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Synthesis and evaluation of the radiosensitising potential of iron oxide nanoparticles functionalized by a platinum complex

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Introduction

Since 2004, metallic nanoparticles (NPs) have been extensively studied as radiosensitizing agents considering their ability to enhance the effectiveness of radiation therapy by increasing the rate of tumor cell death in response to radiation. To date, most research focused on high Z metallic NPs as candidates for radiosensitizing purposes. However, our team has recently evidenced the in vitro radiosensitizing properties of iron oxide NPs (IONPs), which appear as a promising theragnostic nanoplatform due to their biocompatibility and magnetic properties [1]. To further improve our previously described nanoplatform, we considered to study the impact of a new platform functionalized with platinum complex (IONPs@Pt(II)) structurally similar to carboplatin, which is widely described as having radiosensitizing properties, inspired by a procedure described in the literature [2,3].

Methods

Synthesis of IONPs@Pt(II)
The synthesis of the magnetic cores has been done through co-precipitation in polyol method with a subsequent organosilanization/PEGylation process
The complexation of platinum complex was possible due to the carboxylic acid functions exposed by IONPs.

Stability in biological fluids assays
The complexation stability was assessed by measuring the concentration of free ions over time by ICP-OES in artificial lysosomal fluids (ALF) and simulated body fluid (SBF).

Clonogenic assays
The radiosensitizing effect of IONPs ([Fe] = 25 µg/ml), was determined by measure of the amplification factor (AF) at 2 Gy of 225 kV X-rays.
The colonies (>30 cells) were stained with crystal violet and counted under a brightfield microscope.

Comet assays
Alkalyne comet assays have been conducted on A549 cells exposed or not exposed to IONPs ([Fe] = 25 µg/ml).
H_2O_2 exposure (500 mM ; 30 min) has been used a DNA breaking condition.

Results

Synthesis and characterization of IONPs@Pt(II)

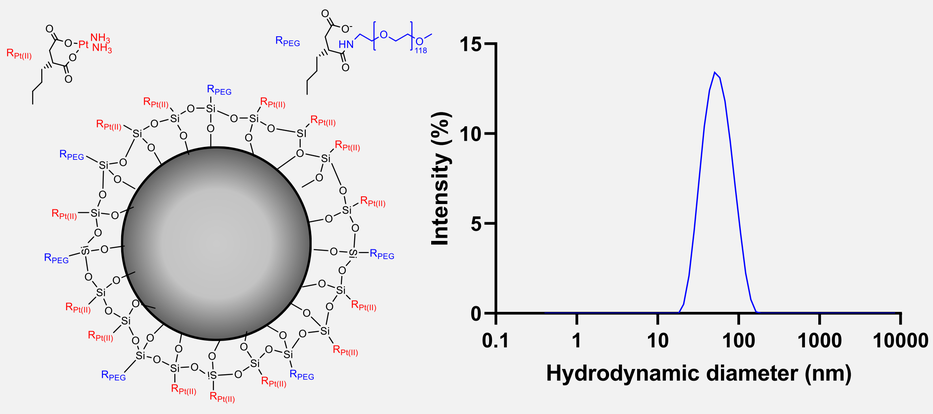

Figure 1

- The synthesis of IONPs@Pt(II) followed a 4-step process including (A) synthesis of the magnetic core [1] ; (B) organosilanization of the core [1]; (C) PEGylation of the core [1] and (D) complexation of cis-diammine diaquaplatinum to the carboxylic acids functions exposed by IONPs [2] (Fig.1). This strategy led to the synthesis of a monodisperse in size formulation of IONPs harboring carboplatin-like motifs.

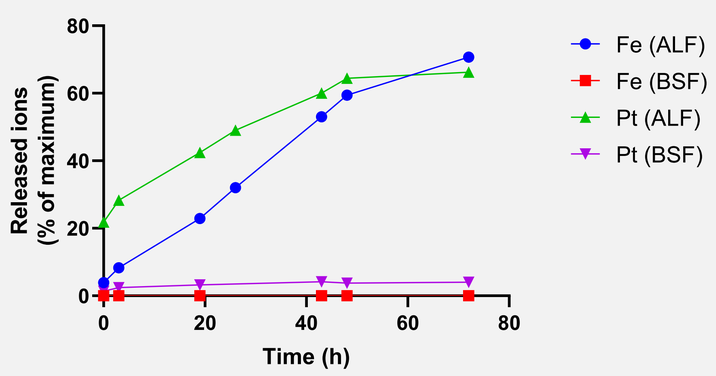

Figure 2

- The stability of the complexation between Pt(II) and the IONPs has been studied in artificial plasmatic fluid (SBF) and artificial lysosomal fluids prepared as described in the literature [4]. We observed a minimal degradation of IONPs@Pt(II) into iron and platinum ions overtime in SBF. In the other hand, IONPs@Pt(II) are subject to rapid degradation in ALF (Fig 2).

