Nano-theranostic chelating agents: an innovative approach to regulate intracellular iron in brain

Eleonora Ficiarà (1), Shoeb Ansari (1), Silvia Gazzin (2), Sri Jayanti (2), Caterina Guiot (1) and Roberta Cavalli (3)

(1) Department of Neurosciences "Rita Levi Montalcini", University of Torino
(2) Fondazione Italiana Fegato-Onlus, Bldg. Q, AREA Science Park, ss14, Km 163.5, Basovizza, 34149 Trieste, Italy
(3) Department of Drug Science and Technology, University of Torino
**e-mail contact: eleonora.ficiara@unito.it*

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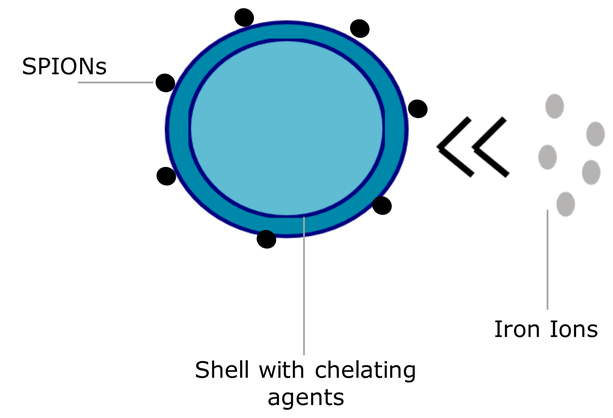
BACKGROUND AND AIM

Excess iron is considered a possible trigger of neurodegenerative diseases due to the involvement in the oxidative stress and toxic proteins aggregation.
Chelation therapies are limited by systemic delivery and innovative approaches for an effective local administration and monitoring are currently under investigation[1].
We developed new formulations of nanobubbles (NBs), with shell composed of different polymers: glycol-chitosan, glycol chitosan + deferoxamine (DFO), glycol chitosan + DFO to optimize the chelation capacity.
Furthermore, we manufactured magnetic chelating NBs with the addition of superparamagnetic iron oxide nanoparticles (SPIONs) in the shell, to explore the theranostic properties of the system.



NBs FORMULATION AND CHARACTERIZATION

Structural Characterization of synthesized derivative of Glycol-Chitosan + DFO

- FT-IR Spectroscopy and Nuclear Magnetic Resonance (1HNMR)

