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Hybrid inorganic (nonporous silica)/organic (alginate) core-shell platform for targeting a cisplatin-based Pt(IV) anticancer prodrug

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ABSTRACT

Nonporous silica nanoparticles with an external shell containing the 3-aminopropyl arm (**SiNP**) were further decorated with alginic acid (**SiNP-ALG**) as a potential biocompatible delivery system for Pt antitumor agents. Such particles were coupled with the prodrug (*OC*-6-44)-acetato(β-alaninato)diamminedichloridoplatinum(IV), **1**, through the formation of amide bonds between the pendant carboxylate groups on **SiNP-ALG** and the free amino group of the complex. Cytosol extracted from tumor cells was able to quickly and efficiently reduce the Pt (IV) prodrug, and produces the active metabolite cisplatin. **SiNP-ALG-Pt** conjugate was more active than both cisplatin and **1**, due to its more efficient cell uptake, whereas the **SiNP-ALG** unplatinated nanoparticles were deprived of any nonspecific toxicity.

1. Introduction

Nowadays, nanoparticles (NPs) are widely employed in biomedicine [1–4]. Their specific chemical and morphological features make them appropriate to deliver diagnostic or therapeutic agents or a simultaneous combination of these payloads in the emerging *theranostic* framework. In particular, NPs are used in anticancer therapy to deliver physisorbed or covalently attached chemotherapeutics to tumor tissues, by a preferential accumulation due to their imperfect vascular and lymphatic systems (*i.e.* Enhanced Permeation and Retention, EPR, effect) [3,5,6]. Several different materials (inorganic, organic or hybrid) have been proposed for the selective delivery of platinum drugs to cancer tissues, in order to minimize the toxicity of this well-known and used class of anticancer drugs, and to maximize the drug efficacy [7–9].

Silica NPs (SiNPs) represent one of the most employed and studied material, albeit, despite the many optimistic statements regarding a bright future as vectors, no SiNPs have been so far approved for clinical use by FDA. SiNPs can be synthesized with fast, straightforward chemical routes, being their inner cores bulky and compact (nonporous SiNPs) [10,11], or featured with different sized channels and cavities (microporous and mesoporous SiNPs) [12]. In general, nonporous SiNPs are deemed safer than the high-surface-area microporous and mesoporous counterparts [13].

In mesoporous SiNPs the pharmaceutical cargo is generally loaded inside the core, whereas nonporous SiNPs allow loading of the drug on the outer surface by means of functionalized arms (decoration) due to the compact nature of the bulky core. The release of the drug in the right place at the right time is the critical point. In the case of the antitumor cisplatin attached to nonporous SiNPs, the linker between the Pt moiety and the vector should contain a hydrolysable or a stimuli-responsive group to guarantee the release of the drug [14].

The use of octahedral Pt(IV) antitumor prodrugs may overcome many of the synthetic difficulties concerning the chemical/biochemical requirement of the arm. Indeed, Pt(IV) prodrugs are activated by reduction to their cytotoxic Pt(II) metabolites in the hypoxic (reducing) tumor milieu releasing the two axial ligands [15–17]. For this reason, any suitable axial ligand in Pt(IV) conjugates constitutes an ideal linker, since it will be released upon reduction, without altering the overall activity of the final Pt(II) metabolite. Such an axial ligand usually consists of a dicarboxylic acid that forms an amide bond with an amine on the decorated nonporous SiNPs.

In this context, two amino-decorated SiNPs have been recently obtained by using the silane coupling agents (3-aminopropyl)triethoxysilane (APTES), or *N*-(6-aminohexyl)aminomethyltriethoxysilane (AHAMTES). Such SiNPs were further conjugated with Pt(IV) prodrugs containing a −COOH group in axial position. Unfortunately, both the arms showed some weak spots: the former undergoing partial hydrolysis with preventive, unwanted detachment of the anticancer payload,

