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Polyhedral octasilsesquioxanes labelled with the photosensitive cationic phenosafranin dye as a new nanocarrier for therapy and cellular imaging

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ABSTRACT

New fluorescent nanocarriers based on the POSS (polyhedral octasilsesquioxanes) nanocage labelled with the photosensitive cationic phenosafranin dye (PSF) provide a useful means for in vitro imaging. In this study, a simply reaction for preparing biocompatible POSS-PSF nanohybrids was successfully developed using a coupling (*N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride) agent in a one-step reaction between the amino group of dye molecules and anhydride residues of POSS. The first time are reported fluorescent nanodots as a unique POSS-PSF nanostructure easily transferable by cellular pores due to their cationic charges, which are new intercalative DNA agents useful in anticancer therapies and cellular imaging.

1. Introduction

The octakis-[3-(2-succinic anhydride)propyl, dimethylsiloxy]-octasilsesquioxane (POSSAn) was synthesized from commercially available materials by hydrosililation of allyl succinic anhydride with an octakis (hydridodimethylsiloxy)octasilsesquioxane (HPOSS) [1]. Due to their nanoscale size (6 $Å^3$) [2a] these materials can be easily transferred through vascular pores, resulting in increased tissue uptake [2b]. Thus, the POSS embedded with reactive groups at each corners for possible chemical modification [3] and tunable functionalization [4] allows for simultaneous delivery of different moieties and it is regarded as a next generation material in biomedicine [5,6]. Such nanomaterials [7,8] have gained considerable attention in biomedical applications especially as nanocarries for a nonsteroidal anti-inflammatory system by using ibuprofen or acetaminophen [9] and chemotherapeutic agents (e.g. daunorubicin [1], doxorubicin [10,11]) useful in effective drug targeting [12]. Moreover, they can be powerful tools used as activatable fluorescent nanoprobes in biocompatible imaging [13,14] for in vivo diagnosis due to their ability to overcome interference from cellular autofluorescence [15]. Additionally, POSS with covalently attached chlorin e6 [16] or porphyrin IX [17] fulfills a crucial role in the development of theranostic agents in photodynamic therapy, which has drawn extensive attention as a promising cancer treatment modality.

Herein, we describe the synthesis of chemically inert and physically stable a new type of fluorescent organic-dye-POSS nanodots with uniform shape and size suitable for dual detection methods (spectrophotometric and fluorescence) in imaging systems. The phenosafranin dye (PSF; 3,7-diamino-5-phenyl phenazinum chloride) was covalently bound to reactive groups of the POSSAn nanoparticle via the *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC) method, thus facilitating tracking it in visible light (Scheme 1).

Moreover, the PSF molecule embedded with the cationic chromophore is used as a fluorescent light-triggered agent (photosensitizer) and a biological probe that acts also as a nucleic acid intercalator and human ribonuclease reductase inhibitors [18a,b,c]. Additionally, phenazinum dyes (e.g. methylene blue) has been applied in phothodynamic therapy (PDT) against AIDS-related Kaposi's sarcoma [19]. Thus, the aim of our work is the fabrication of organic-inorganic POSS-PSF molecules which are ideal for utilization in fluorescent imaging and potentially useful in photodynamic anticancer therapy. In vitro studies in HeLa cells culture revealed that new photosensitive nanocages could be a prospective candidate in a theranostic system because they are tunable to strong NIR absorption and have low cytotoxicity. The strategy for the synthesis of innovative nanocages based on the cationic phenosafranin fluorophore offers a promising application in medicine including drug delivery and the both in vitro/in vivo diagnostics. These aspects make the biocompatible POSS-PSF nanocages unique among currently available biomarkers and we expect that the new conjugate can be applicable as an intercalator in tumor treatment.

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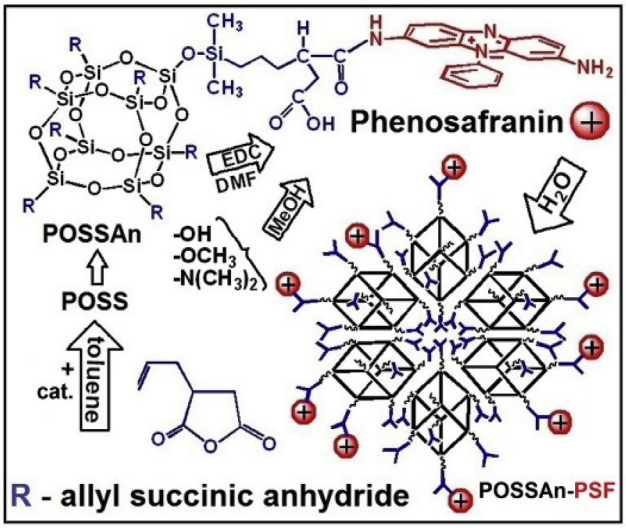

Scheme 1. General illustration depicting the synthesis of POSS-PSF fluorescent nanocages and their self-assemble in aqueous environments.

