Radiolabelled Porphyrin Metalla-assemblies in Cellulose Nanocrystals as PDT Agents

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INTRODUCTION

Cancer is one of the biggest set of diseases to concern our society, meaning that an urgent need of new drugs and practices to treat it. Consisting of the combined work between a photosensitizer, light and oxygen, photodynamic therapy (PDT) offers a selective therapy methodology and achieves interesting antiproliferative results, mainly due to the production of localized reactive oxygen species inside cells.[1] Early diagnosis undertakes, alongside treatment, a critical role for the desired effect. In this case, imaging is an extremely intriguing tool since it allows not only for diagnosis but to follow treatment as well. We have synthesized and radiolabeled photo-responsive molecules in cellulose nanocrystals, therefore creating theranostic agents.

01 OVERVIEW

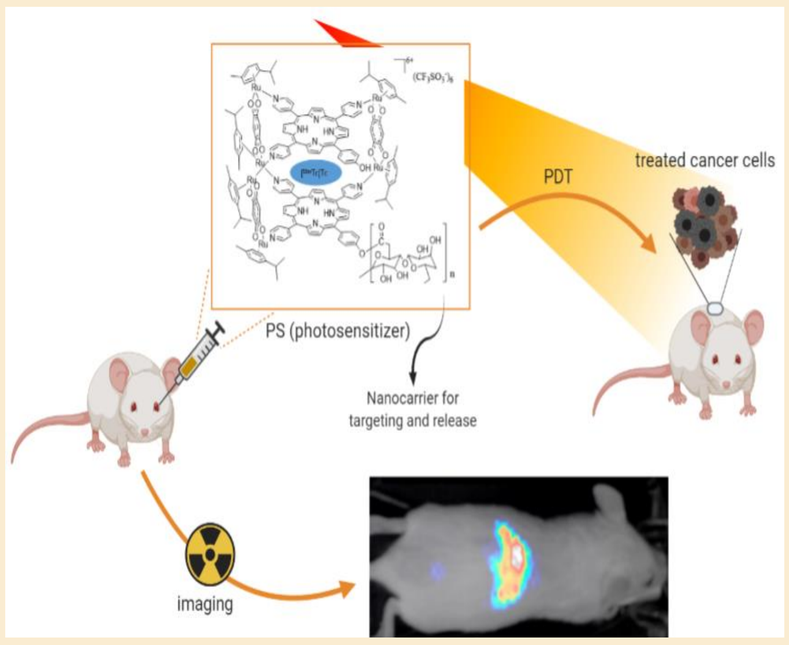

Figure 4 – Overview of the PDT and SPECT imaging for the cellulose-metal-porphyrin assemblies

Table 1 – IC_{50} values (μM) ± SD values of **1** and corresponding metalla-assembly with **(5)** and without CNC **(3)** against cancer cell lines with and without irradiation.

Metabolic Activity	A2780		HEK	
	not irradiated	irradiated	not irradiated	irradiated
1	52.3 ± 4.5	1.3 ± 0.1	43.8 ± 4.0	3.5 ± 0.4
3	4.5 ± 0.3	0.3 ± 0.1	27.1 ± 1.5	0.5 ± 0.1
5	16.7 ± 2.3	0.1 ± 0.0	1.9 ± 0.7	0.2 ± 0.0

04 CONCLUSION

After synthesis, in vitro assays were performed to determine the IC50 (Figure 3, Table 1) and whether the PDT agents were not only efficient PDT agents but also if they were selective towards cancer cell lines. At the same time, it allowed to calculate the initial concentration for the in vivo experiments. Finally, radiolabelling with technetium-99m and administration of these compounds in SCID mice allowed to follow their biodistribution for 24 h through SPECT imaging, showing preferential accumulation in the liver for non-tumor bearing mice (Figure 4).

We can conclude, that a multimodal agent combining an optimized photosensitizer for PDT treatment was synthesized and coupled to a radioactive agent for SPECT imaging. Furthermore, in vitro data confirmed that these compounds are interesting agents for PDT. A multimodal agent Lastly, in vivo experiments are currently being performed to confirm that such molecules can be used for imaging, and later for the diagnosis of cancer as well.