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and the latter scarcely decorating the SiNPs [18,19]. To by-pass this inconvenience and to improve bio-distribution, attempts at wrapping silica cores with bio-active and bio-compatible folders have been reported in literature, either by supramolecular complexation or by covalent attachment of bio-mimetic layers to a chemically modified silica surface [20,21]. As a matter of principle, appropriate bio-coatings consist of carbohydrate polymers (*e.g.* cellulose, arabic gum, xanthan gum, dextran, chitosan, hyaluronic acid, *etc.*) [22]. In this context, alginate (**ALG**) has been established among one of the most versatile biopolymers. Because of its biocompatible and biodegradable nature, **ALG** has been used as a carrier to immobilize or encapsulate drugs, bioactive molecules, and proteins. The presence of carboxylic groups makes possible the use of popular conjugation reagents in both aqueous and organic solvents; moreover, the excess of reagents and the by-products of the coupling reaction can be easily removed by dialysis or gel-filtration [23–25].

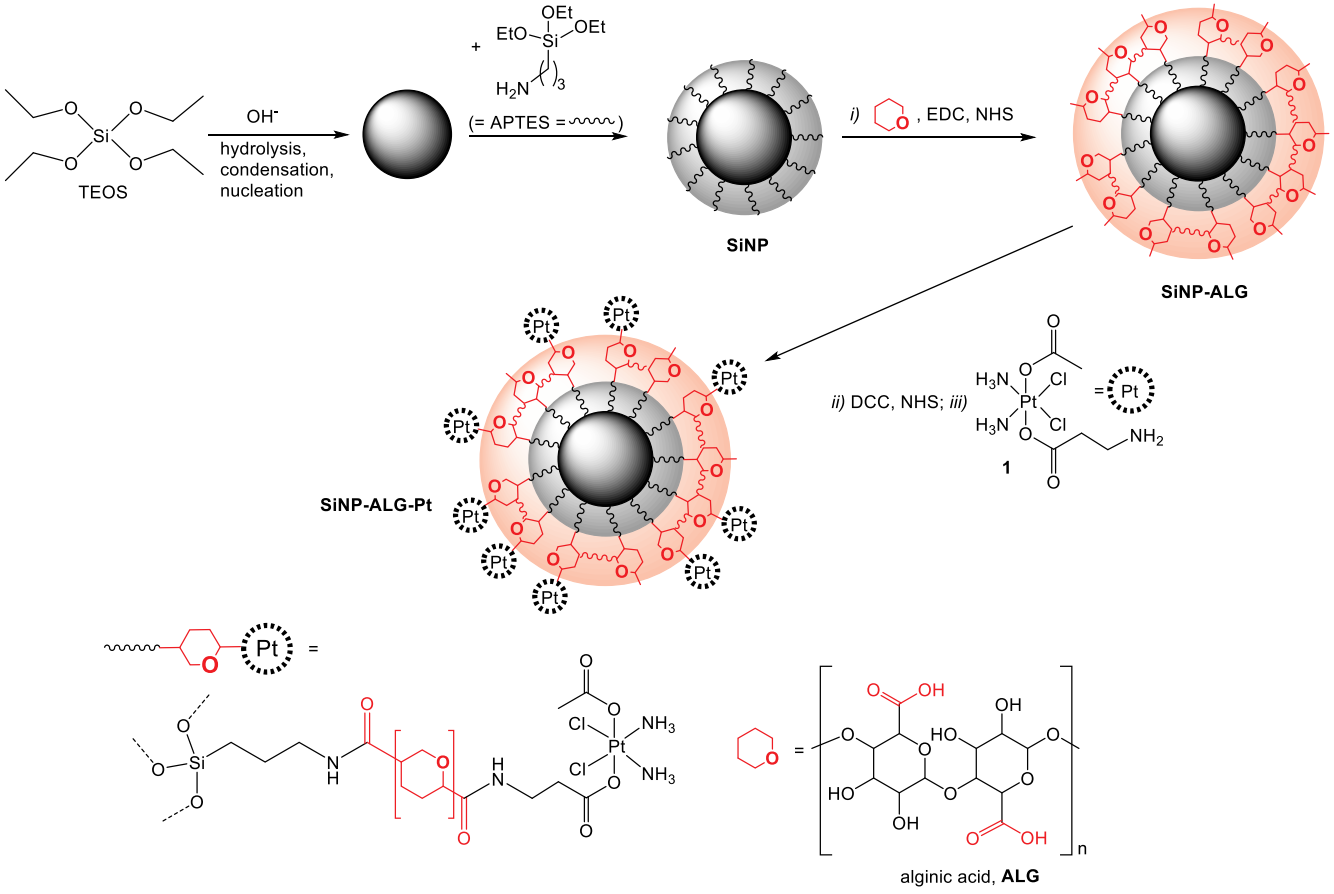
In this work, hybrid core-shell NPs were obtained by covalently coating an amine-functionalized silica core with **ALG**. **ALG** is a linear polymer consisting of D-mannuronic acid and L-guluronic acid residues. In the presence of divalent cations (especially Ca^{2+}) **ALG** tends to form hydrogels able to incorporate pharmaceuticals, albeit they have shown disadvantages as low drug entrapment and scarcely controllable release [26,27]. The assembly between SiNPs and **ALG** provides a nanomaterial scaffold still containing −COOH groups able to link (*via* a further amide bond) cisplatin-based Pt(IV) anticancer prodrugs, namely (*OC*-6-44)-acetato(β-alaninato)diamminedichloridoplatinum(IV), **1** [28], having an amine functionality in the axial ligand (Scheme 1).