2. Materials and methods

2.1. Materials

Octakis(hydridodimethylsiloxy)octasilsesquioxane (HPOSS) from (HybridPlastics), POSS-(3-Mercapto)propyl-heptaisobutyl substituted (iBu-POSS) and organic dye phenosafranin hydrochloride (PSF) (CAS No. 81-93-6), color index number: 50200, dye content 80%) (3,7-diamino-5-phenylphenazinum chloride), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide (EDC), allyl succinic anhydride, platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane catalytic complex solution in xylene (Karstedt"s catalyst, Pt 2%) and all organic solvents used (dimethylformamide (DMF), methanol, toluene) were of analytical grade. All reagents were supplied from Sigma-Aldrich Chemical Co., USA (St. Louis, MO). The reactions were carried out in argon atmosphere in Schlenk flask. The melting point (mp) was measured in open glass capillary using a VEEGO Melting point apparatus.

2.2. Synthesis of POSS-PSF

2.2.1. The synthesis of POSSAn-PSF

The octakis[3-((2-succinic anhydride)propyl, dimethylsiloxy)octasilsesquioxane (POSSAn) was obtained by hydrosililation of allyl succinic anhydride with octakis-(hydridodimethylsiloxy) octasilsesquioxane. This method is precisely described in our earlier work [1]. The POSSAn-PSF conjugates were synthesized from obtained POSSAn (0.214 g, 1.0×10^{-4} M) dissolved in 20 ml of dry dimethylformamide (DMF) with the addition of the phenosafranin hydrochloride dye (PSF, 0.290 g, 7.2×10^{-4} M) dissolved in 20 ml of DMF in the presence of *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide (0,204 g, 1.3×10^{-3} M) and triethylamine (Et_3N, 0.162 g, 1.6×10^{-3} M) (the mixture was adjusted to pH = 8–9). The reaction was stirred overnight at 15 °C in the dark. After that, the solvent and unreacted Et_3N were evaporated under reduced pressure and the resulting conjugate was dissolved in methanol and precipitated in hexane. A red product was purified by dialysis against methanol by using bags that cut off a molecular weight (MWCO) of 1000 Da to remove urea and a plain dye, then dried under vacuum. The product was obtained in 90% yield, mp: 194–195 °C. We determined the structure of POSSAn-PSF using the following ^{1}H and ^{13}C NMR assignments:. ^{1}H NMR (DMSO) δ(ppm): 0.08 (6H, C$\underline{H}_3$−Si), 0.53 (2H, m, C$\underline{H}_2$−Si), 1.28 (2H, m, C$\underline{H}_2$CH$_2$Si), 1.40–1.54 (2H, m, CHC$\underline{H}_2$CH$_2$), 2.31–2.40 (2H, m, C$\underline{H}_2$C=O), 3.18 (1H, m, C$\underline{H}$C=O), 5.81 (d, J = 2.15 Hz, C$\underline{H}$−CNH$_2$), 5.91 (s, C$\underline{H}$−CNH), 7.21 (dd, J = 9.38 and 2.15 Hz, CH−C$\underline{H}$−CNH$_2$), 7.63 (m, CHC$\underline{H}$−CNH), 7.65–7.90 (m, benzene ring), 7.92 (d, J = 9.37 Hz, CC$\underline{H}$CH), 8.09 (d, J = 9.37, CC$\underline{H}$CH), 7.51 (m, NH), 3.35 (m, C$\underline{H}_3$O). ^{13}C NMR (DMSO) δ(ppm): 0.17 (CH$_3$−Si), 17.96 (CH$_2$−Si), 19.90 (C$\underline{H}_2$CH$_2$−Si), 35.07 (CHC$\underline{H}_2$CH$_2$), 36.60 (CHC$\underline{H}_2$C=O), 41.51 (C$\underline{H}$C=O), 92.77 (C$\underline{H}$−CNH$_2$), 93.50 (C$\underline{H}$−CNH), 120.90 (CHC$\underline{H}$−CNH$_2$), 125.1 (CHC$\underline{H}$−CNH), 127,0 131.0, 130.61 (benzene ring), 133.86 (CHC$\underline{H}$−C), 134.62 (CHC$\underline{H}$C), 134.20 (CHC−N), 134.80 (CHC−N), 136.14 (C−N), 141.6 (CHC−N), 157.1(C−NH$_2$), 173.7(C=O); *m/z* (MALDI-TOF MS) found: [M + H]$^+$ 2421.8, molecular formula $C_{90}H_{136}N_4O_{44}Si_{16}$ requires 2423.48.