Study of the biological impact of IONPs@Pt(II)

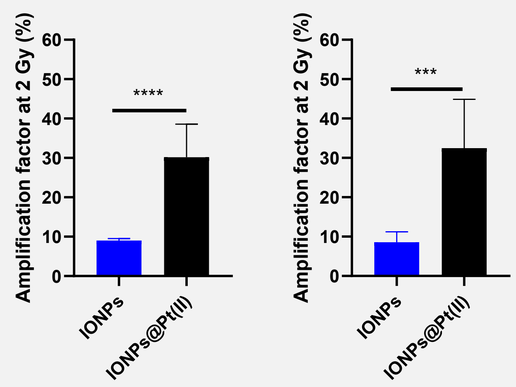

Figure 3

- Radiosensitizing potential has been measured through the amplification factor at 2 Gy (AF_{2Gy}) via clonogenic assay after 24 (Fig. 3A) and 48 hours (Fig. 3B) of exposure to IONPs before irradiation of the cells. A significant increase of the AF_{2Gy} was detected for IONPs@Pt(II) in comparison of IONPs for both 24 h and 48 h of exposures.

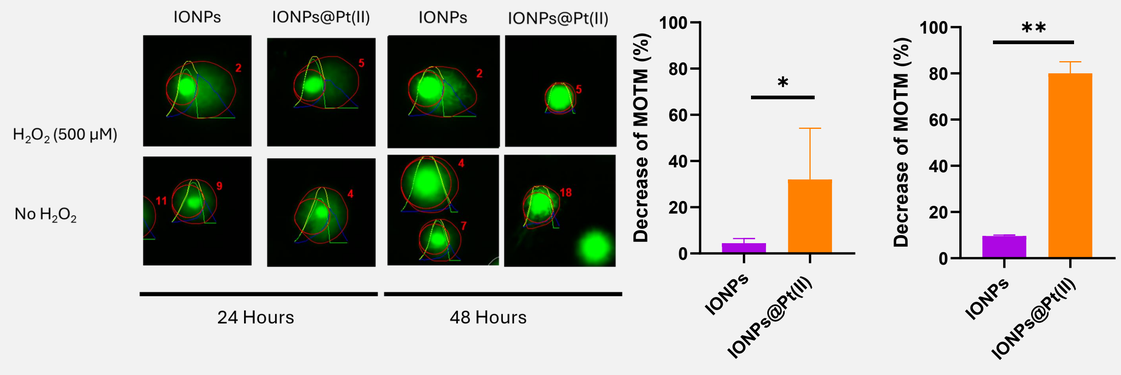

Figure 4

- Alkalyne comet assay showed no significant induction of DNA strand break as a consequence of the exposure of cells to IONPs or IONPs@Pt(II). However, we detected high levels of DNA adduct through a significant decrease in Mean Olive Tail moment (MOTM) for the cells exposed to IONPs@Pt(II) after 24 (Fig. 4B) and 48 hours (Fig. 4C).

Discussion And Conclusion

Our results showed a stable association between our IONPs and the platinum complex in simulated body fluid (SBF), but rapid dissociation in artificial lysosomal fluid (ALF) (Fig. A). In addition, exposure of lung cancer cells (A549; 25 µg of Fe per ml) to IONPs@Pt(II) has been shown to induce a significantly enhanced radiosensitizing effect compared with cells exposed to IONPs not functionalized with platinum complex (Fig. B). As it is widely accepted that platinum complexes exert a biological effect mainly via the formation of DNA adducts, we carried out comet assays to assess the DNA integrity of cells exposed to both IONPs and IONPs@Pt(II). We found that cells exposed to IONPs@Pt(II) had significantly higher DNA adduct levels than cells exposed to IONPs, explaining at least partially the enhanced radiosensitizing behavior of this new formulation (Fig. C).

References And Acknowledgement

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