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Report on Ms Mathilde Ponchelle's doctoral thesis

Ms Mathilde Ponchelle wrote a doctoral thesis entitled: Design and synthesis of micellar nanocarriers for internal radiotherapy. This PhD work was carried out both at Service de Chimie Bioorganique et de Marquage (SCBM) at CEA Saclay (France) under the supervision of Dr Eric Doris and at the National Centre for Nuclear Research/NOMATEN (Poland) under the co-supervision of Dr Marek Pruszynski. The thesis was completed under joint supervision of the ICHTJ (Poland) and Paris-Saclay University.

Ms Ponchelle's thesis focuses on the development of nanoparticles applied to improving radiotherapy in cancer treatment. The manuscript begins with a general introduction ended by the problematic of her PhD work, and is followed by two chapters presenting Ms Ponchelle's work and results, and ends with a general conclusion, an experimental section and an annex with summaries in English, French and Polish. The manuscript is well written, easy and pleasant to read.

The manuscript starts by a general introduction that provides the reader with a set of information relating to the main key points associated with the subject of this PhD work, namely: 1) the characteristics of cancer and the main methods of diagnosis and treatments; 2) a focus on nuclear medicine with the main type of irradiations that can be used, how radioisotopes can be produced and their application in imaging; 3) the physical principle of radiotherapy, what radiosensitivity and radioresistance are; 4) the type of nanoparticles that can be used in nanomedicine, their *in vivo* fate, and how they can be used in cancer treatment and in radiotherapy. This introductory chapter concludes with a presentation of the objective of this thesis, in which Ms Ponchelle plans to improve the effectiveness of internal radiotherapy by designing and preparing nanocarriers containing both oxygen and gold nanoparticles, two key elements to be delivered in the tumor environment. The impact of a third element, lutetium 177,

a β^- emitter, will be also assessed in this work to further improve the efficacy of the treatment and to follow the biodistribution of the nanocarrier *in vivo*.

The second chapter, entitled: "Fluorinated micelles encapsulating gold nanoparticles for radiosensitization", begins by providing a series of information on the nanocarriers that will be prepared in this thesis. These are micelles, composed of amphiphilic molecules made of both a hydrophobic alkyl chain and a polar head (being either anionic, cationic or nonionic) that can spontaneously self-assemble above the critical micellar concentration to form spherical or cylindrical structures. As hydrophobic chains, Ms Ponchelle decided to prepare micelles composed of fluorinated alkyl chain, as they allow oxygen to be encapsulated, an element present in low quantities in hypoxia tumors limiting the effectiveness of radiotherapy. The second partner to be co-encapsulated into the micelles is gold nanoparticles (AuNPs). Among their different properties (surface plasmon resonance, surface enhanced Raman scattering), thanks to their high atomic number (Z = 79), AuNPs have the ability to enhance the effect of radiations, notably X-rays, by increasing the amount of reactive oxygen species (ROS). The second part of this chapter presents the results obtained by Ms Ponchelle. First of all, they concern the synthesis of an amphiphilic molecule (PFTD-PEG), composed of an aliphatic chain of 13 carbon atoms bearing 27 fluorine atoms and a hydrophilic chain composed of a 2 kDa polyethylene chain obtained in 2 steps and 64% yield. When dispersed in water above the CMC (0.056 mg/mL) and using ultrasonic treatment, this molecule self-assembles to form micelles of 11 nm (hydrodynamic diameter) and a neutral surface potential (ζ = 0 mV). The ability of these micelles to encapsulate oxygen (realized at different pressure, from 0.25 to 2.5 bar of O_2) was monitored using $^1H/^{19}F$ nuclear magnetic resonance, exploiting the paramagnetic properties of molecular oxygen enhancement which modulates the relaxation of nuclei (mostly of ^{19}F) through the paramagnetic resonance enhancement (PRE) effect. The second part of this chapter concerns the synthesis of AuNPs and to this end, three methods were tested: Brust-Schiffrin method using gold (III) salt, tetraoctylammonium bromide (TOAB), a reducing agent ($NaBH_4$) to convert gold (III) to (0) and a perfluorooctane thiol (PFSH) to functionalize to surface of AuNPs. After encapsulation in PFDT-PEG micelles, it forms nanohybrids of 5 nm (determined by TEM), but unfortunately their cytotoxicity even at low concentration (0.004 mg/mL) prevents their further use. Two other methods have been tested (Turkevich and Stellacci), and the most interesting one in terms of application is Stellacci's synthesis, which is prepared in one step in ethanol starting from $HAuCl_4$, $NaBH_4$, PFSH and ends with their encapsulation into the fluorinated micelles. This method allows the formation of nanohybrids of ~18 nm (by DLS) and ~3.8 nm by TEM, with no sign of cytotoxicity on SKBR3 cell lines even after 72 hours of incubation.

