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Nanoscale Coordination Polymers for Platinum-Based Anticancer Drug Delivery

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Nanoscale coordination polymers (NCPs) are a class of "soft" materials constructed from metal ion connectors and polydentate bridging ligands. Owing to an essentially limitless choice of building blocks, they have the potential to be engineered for a wide range of applications, such as heterogeneous catalysis,[1] imaging,[2] and sensing.[3] Yet, challenges remain in the design of functional NCPs that meet the stringent requirements of biological systems, including stability in an aqueous environment and at high concentrations of competing ligands. Although the stability of NCPs can be enhanced via a judicious choice of metal connectors and bridging ligands, they can alternatively be designed for controlled release of biologically functional species, such as imaging or therapeutic agents, by exploiting their inherent solubility in an aqueous environment. The key to the latter approach lies in our ability to stabilize the NCPs so that sustained release of imaging or therapeutic cargoes can be achieved upon their delivery to the intended tissues.

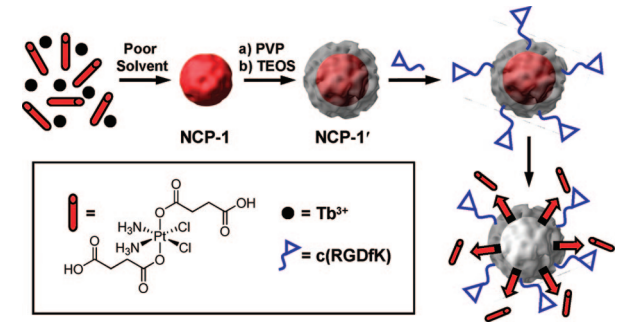
Over the past decade, the clinical value of materials on the nanometer-scale has become increasingly evident, particularly in the context of particle-mediated drug delivery for cancer therapy.[4] Nanotherapeutic formulations offer numerous advantages over their small molecule counterparts, including enhanced drug accumulation in tumor tissue, reductions in systemic toxicity, and the ability to be surface-functionalized with passivating and targeting moieties.[4] The nanotherapeutic approach has been validated in the clinic with the FDA approval of several nanoparticle-based anticancer drugs,[4] and given that Pt-based drugs are still used as the frontline treatment for a number of cancers, there has been a significant interest in the development of nanoparticle formulations for their effective delivery to tumors.[5] Herein we wish to report a novel and general strategy for the delivery of Pt-based drugs to cancer cells via their inclusion into NCPs. The Pt-based NCPs are stabilized with shells of amorphous silica to prevent rapid dissolution and to effectively control the release of the Pt species. Furthermore, the anticancer efficacy of the NCPs is demonstrated on multiple cancer cell lines *in vitro*.

NCPs constructed from Tb^{3+} ions and *c*,*c*,*t*-(diamminedichlorodisuccinato)Pt(IV) (DSCP, abbreviated for disuccinatocisplatin) bridging ligands, **NCP-1**, were precipitated from an aqueous solution of the components via the addition of a poor solvent (Scheme 1). In a typical experiment, the pH of a solution of $TbCl_3$ (15 mM) and DSCP (10 mM) was adjusted to ~5.5 with dilute NaOH. Methanol was quickly poured into the precursor solution with vigorous magnetic stirring to induce the formation of **NCP-1** particles, which were subsequently isolated by centrifuge, washed with methanol and ethanol, and redispersed in ethanol via ultrasonication. The **NCP-1** particles were characterized by inductively coupled plasma-mass spectrometry (ICP-MS), thermogravimetric analysis (TGA), TEM, SEM, dynamic light scattering (DLS), and powder X-ray diffraction (PXRD).

