Enhanced Radiotherapy through Silver-Doped Iron Oxide Nanoparticles Functionalized with Bisphosphonates

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Introduction

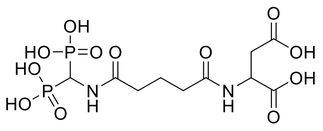
Iron oxide nanoparticles (IONPs) have been extensively investigated as magnetic resonance imaging (MRI) contrast agents[1]. More recently, growing evidence has highlighted their potential to enhance the radiosensitivity of cancer cells. Notably, when combined with radiotherapy, IONPs promote the inhibition of thioredoxin reductase (TrxR), a key enzyme involved in maintaining cellular redox homeostasis[2]. Interestingly, silver ions are also strong inhibitors of TrxR[3].

Based on these observations, the combination of IONPs with silver ions emerges as a promising strategy to improve the efficacy of conventional radiotherapy treatments.

Methodology

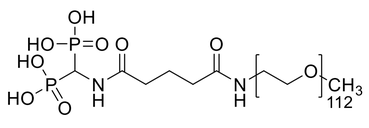
1 *Synthesis of bisphosphonate (BP) derivatives*

Compound **A (BP-Asp)**



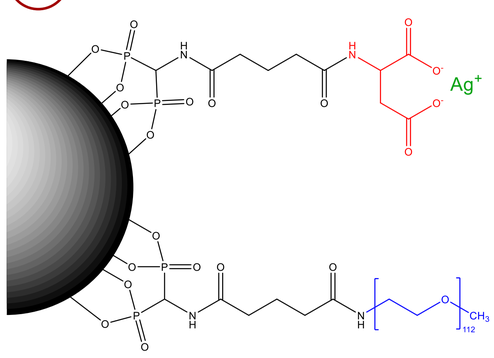
Aspartic acid moiety for silver ions **complexation**

Compound **B (BP-PEG)**



PEG moiety for **stability**

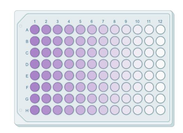
2 *IONPs functionalization & Ag^+ complexation*



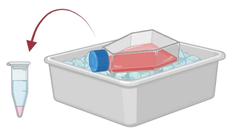
Characterizations :

- Hydrodynamic diameter by DLS
- Relative composition by FT-IR
- Silver ions concentration by ICP