The ability of the AuNPs-loaded micelles to improve the efficacy of radiotherapy was investigated using external electron beam irradiation (at 1, 2 or 4 Gy) on two cell lines, B16F10, a more radioresistant cell line, and SKBR3 which is less resistant to radiation. Various quantitative parameters (SER, DMR, SF and RER) confirmed the higher performances of the formulations (higher sensitizing values) for micelles containing AuNPs, compared to empty micelles. These *in vitro* experiments demonstrate that gold nanoparticles can improve the radiosensitivity in cancer cell treatments when incorporated into fluorinated micelles. The *in vivo* application of this nanocarrier will be addressed in the next chapter.

The final chapter of this thesis is devoted to the radiolabeling of this nanocarrier in order to evaluate its pharmacokinetic, biodistribution and efficacy in tumor treatment. Among the possible radioisotopes, lutetium (^{177}Lu) was chosen. This lanthanide (which has a half-life of 6.6 days) emits β^- particles (498 keV) and has short tissue penetration (~1 mm), which allows it to induce local DNA damage in the tumor area while limiting side effects on healthy tissue. Its ability to also emit γ photons, allows it to be detected using SPECT or gamma cameras, and makes ^{177}Lu a theranostic element. To date, two radiopharmaceuticals labeled with ^{177}Lu have been approved by the FDA and EMA: Lutathera® for the treatment of neuroendocrine tumors and Pluvicto™ for the treatment of prostate cancer (168 clinical trials). All these aspects were reviewed by Ms Ponchelle in 2025 in Small Science, of which she is the first author.

To improve the radiotherapeutic effect of ^{177}Lu, its association with AuNPs encapsulated in the fluorinated micelles, developed in the previous chapter was studied. The grafting of a Lu chelating moiety (DOTA) on fluorinated amphiphilic molecule (PFTD-DOTA) was realized in seven steps. This molecule was then mixed with PFTD-PEG prepared in the previous chapter, in a 1/9 ratio, to self-assemble into mixed micelles of 11 nm (by DLS) after ultrasonic treatment. The experiments of radiolabeling of PFTD-DOTA with ^{177}Lu were carried out at the NCBJ Institute (Poland). ^{177}Lu was produced with an average specific activity of 850 GBq/mg starting from ^{176}Lu irradiated with neutrons in a hydrochloric acid solution using the carrier-added pathway.

As the radiolabeling of the preformed micelles PFTD-PEG/PFTD-DOTA with ^{177}Lu was not successful, Ms Ponchelle decided to study the radiolabeling of the amphiphilic PFTD-DOTA molecule. A series of reactions was realized with a fixed lutetium activity (20, 100 or 300 MBq) and increasing equivalents of PFTD-DOTA (from 1 to 200 equiv). Radiochemical yields (RCY) were determined on thin layer chromatography (iTLC) and the best RCYs were obtained using 50 equivalents of PFTD-DOTA. After purification on exclusion chromatography column to remove Lu impurities, mixed micelles were formulated by adding either PFTD-PEG alone or AuNPs-loaded micelles. The radiolabeling stability of the Lu micelles was assessed in different media (water, PBS, FBS and cell culture medium) at room temperature and 37°C over 10 days using iTLC. The best radiolabeled stabilities were observed in FBS and cell culture media. Their cytotoxicity was then evaluated in two cancer cell lines. Both formulations showed a cytotoxic effect, but the presence of gold nanoparticles in the micelles enhanced the cytotoxicity, particularly at higher concentrations and radioactivity levels of ^{177}Lu.

A first biodistribution study was performed in healthy mice with intravenously injected ^{177}Lu-AuNPs-loaded micelles. The distribution profile in different organs was consistent with that of other nanometric carriers, with urinary and hepatobiliary excretion pathways. Tumor retention was evaluated in mice with melanoma after intratumoral injection of both nanocarriers. ^{177}Lu-AuNPs-loaded micelles showed superior long-term retention. In addition, some reduction in tumor growth was observed in mice treated with ^{177}LuAuNPs-loaded micelles. This suggests a synergistic radio-enhancement effect of gold in potentiating the effect of ^{177}Lu. These preliminary *in vivo* results are very encouraging.

The manuscript concludes with a general conclusion highlighting the main results obtained in this doctoral work, followed by the bibliographical references, a list of figures, the experimental section, an appendix and summaries.

As part of her PhD work, Ms Ponchelle conducted various studies ranging from the molecular to the nanoscale, as well as on self-assembly in supramolecular scaffolds. In addition to the synthetic part, Ms Ponchelle carried out numerous physicochemical experiments (NMR, DLS, UV, IR, TEM) in order to fully characterized her samples. Ms Ponchelle also participated in radiochemistry work and *in vitro* and *in vivo* experiments on cells and mice. Working on different topics is never easy due to skill and time constraints, but Ms Ponchelle has largely succeeded in producing high-quality work within the limited timeframe of a PhD thesis and in two countries.

Given the scope, diversity and quality of her work and its results, I am pleased to recommend Ms Ponchelle's thesis for doctoral degrees from both Paris-Saclay University (France) and ICHTJ (Poland).

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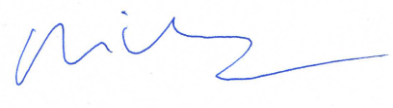

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