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Water-soluble nickel-bis(dithiolene) complexes as photothermal agents

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The remarkable photothermal properties of pegylated nickel-bis(dithiolene) complexes in water can induce cell death under laser irradiation in the near infrared (NIR) region.

Photothermal therapy (PTT) is a minimally invasive and harmless therapeutic methodology which employs hyperthermia generated by photothermal agents from laser energy to kill malignant cells. Such a PPT prefers near-infrared (NIR, λ = 700–1100 nm) radiation with the typical tissue penetration of several centimeters in biological tissues[1] and has attracted much attention in recent years as a promising alternative or supplement to traditional cancer therapies. Numerous photothermal agents with strong absorbance of NIR laser have been extensively explored to increase the photothermal conversion efficiency and correspondingly, enhance the thermal lethality in tumors. Currently, four families of photothermal agents have been extensively developed, resulting in great contributions to PTT therapy. First, organic compounds such as indocyanine green (ICG) dye and polyaniline nanoparticles,[2] suffer from limitations such as photobleaching and/or unsatisfactory photothermal conversion efficiency. Besides, carbon-based materials, including carbon nanotubes (CNTs) and graphene, have relatively low absorption coefficient in the NIR region.[3] The third family consists in metal (particularly gold) nanostructures (supramolecularly assembled

nanoparticles, nanorods, nanoshells, hollow nanospheres, nanocages, nanocrosses, and nanostars).[4] These noble metal–based nanostructures exhibit intense NIR photoabsorption and are the most studied photothermal agents, but the intense NIR absorption of these materials is attributed to the surface plasmon resonance (SPR), and their maximum absorption is therefore related to the dielectric constant of the surrounding matrix, leading to a difference of the maximum absorption between *in vitro* and *in vivo*. These gold nanomaterials also suffer from relatively low thermal stability during prolonged laser irradiation. The last type is found in copper chalcogenide semiconductors, including CuS[5] and $Cu_{2-x}Se$[6] nanoparticles, but the main limitation of these compounds is their unsatisfactory photothermal conversion efficiency. To meet the severe requirements of future PTA therapy, it is necessary to search for more stable and biocompatible agents displaying high photothermal conversion efficiency in the NIR region.

Nickel-bis(dithiolene) are strong near-IR absorbers with high thermal- and photo-stability (Figure 1). In their neutral state, they display high absorption coefficients (around 30 000 $M^{-1}.cm^{-1}$) in organic solvents in a wide range of NIR absorption maxima that are tunable from 600 nm to 1200 nm depending on the dithiolene substituents. Furthermore, these complexes are non-luminescent, implying that all the absorbed energy is released in the environment as heat. This peculiar feature has found applications for photothermal laser printing on thin films.[7] Recently, it has also been demonstrated that the photothermal properties of metallogelators built around nickel-bis(dithiolene) cores can efficiently be used to control the degelation of robust organogels.[8] Thus, properly functionalized nickel-bis(dithiolene) could be good candidates as photothermal therapeutic agents under near-IR laser irradiation, if they were water-soluble and biocompatible.

In the present work, we report the design and the synthesis of pegylated nickel-bis(dithiolene) complexes displaying photothermal activities in water and their *in vitro* ability to act as photothermal agents on human renal carcinoma cells.

Three pegylated nickel-bis(dithiloene) derivatives were considered, depending on the nature of the dithiolate ligand and the number of polyethylene glycol chains (Figure 1). Polyethylene chains have been selected for their good solubility in water and their biocompatibility.[9] Preparation of complex **1** was achieved by treatment of the corresponding pegylated 1,3-dithiole-2-thione derivative with sodium methoxide, followed by addition of a solution of nickel(II) chloride hexahydrate. The bis(oligo(ethyleneoxide)) substituted 1,3-dithiole-2-thione precursor was obtained by direct alkylation of the bis(2-thioxo-1,3-dithiole-4,5-dithio)-zincate salt $[Zn(dmit)_2]^{2-}$ with 2-methoxy(2-ethoxy(2-ethoxyethyl)) *p*-toluenesulfonate in refluxing acetonitrile. For the preparation of complexes **2** and **3**, 4,4'-dihydroxybenzil and 3,3',4,4'-tetrahydroxybenzil were first alkylated with 2-methoxy(2-ethoxy(2-ethoxyethyl)) *p*-toluenesulfonate in refluxing DMF in presence of K_2CO_3. The pegylated benzil derivatives were then sulfurated with P_4S_{10} in dimethylimidazolidinone (DMI) before addition of an aqueous solution of $NiCl_2 \bullet 6H_2O$.[10] All neutral complexes were isolated after purification as dark green waxy solids after drying. Full experimental details of the synthetic process and characterizations are presented in the Electronic Supplementary Information (ESI).