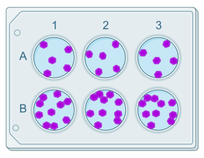
3 *In vitro tests*



MTT assay
Evaluation of the cytotoxicity



TrxR assay
Evaluation of the residual enzymatic activity

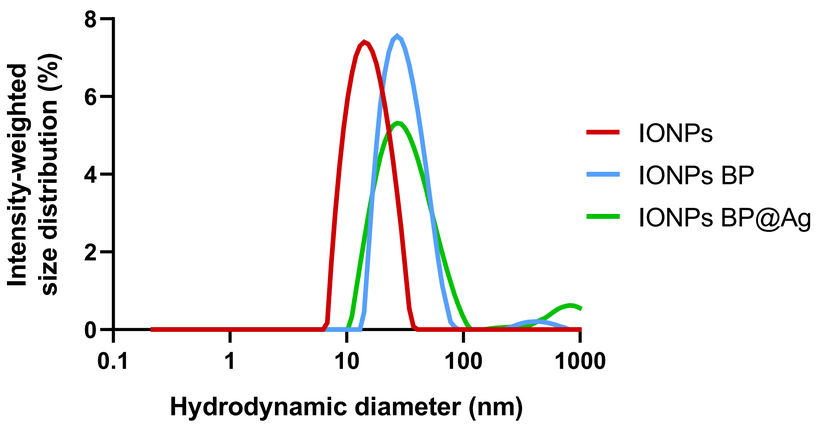


Clonogenic assay
Evaluation of the cytotoxicity after X-ray irradiation

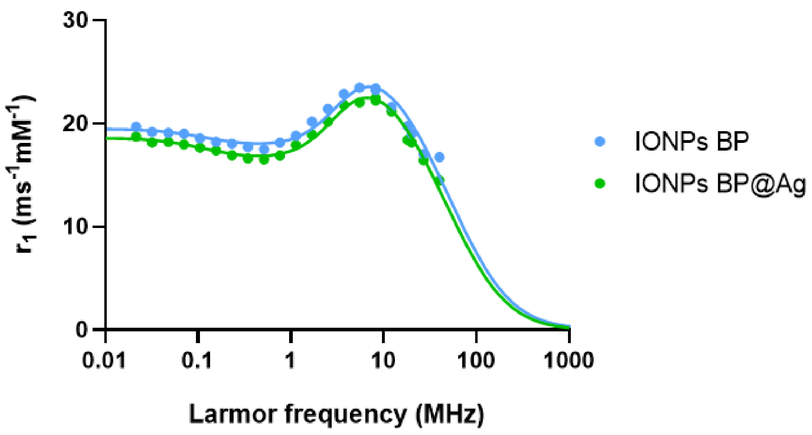
Results

IONPs functionalization and Ag^+ complexation

- DLS measurements
  - → Slight increase in hydrodynamic diameter after BP derivatives addition
  - → Constant hydrodynamic diameter after complexation and purification by dialysis

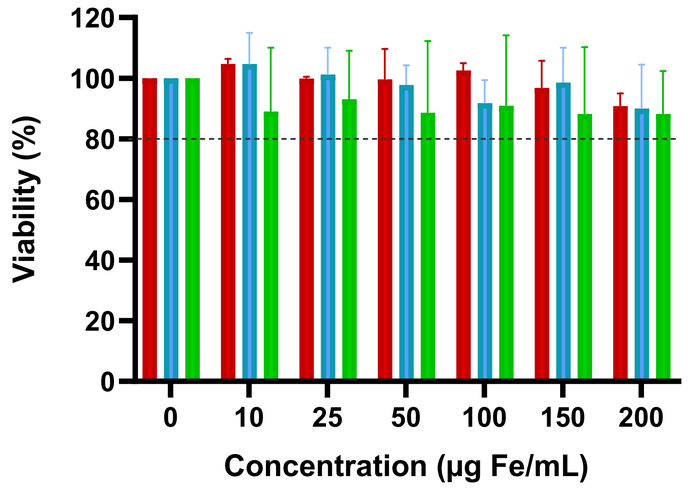


- ICP analysis
  - → Mean Ag/Fe ratio of 0.4%
- NMRD profiles
  - → Overlapping curves confirmed complex stability