2. Experimental

2.1. General procedures

All the chemicals (Johnson Matthey, Co., Sigma Aldrich and Gelest, Inc.) were used without further purification. Elemental analyses were carried out with an EA3000 CHN Elemental Analyzer (EuroVector, Milano, Italy). Chromatographic analyses were carried out using a C18 Phenomenex Phenosphere-NEXT (5 μm, 250 × 4.6 mm ID) column on a Waters HPLC-MS instrument (equipped with Alliance 2695 separations module, 2487 dual lambda absorbance detector, and 3100 mass detector). The mobile phase employed was a mixture containing 30% methanol/70% aqueous 15 mM formic acid; the flow rate was 0.5 mL min^{-1} and the UV–visible detector was set at 210 nm. Mass spectra were recorded using source and desolvation temperatures set to 150 and 250 °C, respectively, with nitrogen used both as a drying and as a nebulizing gas. The cone and the capillary voltages were usually +30 V (positive ion mode) and 3.00 kV, respectively. Quasi-molecular ion peaks $[M+H]^+$ were assigned on the basis of the *m/z* values and of the simulated isotope distribution patterns. Purity of the Pt complexes was > 95% (assessed by HPLC analysis).

The NMR spectra were measured on a NMR-Bruker Avance III operating at 500 MHz (^{1}H), 125.7 MHz (^{13}C), 107.2 MHz (^{195}Pt), and 50.7 MHz (^{15}N), respectively. ^{195}Pt NMR spectra were recorded using a solution of $K_2[PtCl_4]$ in saturated aqueous KCl as the external reference. The shift for $K_2[PtCl_4]$ was adjusted to −1628 ppm from Na_2PtCl_6 (δ = 0 ppm). ^{15}N NMR spectra were recorded using a solution of $^{15}NH_4Cl$ in 1 M HCl as the external reference. [^{1}H, ^{15}N] HSQC spectra (Heteronuclear Single Quantum Correlation) were obtained with the standard Bruker sequence hsqcetgpsiz.



Scheme 1. Synthetic pathway for the production of amino-decorated (with (3-aminopropyl)triethoxysilane, APTES) nonporous silica nanoparticles (**SiNP**), their coating with alginic acid (**ALG**) to obtain **SiNP-ALG**, and final loading with Pt(IV) prodrug **1** to produce the **SiNP-ALG-Pt** conjugate. EDC = 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride, DCC = *N*,*N*′-dicyclohexylcarbodiimide, NHS = *N*-hydroxysuccinimide.