This general synthetic approach is based on the premise that NCPs are significantly less soluble in the precipitating solvent than the individual components. For instance, neither $TbCl_3$ nor the di(methylammonium) DSCP salt precipitates out of an aqueous solution upon addition of methanol; however, when in solution together, a clear dispersion with a distinct bluish-white hue immediately forms, which is indicative of nanoparticle formation. Analogous precipitation methods have recently been used by several groups to formulate a small variety of coordination polymer nanoparticles.[1a,6]

As shown in Figure 1A, the as-synthesized NCPs exhibited a spherical morphology, and they were structurally amorphous, yielding no PXRD peaks that would indicate a crystalline phase. DLS measurements gave a diameter of 58.3 ± 11.3 nm for the **NCP-1** particles in ethanol. ICP-MS measurements consistently gave a Tb/Pt molar ratio of slightly higher than 2:3 for **NCP-1**, suggesting the NCPs were Tb-terminated. TGA confirmed

Scheme 1



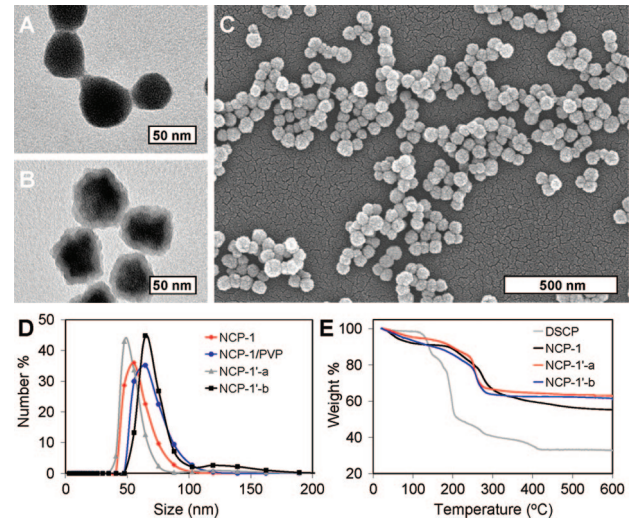


Figure 1. (A) TEM micrograph for as-synthesized **NCP-1**. (B) TEM and (C) SEM micrographs for **NCP-1′-b**. (D) DLS curves for **NCP-1**, PVP-functionalized **NCP-1**, and **NCP-1′**. (E) TGA curves for DSCP, **NCP-1**, and **NCP-1′**.

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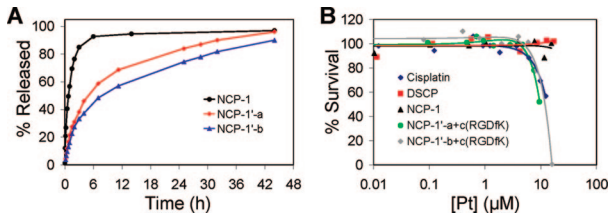

Figure 2. (A) Release profiles for as-synthesized **NCP-1**, **NCP-1′-a**, and **NCP-1′-b** obtained by plotting the % Pt released against time. (B) *In vitro* cytotoxicity assay curves for HT-29 cells obtained by plotting the % cell viability against the Pt concentration of various samples and cisplatin control.

$Tb_2(DSCP)_3(H_2O)_{12}$ as the correct empirical formula for **NCP-1**, with a 9.2% (calcd ∼9.7%) and 36.0% (calcd ∼36%) weight loss for the water and organic moieties, respectively.

While the NCPs were stable and readily dispersible in most organic solvents, nanoparticle formation was reversible if excess water was added to the reaction mixture or to the isolated material. In order to stabilize the NCPs against rapid dissolution in water, we have encapsulated them in shells of amorphous silica.[3,7] Silica as a surface coating offers numerous advantages, including enhanced water dispersibility, biocompatibility, and its ease of further functionalization with a variety of silyl-derived molecules. In the coating procedure, polyvinylpyrollidone (PVP)-functionalized **NCP-1** intermediates were treated with tetraethyl orthosilicate (TEOS) in a 4% (v/v) aqueous ammonia/ethanol mixture to yield silica-coated particles **NCP-1′**. The silica shell thickness could be tuned by varying the reaction time or the amount of TEOS. For example, 2 h of TEOS treatment afforded **NCP-1′-a** with a silica shell thickness of ∼2 nm. These particles had a DLS diameter of 52.8 ± 8.1 nm. Longer TEOS treatment (4 h) afforded **NCP-1′-b**, which had a silica shell thickness of ∼7 nm (Figure 1B) and a DLS diameter of 68.6 ± 10.2 nm. Moreover, TGA gave a 7.0 and 8.5% reduction in the total weight loss for **NCP-1′-a** and **NCP-1′-b**, confirming the presence of the silica shell. The shell thicknesses were highly reproducible, varying to only a small degree when the same reaction conditions were used from sample to sample.