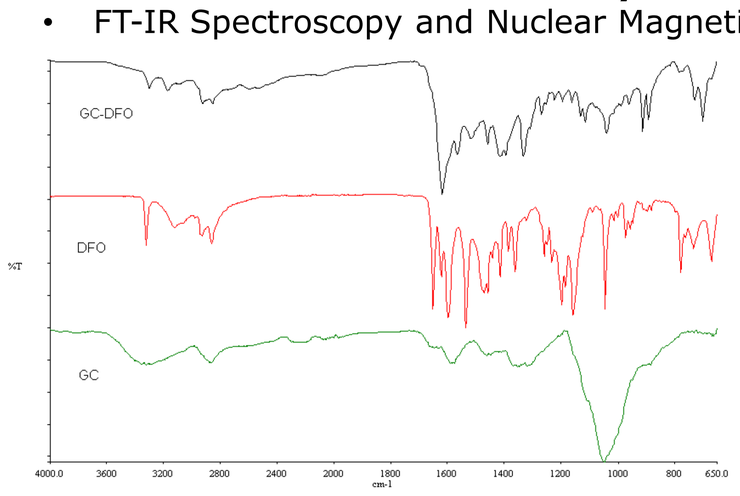


FIGURE 1A. FT-IR Spectra of GC, DFO, and GC-DFO

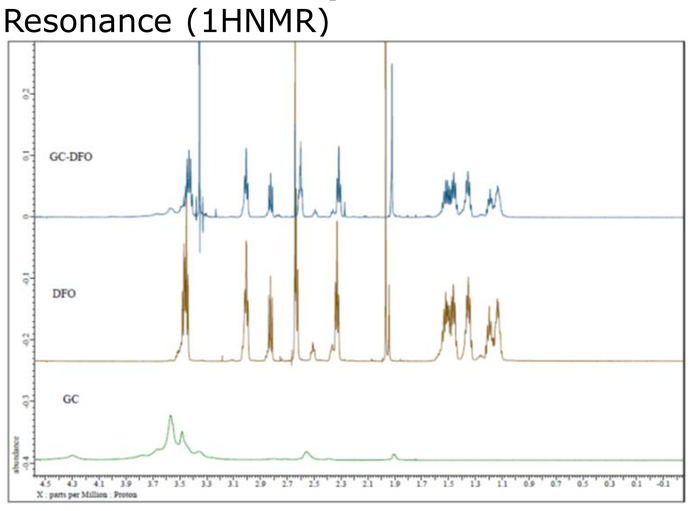
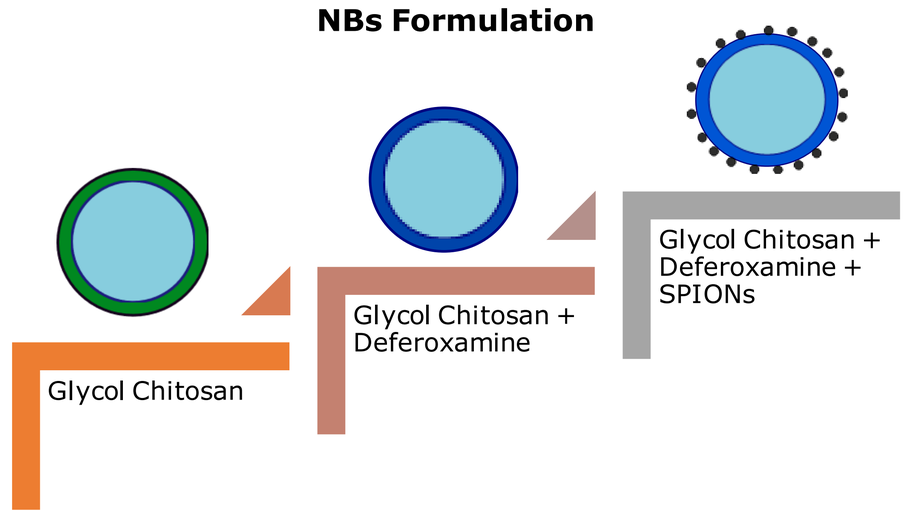


FIGURE 1B. 1HNMR Spectra of GC, DFO and GC-DFO

NBs Formulation



Iron Chelation Capacity

- Ferrozine assay for Fe^{+2}
- Deferoxamine assay for Fe^{+3}

Physico-chemical Characterization of NBs

Formulation NBs	Mean Diameter ± SD (nm)	PDI ± SD	ζ ± SD (mV)
GC	474.0 ± 8.3	0.336 ± 0.124	-27.58 ± 8.75
GC-DFO	477.9 ± 16.8	0.254 ± 0.014	-40.62 ± 1.12
GC-DFO+SPIONs	484.6 ± 7.6	0.298 ± 0.018	-19.71 ± 8.61

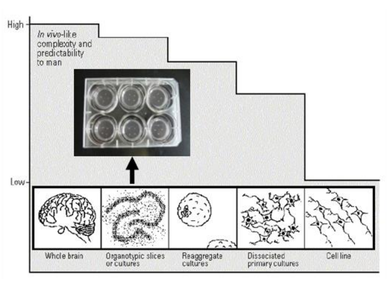
TABLE 1. Mean diameter, Polydispersity Index (PDI) and Zeta Potential (ζ) for different formulations of NBs.

Formulation NBs	% Of Fe^{+3} chelation	% Of Fe^{+2} chelation
GC	29.43	55.32
GC-DFO	94.32	52.16
GC-DFO+SPIONs	93.63	58.19

TABLE 2. % Iron chelation for NBs formulations

In vitro CYTOTOXICITY

- Organotypic brain culture cells (slices of substantia nigra)
- Cells treated with GC NBs, GC-DFO NBs, GC-DFO-Fe (NBs in iron solution), GC-DFO-SPION NBs in dilutions 1:8 , 1:64, 1:192
- After 24h incubation, toxicity evaluated by 2,3-bis[2-methoxy-4-nitro-5sulphophenyl]-2Htetrazolium-5carboxanilide (MTT) assay and lactate dehydrogenase (LDH) assay.