2.2.2. The synthesis of iBu-POSS−PSF

We used the similar procedure as described earlier to covalently bound the PSF molecule to 3[(3-succinic anhydride propyl)thiopropylheptaisobuthyl octasilsesquioxane (iBu-POSSAn). The anhydride terminal groups were introduced by thiol-ene addition of allyl succinic anhydride to mercaptopropylisobutyl-POSS (iBu-POSS−SH) using 2,2′-azoisobutyronitryl (AIBN) as an initiator according with the recipe described in our work [20*a*]. The iBu-POSS−PSF conjugate was obtained in the reaction of PSF (0.090 g, 2.2×10^{-4} M) with iBu-POSSAn (0.260 g, 2.5×10^{-4} M) dissolved in 8 ml of DMF in the presence of EDC (0.027 g, 1.4×10^{-4} M) and Et_3N (0.056 g, 5.5×10^{-4} M). The synthesis was carried out at room temperature for 24 h (in 78% yield) then the product was purified by dialysis against methanol (using dialysis tubing with MWCO 1000 Da). mp: 197–198 °C. We determined the structure of iBu-POSS−PSF using the following ^{1}H and ^{13}C NMR assignments:.^{1}H NMR (DMSO) δ(ppm): 0.57 (2H, s, C$\underline{H}_2$−Si), 0.68 (2H, s, C$\underline{H}_2$−Si), 0.91 (6H, s, C$\underline{H}_3$−CH), 1.56 (2H, m, C$\underline{H}_2$CH$_2$Si), 1.69 (2H, m, CH$_2$C$\underline{H}_2$CH$_2$), 1.78 (1H, m, C$\underline{H}$−CH$_3$), 1.98 (2H, C$\underline{H}_2$CH), 2.44 (2H, m, CH$_2$−S), 2.46 (2H, m, CH$_2$−S), 2.72 (2H, m, C$\underline{H}_2$C=O), 2.98 (1H, m, C$\underline{H}$C=O), 5.85 (d, J = 2.15 Hz, C$\underline{H}$−CNH$_2$), 5.99 (s, C$\underline{H}$−CNH), 7.20 (dd, J = 9.37 and 2.15 Hz, CHC$\underline{H}$−CNH$_2$), 7.51 (m, NH), 7.63 (m, CHC$\underline{H}$−CNH), 7.65–7.90 (m, benzene ring), 7.93 (d, J = 9.37 Hz, CC$\underline{H}$CH), 8.11 (d, J = 9.37, CC$\underline{H}$CH), 3.35 (m, CH$_3$O). ^{13}C NMR (DMSO) δ(ppm): 10.5 (CH$_2$−Si), 21.80 (CH$_2$−Si), 22.50 (C$\underline{H}_2$CH$_2$−Si), 23.3 (C$\underline{H}$CH$_3$), 25.2 (C$\underline{H}_3$−CH), 30.1 (CH$_2$−S), 33.1 (CH$_2$−S), 35.2 (CHC$\underline{H}_2$CH$_2$), 35.70 (CHC$\underline{H}_2$C=O), 42.21 (C$\underline{H}$C=O), 92.72 (C$\underline{H}$−CNH$_2$), 93.44 (C$\underline{H}$−CNH), 121.0 (CHC$\underline{H}$−CNH$_2$), 125.94 (CHC$\underline{H}$−CNH), 127, 131.0, 130.61 (benzene ring), 133.86 (CHC$\underline{H}$C), 134.62 (CHC$\underline{H}$C), 134.65 (CHC−N), 136.14 (C−N), 141.5 (CHC−N), 157.1(C−NH$_2$), 173.7 (C=O); *m/z* (TOF MS ESI) found: 1317.4519, molecular formula $C_{56}H_{93}N_4O_{15}Si_8S$ requires 1317.4512.

3. Measurements

3.1. Mass spectrometry

The matrix assisted laser desorption ionization time of flight mass spectra (MALDI-TOF MS) were recorded on a Voyager Elite mass spectrometer (PerSeptive Biosystems Inc.,Framingham, MA) equipped with a nitrogen laser (337 nm) in a linear, positive mode at an acceleration voltage of 20 kV and with delayed extraction. All mass spectra were accumulated from at least 100 laser shots and processed by Data Explorer ver. 4 software (Applied Biosystems, Foster City, CA). The atmospheric pressure photoionization mass spectra (APPI-MS) were recorded using Synapt G2-Si mass spectrometer (Waters) equipped with a quadrupole-time-of flight mass analyser. The mass spectrometer was operated in the positive ion detection mode. The results of the measurements were processed using the MassLynkx: 4.1 sofware (Waters) incorporated with the instrument.

3.2. Hela cells culture, microscopic evaluation of the cellular uptake of POSS-PSF

HeLa (human, cervical carcinoma) cells were cultured in RPMI