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02 METHODS

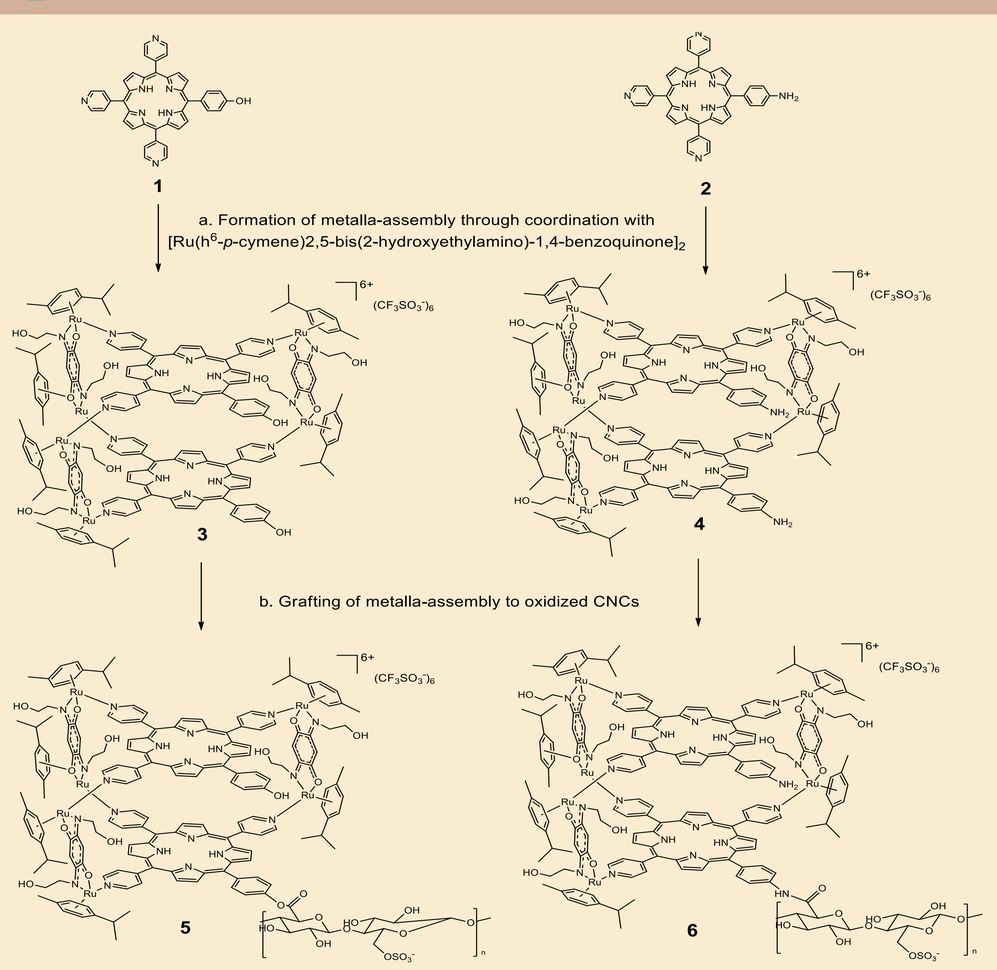

Figure 1 - Overview of the synthetic route for the cellulose-metal-porphyrin assemblies

Several steps were taken to synthesize these PDT agents (Figure 1) and to radiolabel them for diagnostic purposes using SPECT imaging (Figure 2). First, photo-responsive molecules were prepared[2] and characterized. These were then used to construct metalla-assemblies through coordination with ruthenium dimers.[3] They were subsequently linked to CNCs,[4] for better solubility, targeting, and transport to biological targets. The resulting photo-responsive compounds were then radiolabeled with technetium-99m by, first, reducing [99mTc]Tc-Pertechnetate with SnCl2 and then using it for the direct radiolabelling of our PDT agents, allowing them to also be used as imaging probes. Their cytotoxicity and potential therapeutic effect were evaluated on a cancer (A2780) and a normal cell line (HEK293T) through a metabolic activity assay. Afterwards, the radiolabeled compounds were used for SPECT imaging in SCID (Severe combined immunodeficiency) mice at several timepoints (1, 2, 4 and 24 h).

03 RESULTS & DISCUSSION

These compounds are composed of 5-(4-aminophenyl or 4-hydroxyphenyl)-10,15,20-tri(4-pyridyl)-21H,23H-porphine that due to their peculiar electronic resonance, give us the necessary photosensitizer properties for PDT, and ruthenium metalla-clips that offer stability and inherent cytotoxicity through reduction in the cancer site.[5] One of the biggest advantages of these compounds is their size, which helps with the EPR (Enhanced Permeability and Retention) effect, allowing us to take advantage of the characteristic vasculature of tumors, culminating in the accumulation of these molecules in the cancer site and indirect targeting. Furthermore, the CNCs helps with biological compatibility for the necessary prolonged blood circulation time.