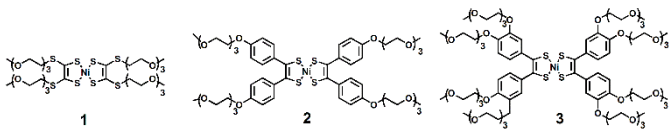

Figure 1. Chemical structures of the three pegylated nickel-bis(dithiolene) derivatives synthesized.

First of all, the solubility of the three complexes was evaluated in water. Complex **2** was found to be completely insoluble. Complex **1** is partially soluble and only a slight coloration of the solution was observed together with the presence of insoluble. On contrary, complex **3,** carrying eight polyethylene glycol chains, is well soluble in water, giving rise to a marked green coloration (Figure 2 left). The solubility was determined to be above 10 $mg.mL^{-1}$ (5 $mmol.L^{-1}$). Partitioning experiments in water/octanol mixtures have also been performed with complex **3**. After phase separation, no trace of complex **3** has been detected

by UV-visible-NIR measurements in the aqueous phase. These results highlight that complex **3** has strong lipophilic character which is in line with its relatively weak solubility in water.

The UV-visible-NIR absorption spectra of complex **3** in water display a strong absorption band centred at 936 nm, extending from 700 nm up to 1200 nm with high extinction coefficients ($\varepsilon \approx 14\ 200\ M^{-1}.cm^{-1}$) in the near-IR region, a characteristic of neutral Ni-bis(dithiolene) complexes (Figure 2 right). This low-energy absorption band is attributed indeed to an electronic transition from the HOMO ($L\pi$) of b_{1u} symmetry to the LUMO ($L\pi^*$-αd_{xy}) with a metallic character of b_{2g} symmetry.[11] Upon dilution, the absorbance perfectly follows the Beer-Lamber law, indicating the absence of intermolecular interactions below 500 $\mu g.mL^{-1}$ in water (insert Figure 2). Spectroscopic data obtained in dichloromethane with complexes **1-3** are gathered in table S1. In CH_2Cl_2, the NIR-absorption maxima of complex **3** is slightly red-shifted to 948 nm but the extinction coefficient strongly increases ($\varepsilon \approx 36\ 000\ M^{-1}.cm^{-1}$).

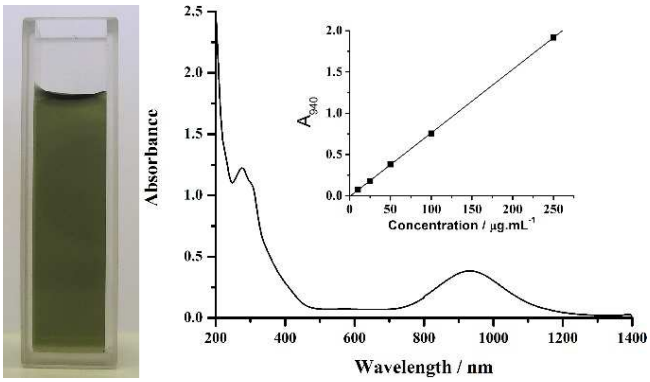

Figure 2. Picture of a dark-green solution of complex**3** in water (c = 500 $\mu g.mL^{-1}$) (left) and UV-vis-NIR spectra of complex **3** in water (c = 50 $\mu g.mL^{-1}$) (right) (insert: evolution of the absorbance at 940 nm as a function of the concentration).