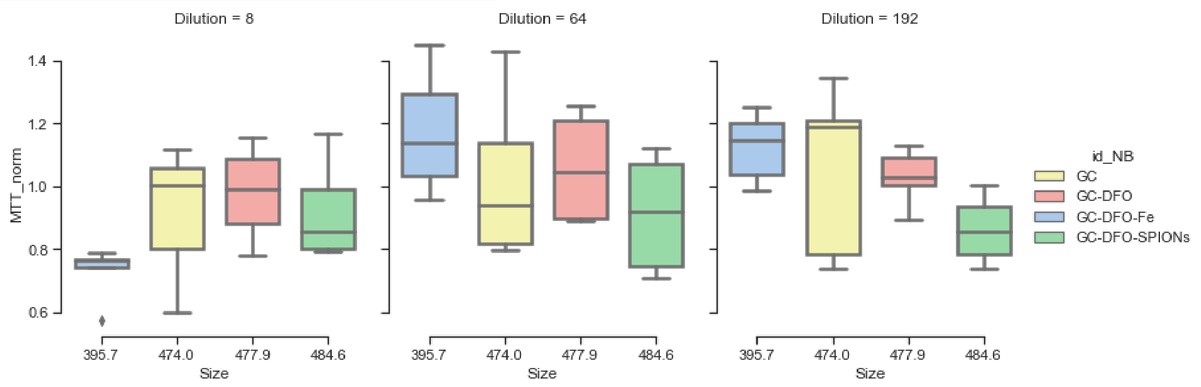


FIGURE 2. MTT assay of different NBs formulations.

No sign of NBs toxicity was present in MTT. LDH revealed toxicity at 1:8 dilution for GC and GC-DFO ($p<0.01$ and $p<0.05$ vs DMSO, respectively).

Toxicity vs. Physico-chemical properties

- Size vs MTT excluding 1:8 data (Spearm coef= -0.46, p=0.001; Pearson coeff: -0.40, p=0.006)
- Negative correlation LDH and Zeta Potential (Pearson coeff= -0.48)

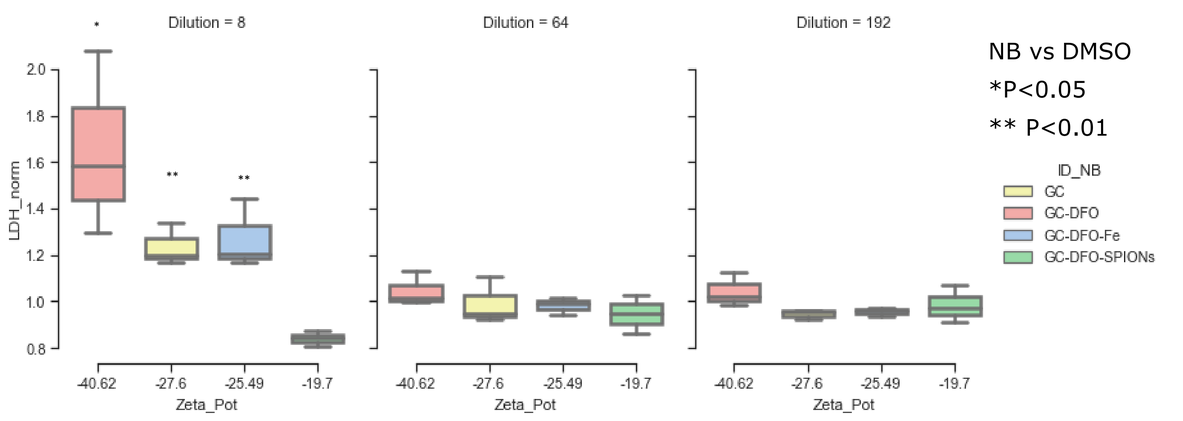


FIGURE 3. LDH assay of different NBs formulations.

FUTURE DIRECTIONS AND CONCLUSIONS

- Deep investigation of magnetic properties of NBs before and after chelation (by MRI and Alternating Gradient Force Magnetometer[2])
- Novel system of chelation through the use of NBs conjugated with SPIONs, able both to be drivable toward precise target and to chelate excess iron simultaneously.

References

[1] E. Ficiarà et al., "Beyond oncological hyperthermia: Physically drivable magnetic nanobubbles as novel multipurpose theranostic carriers in the central nervous system," Molecules, vol. 25, no. 9, 2020, doi: 10.3390/molecules25092104.

[2] Ansari et al. «Step-by-Step Design of New Theranostic Nanoformulations: Multifunctional Nanovectors for Radio-Chemo-Hyperthermic Therapy under Physical Targeting.» *Molecules* **2021**, *26*, 4591. https://doi.org/10.3390/molecules26154591