To investigate the controlled release behavior of the silica-coated NCPs, we dialyzed samples against HEPES buffer (pH 7.4) at 37 °C. As shown in Figure 2A, we were able to efficiently control the release of the Pt species by varying the silica shell thickness. The half-lives of dissolution for **NCP-1′-a** and **NCP-1′-b** were determined to be ∼5.5 and ∼9 h, respectively, whereas the as-synthesized **NCP-1** particles gave a $t_{1/2}$ of ∼1 h. These rates of release would allow sufficient time for the Pt-based NCPs to circulate throughout the body and accumulate in tumor tissue.[8]

Our abilities to formulate NCPs from Pt compounds and to control the release of the DSCP species from the silica-coated NCPs prompted us to evaluate their anticancer efficacies. First, we performed *in vitro* cytotoxicity assays on the angiogenic human colon carcinoma cell line HT-29. Treatment of HT-29 with DSCP, **NCP-1**, and **NCP-1′** did not lead to any appreciable cell death after 72 h of incubation, presumably because the DSCP species released from **NCP-1** and **NCP-1′** does not have a pathway to enter the cells effectively, and there are no reductants in the media under *in vitro* conditions to transform DSCP into the active Pt(II) species. However, the released DSCP would become active *in vivo* via reduction to the Pt(II) species by endogenous biomolecules such as glutathione. Recent studies have shown that satraplatin, a similar Pt(IV) complex under phase III clinical investigation, has a very short half-life of only 6.3 min in whole blood as a result of rapid reduction to the Pt(II) species.[9] In order to enhance the cellular uptake of **NCP-1′** *in vitro*, we grafted silyl-derived c(RGDfK) onto its surface. C(RGDfK) is a small cyclic peptide sequence exhibiting high binding affinity for the $\alpha_v\beta_3$ integrin upregulated in many angiogenic cancers (such as HT-29). As shown in Figure 2B, c(RGDfK)-targeted **NCP-1′-a** and **NCP-1′-b** gave IC_{50} (50% Inhibitory Concentration) values of 9.7 and 11.9 μM, respectively, while our cisplatin standard had an IC_{50} value of only 13.0 μM. These results suggest the targeted NCPs are sufficiently internalized presumably via receptor-mediated endocytosis. Once inside the cells, the DSCP species released from the silica-coated NCPs could then be reduced to the active Pt(II) species by intracellular reductants that are present in high concentrations.

We also performed *in vitro* cytotoxicity assays on a human breast carcinoma cell line (MCF-7) that does not overexpress the $\alpha_v\beta_3$ integrin. In contrast to HT-29, the Pt(IV) complex was active against MCF-7. As expected, DSCP and the silica-coated NCPs gave IC_{50} values on the same order of magnitude as the cisplatin standard in our studies (Supporting Information).

In summary, we have developed a general strategy to formulate highly degradable nanoparticles based on Pt-containing nanoscale coordination polymers. We can control the release of the Pt drug by encapsulating the NCPs in shells of amorphous silica, and we demonstrate the anticancer efficacies of Pt-based NCPs *in vitro*. The generality of this approach should allow for the design of NCPs as effective delivery vehicles for a variety of biologically and medically important cargoes such as therapeutic and imaging agents.

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Supporting Information Available: Experimental procedures and six figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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