A quantitative study of the photothermal performance of complex **3** in water was conducted. The temperature elevation of water solutions at different concentrations (0-500 μg.mL^{-1}) was measured under 940 nm laser irradiation with a power of 1 W.cm^{-2} for 10 min (Figure 3). An obvious concentration-dependent increase was observed after addition of complex **3** under laser irradiation. Below 25 μg.mL^{-1}, no noticeable photothermal effects have been detected. The temperature change (ΔT) drastically goes up with an increase of the complex concentration from 25 to 500 μg•mL^{-1}, and then reaches a maximum (+ 20.2 °C). In contrast, the temperature increase in pure water was only 6.5 °C. As expected, the temperature also strongly increases with the irradiation power density. Temperature increase as high as 65 °C has been obtained with a power of 5 W.cm^{-2} for 10 min at 500 μg•L^{-1} (Figure S1-S4).

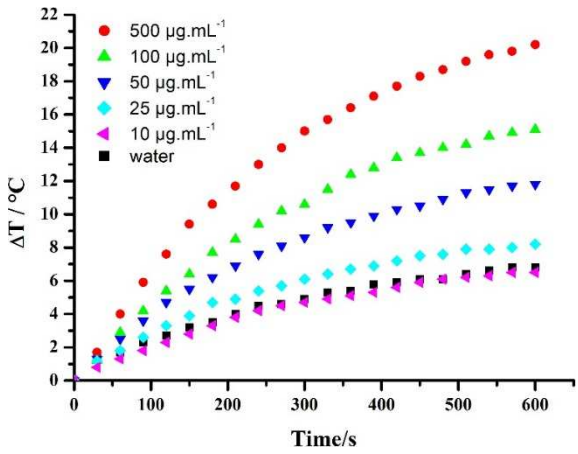

Figure 3. Temperature elevation of solutions of complex **3** in water at various concentrations (0-500 μg•mL^{-1}) as a function of time (0-600 s) under irradiation with a 940 nm wavelength laser at a power of 1 W.cm^{-2}.

The photothermal conversion efficiency (η) was evaluated on solutions of complex **3** in water at 50, 100, 500 μg.mL^{-1} by monitoring the complete temperature profile under continuous irradiation at 940 nm laser

with a power of 1 W.cm^{-2} until 10 min and after turning off of the laser during 10 min (Figure S5-S7). The η value was calculated according to the equation described by Roper and al. based on the energy balance of the system.[12] The η value determined for complex **3** in water is ~ 12 %, which is close to the η values measured with gold nanostructures (13-22%) which are currently the object of great interest for photothermal therapy.[4] NMR experiments and thin layer chromatography performed after NIR irradiation did not evidence any degradation of the complexes, confirming their remarkable photo-thermal stability, even after exposure to laser irradiation for a long time, in contrast to gold nanostructures. It should be noticed that this η value is slightly lower than the one measured recently on cholesterol-substituted nickel-bis(dithiolene) in decane (~ 19 %),[8] highlighting the influence of the substitution pattern around the complex and the nature of the medium/solvent on the absorption and the conversion efficiency.

The good NIR-photothermal conversion efficiency of the octa-pegylated complex **3** in water prompted us to evaluate its potential as a PTT agent *in vivo*. MTT assays on human renal carcinoma cells (786-0) have shown that the viability remained above 80 % after 24 h incubation for concentrations up to 100 μg.mL^{-1} (60 μM) and more than 50 % of the cells survive at 500 μg.mL^{-1} (Figure 4 and S8). Earlier experiments have shown that cells can be killed in 4-6 min for temperatures above 50 °C.[4,13] In regard to the photothermal experiments performed in water (Figure S5-S7), concentrations of complex **3** above 100 μg.mL^{-1} under a power density of 2 W.cm^{-2} or above 50 μg.mL^{-1} under a power density of 5 W.cm^{-2} should be used to reach 50 °C for irradiation performed at 25 °C. The cells were incubated with increasing amount of complex **3** (50, 100, 150 μg.mL^{-1}) for 24 h and then irradiated with a 940 nm NIR-laser for 10 min under power density of 2 or 5 W.cm^{-2}. Upon 5 W.cm^{-2} power density laser irradiation, cell viability significantly decreased for concentrations of complex **3** above 100 μg.mL^{-1} (Figure 4) and after 10 min, less than 20 % of the cells remained alive after irradiation (70 % without irradiation). In contrast, cells without complex **3** incubation were not affected even after 10 min laser irradiation at the highest power density of 5 W.cm^{-2}.

