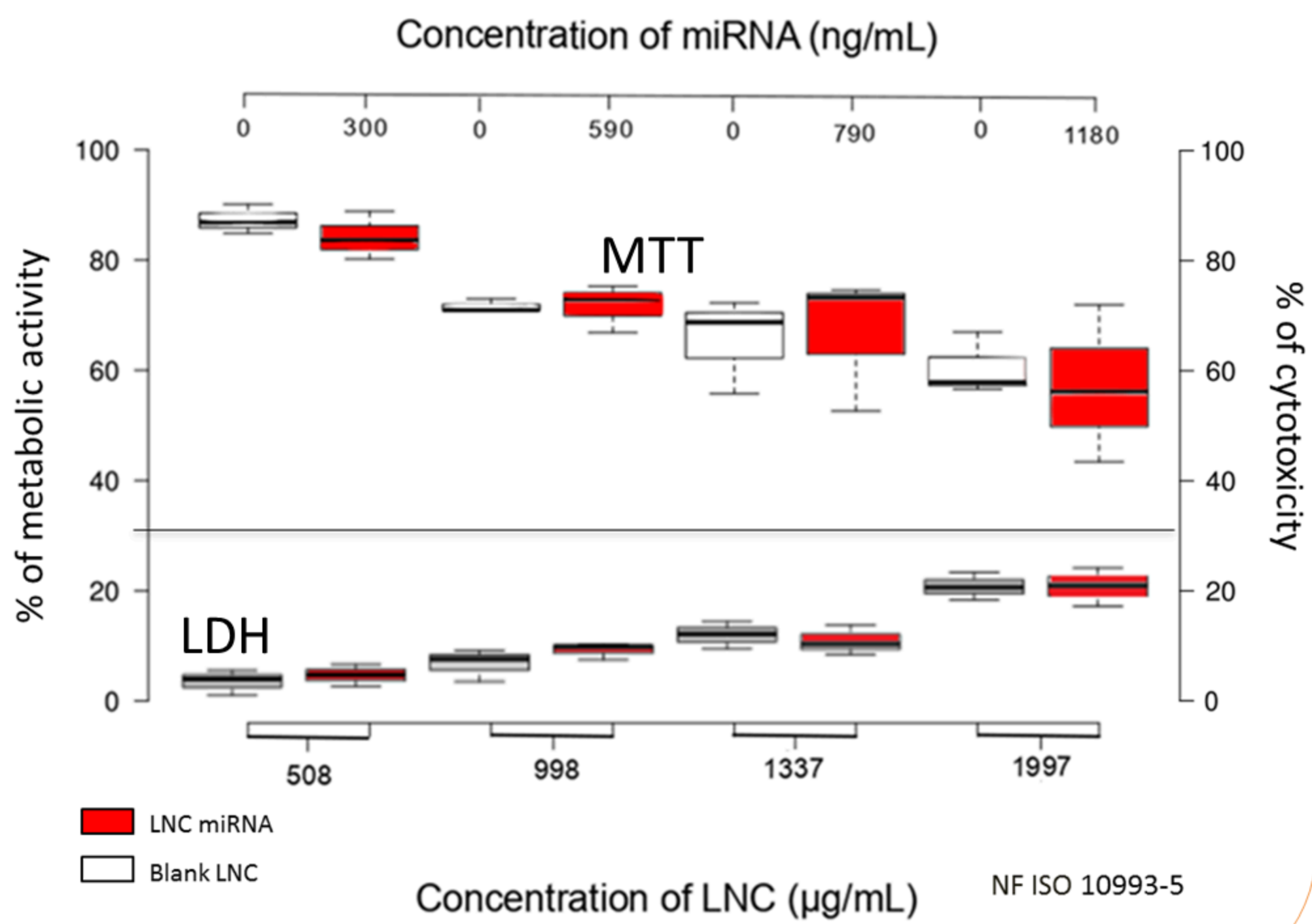


Internalization study

miR-155 alone was slightly internalized after 24 h. LNCs alone were internalized in the cytoplasm after 24 h. miR-155-LNCs showed a cytoplasmic localization. Both LNCs and miRNA were internalized. Furthermore the LNCs and miRNA colocalized after 24 h as observed in “Merge”.
● LNC-DID
● miRNA-Alexa488

Cytocompatibility

After 24 h of incubation with miR155-LNCs and LNCs, hASC cytotoxicity was under the NF ISO 10993-5 recommendation, used for cytocompatible products in human health environment. No significant difference between miR155-LNCs and LNCs were observed, demonstrating no impact of miRNA on cytotoxicity.



Conclusion

LNCs showed promising properties to encapsulate miRNA and to significantly protect the miRNA from degradation, in allowing their uptake by the cells without cytotoxicity. Thanks to these results, miRNA LNCs seem to be a good strategy to encapsulate and deliver miRNA for future use in IVD regenerative medicine. In a foreseeable future, modification of the LNCs in order to specifically target *Nucleus pulposus* cells will be studied. Finally, further experiments will be needed to confirm the interest of this innovative nanoplatform to vectorize new therapeutic in order to counteract IDD.

Acknowledgements

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