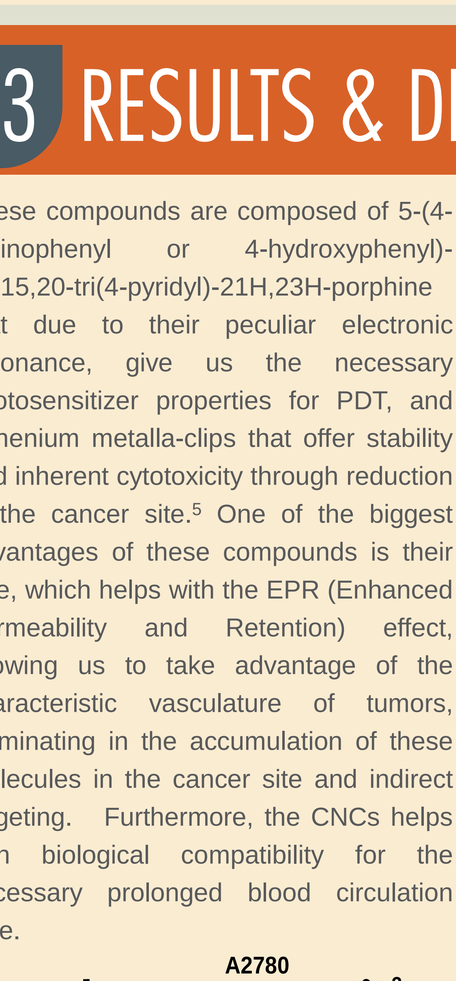

Figure 3 – IC_{50} values (μM) of porphyrin **1** and corresponding metalla-assembly **3** against ovarian cancer cell line A2780 and normal hepatic cell line HEK293T with and without irradiation.

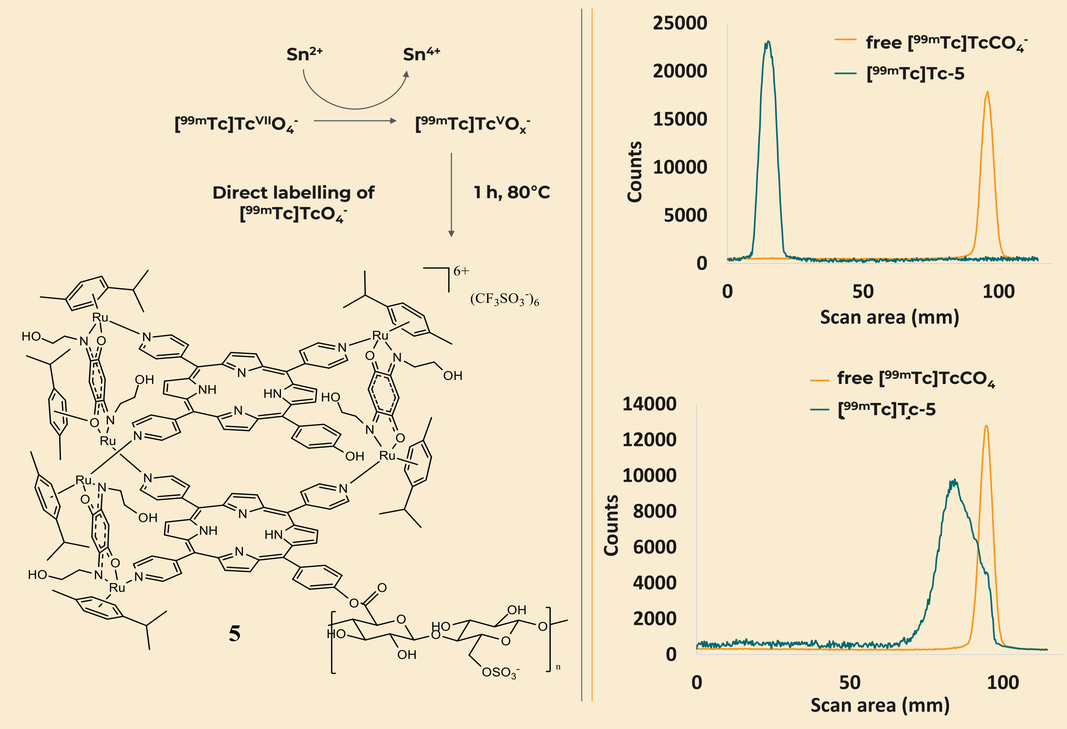

Figure 2 - Direct labelling of **5** with $[^{99m}Tc]TcO_4^-$; radio TLC of free $[^{99m}Tc]TcCO_4^-$ and **$[^{99m}Tc]$Tc-5** developed on Whatman 1MM paper with acetone (top) and 0,1M EDTA (bottom).

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