Power density of 2 W.cm^{-2} was found not to be sufficient to induce marked cell death in 10 min even at 150 µg.mL^{-1}. After one day incubation with complex **3**, the phase contrast observation of the cells was not markedly affected whereas after irradiation, the cells adopted a round shape, a sign of cell death (Figure S9). These preliminary *in vivo* results confirm our expectations that nickel-bis(dithiolene) complexes can indeed act as efficient photothermal agent as they are able to induce cell death under NIR irradiation.

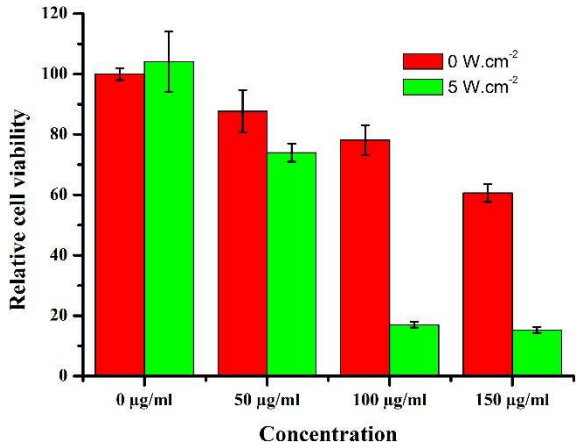

Figure 4. Relative Cell viability 786-0 human renal carcinoma cells treated with different concentrations of complex **3** without or with laser irradiation (940 nm, 5 W.cm^{-2}, 10 min).

In summary, original water-soluble nickel-bis(dithiolene) complexes with good photothermal conversion in water and biocompatibility, were successfully prepared by grafting polyethylene glycol chains on the dithiolene complex core structure. Under NIR irradiation at 940 nm-with a safe power density of 0.5-5 W.cm^{-2}, the temperature of aqueous solutions of complex **3** (25-500 µg.mL^{-1}) can increase by 6.3-65.5 °C in 10 min. Importantly, *the in cellulo* experiments have demonstrated that NIR-laser irradiation at concentrations around 100 µg.mL^{-1} (60 µM) for 5 min can induced cell death. This class of metal complexes provides a new generation of molecular therapeutic agents for photothermal therapy. Proper functionalization of these complexes should improve their photothermal efficiency and their

biocompatibility as well as their targeting abilities. Efforts along these lines are currently under progress in our laboratory.

This study was inspired by earlier discussions with U. T. Müller-Westerhoff in 2008 during a meeting in Philadelphia.[14]

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details, synthesis, Time evolution of the temperature of solution of complex **3** in water at various concentrations (0-500 μg•mL^{-1}) as a function of time (0-600 s) under irradiation with a 940 nm wavelength laser at a power of 0.5, 2 and 5 W.cm^{-2}, temperature profiles and time constants for heat transfer determined on solutions of complex **3** in water at 50, 100, 500 μg•mL^{-1}, Cell viability after 24 h incubation with various concentrations of complex **3** and images of the cells before and after irradiation in presence or not of complex **3**. See DOI: 10.1039/c000000x/

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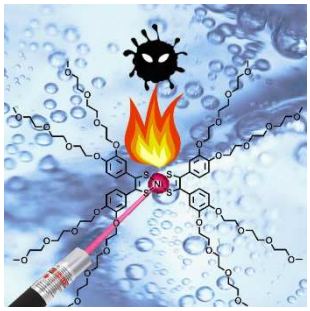

Pegylated nickel-bis(dithiolene) complexes can efficiently convert NIR light into heat in water to induce cells death.