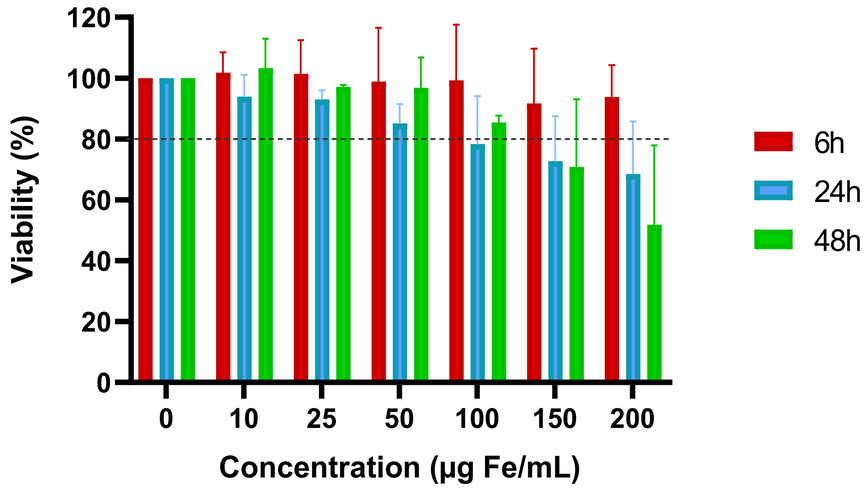
In vitro assays

MTT assay ***IONPs BP***



- No significant cytotoxicity observed after 6h, 24h and 48h

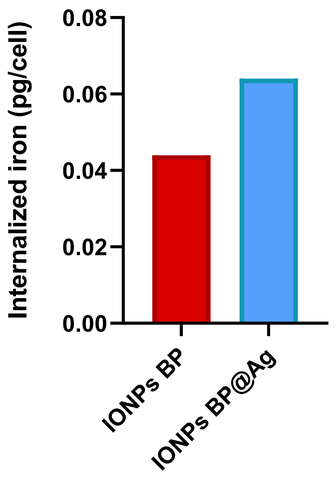
MTT assay ***IONPs BP@Ag***



- Slight cytotoxicity observed at higher concentrations

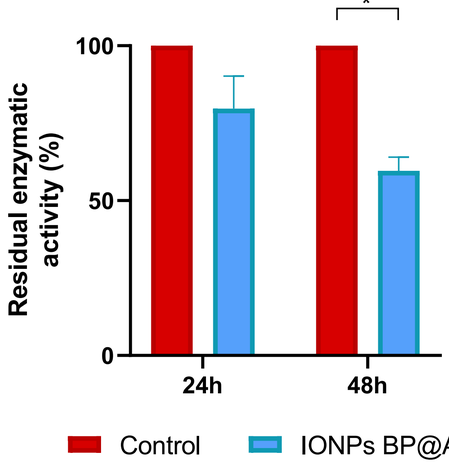
→ Further assays realized with a concentration of **50 µg Fe/mL**

Internalization assay



- Internalized iron after 48h
  - → **Higher iron uptake** in presence of Ag^+

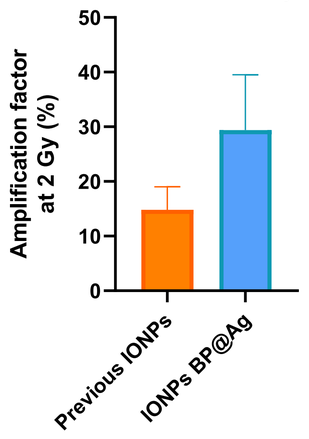
TrxR assay



- TrxR residual activity
  - → **Significant inhibition** after 48h of treatment with IONPs BP@Ag

X-ray irradiation

Amplification Factor (AF): cytotoxicity enhancement induced by radiosensitizers



IONPs BP@Ag were compared with carboxylated IONPs previously synthetized by our team[2]

- → **Increased AF at 2 Gy** for cells treated with IONPs BP@Ag (29,4 ± 10,1 %)

Conclusion

This work investigated IONPs functionalized with bisphosphonate derivatives and doped with Ag^+. These nanoparticles were non-cytotoxic up to 50 µg Fe/mL and they significantly inhibit TrxR. Finally, preliminary X-ray irradiation tests revealed an increased amplification factor, suggesting their potential to improve radiotherapy treatment.

Future work could include evaluation of ROS production, DNA damage and mitochondrial assays, while irradiation tests should be completed.

References

1. Vangijzegem, T. *et al.* Superparamagnetic Iron Oxide Nanoparticles (SPION): From Fundamentals to State-of-the-Art Innovative Applications for Cancer Therapy. *Pharmaceutics* **15**, 236 (2023).
2. Ternad, I. *et al.* Advances in the Mechanistic Understanding of Iron Oxide Nanoparticles' Radiosensitizing Properties. *Nanomaterials* **13**, 201 (2023).
3. Bjørklund, G. *et al.* Thioredoxin reductase as a pharmacological target. *Pharmacol. Res.* **174**, 105854 (2021).

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