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Full Length Article

Chirality-dependent cellular uptake of chiral nanocarriers and intracellular delivery of different amounts of guest molecules

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ABSTRACT

We demonstrate the organic molecules loaded and chiral polymers coated periodic mesoporous organosilica (PMO) to generate chiral nanocarriers that we used to study chirality-dependent cellular uptake in serum and serum-free media and the subsequent delivery of different amounts of organic molecules into cells. Our results show that the amount of internalized PMO and thus the transported amount of organic molecules by nanocarrier PMO into cells was chirality dependent and controlled by hard/soft protein corona formation on the PMO surfaces. Therefore, this study demonstrate that chiral porous nanocarriers could potentially be used as advanced drug delivery systems which are able to use the specific chiral surface–protein interactions to influence/control the amount of (bio)active molecules delivered to cells in drug delivery and/or imaging applications.

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1. Introduction

Functional porous nanometer-scaled materials (NMs) have gained major attention in nano- and bio-technological fields due to their unique size- and shape-dependent physical properties, e.g. nanostructured characteristics, chemical composition, large surface area, tunable porosity, and electronic, magnetic and optical properties, which differentiate NMs from molecules or bulk materials [1,2]. Besides the physical properties of NMs, surface functionalizing them with bioactive molecules has been used as an additional tool to affect and/or control the interaction of NMs with cells. Moreover, previous studies have demonstrated that cell-NM interactions are influenced not only by NM properties but also NM-protein interactions, namely the formation of NM–protein corona complexes [3]. These complexes are formed when NMs interact with biological media, such as blood- or serum-containing cell culture media, and biomolecules e.g. proteins adsorb on the surface of NMs [4–7]. The specific NM-protein interaction is a dynamic process and based on several driving forces, such as hydrogen bonding, van der Waals interactions, and electrostatic forces [8]. With time, the proteins bound onto NM surfaces are replaced with other proteins that have higher affinity to the NM surfaces. Long-term binding of proteins on NM surfaces results in a "hard corona", while short-term binding leads to formation of a "soft corona". The composition of protein corona (hard vs. soft) on NM surfaces defines the bioactivity of NMs [9–13] and alters their characteristics (e.g. size, shape, chemical composition, surface charge, hydrophobicity/hydrophilicity) [14–16].

Recently, we [17–19] and other groups [20–23] functionalized NMs with enantiomers of bioactive molecules to study protein adsorption on chiral NM surfaces and the subsequent impact of nanoscale stereochemistry on cell behavior (as an alternative to molecular chirality [24–27]). We described for the first time the stereocontrolled interactions of cells with 2D enantiomorphous zeolite L [19] and periodic mesoporous organosilica (PMO) [18] monolayers, and we assessed how the stereochemistry of a PMO surface affected cell-surface interactions within 3D nanocomposite hydrogel scaffolds [17]. Our results demonstrate that cells could differentiate the type of enantiomers on the zeolite and PMO surfaces and that they showed different affinities for the enantiomerically functionalized 2D zeolite/PMO monolayers and 3D NC hydrogel scaffolds. Additionally, we showed that cells can more strongly differentiate surface chirality in serum-containing cell culture media than in serum-free media, indicating the fundamental and substantial effects of surface stereochemistry (protein adsorption onto chiral NMs) on cell-artificial surface interactions [17,18].

In another contribution, Yang et al. used chiral mesoporous silica (CMS) for controlled enantioselective release of chiral drug molecules, made possible because of the local chirality on the pore wall surface of CMS [23]. Recently, in pioneering work, Gao et al.

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demonstrated how protein adsorption affected the cellular uptake of enantiomorphous gold nanoparticles (NPs) [21,22]. They showed that the amount of internalized gold NPs in cells varied with the enantiomer that was used on the surface of the NPs and the amount of serum in the cell culture media.

In this respect, to go beyond the current state of the art in cell-chiral NM interactions, in this study we used chiral porous NM as a nanocarrier for chirality-dependent cellular uptake and for the subsequent delivery of different amounts of organic molecules into cells. This dual nature of our chiral porous NMs—namely their chirality-dependent cellular uptake and intracellular (bio)molecule delivery into cells—means they have great potential to be used as advanced drug delivery systems. In this work, we first describe the internal and external surface functionalization of PMO with organic molecules and chiral polymers, respectively, to generate enantiomophous organic molecule-loaded nanocarriers. Then we studied the effect of nanocarrier surface chirality on their cellular uptake and subsequent delivery of organic molecules to cells. These experiments were done in the presence and absence of serum in the cell culture media, to investigate how protein adsorption at chiral nanocarrier surfaces influences the cellular uptake of chiral porous NMs. In addition, we studied how hard/soft corona formation at chiral PMO surfaces affects their cellular uptake.

2. Experimental details

2.1. Materials

Paraformaldehyde (PFA), poly-*L(D)*-Lysine (PLL/PDL), hexadecyltrimethylammonium bromide (CTAB, 98%), 1,2-bis(trimethoxysilyl)ethane (BTME, 96%), *N,N'*-bis(2,6-dimethylphenyl)perylene-3,4,9,10-tetracarboxylicdiimide (DXP), and trypsin, were purchased from Sigma-Aldrich. Ethanol (absolute for analysis), ammonia solution (32%, pure) and hydrochloric acid (32%, for analysis), were purchased from Merck. Phalloidine Alexa Fluor® 488 was purchased by Invitrogen. 4′,6-Diamidino-2-phenylindoledihydro-chloride (DAPI) was acquired from Polysciences Europe GmbH. Trypan blue solution was purchased by Life Technologies GmbH. ER-ID TM green assay kit for endoplasmic reticulum staining was purchased from Enzo. The cell medium (RPMI 1640) supplemented with 10% (v/v) fetal bovine serum (FBS) was obtained from Biochrom, Germany. Primary dermal fibroblast; normal, human, adult (ATCC® PCS-201-012™) cells were purchased from ATCC. BCA (bicinchoninic acid) protein assay kit was purchased by Thermo Scientific.

2.2. Characterization

The morphology of the ^{DXP}PMO–OH, ^{DXP}PMO-PLL, and ^{DXP}PMO-PDL was investigated using a Zeiss 1540 EsB dual beam focused ion beam/field emission scanning electron microscopy (SEM). Infrared spectroscopy (IR) were carried out with Varian 3100 FT-IR Excalibur Series Spectrometer. Zeta potential measurements and dynamic light scattering (DLS) were done with Malvern Zetasizer Nano Series. A time resolving spectrofluorimeter (Edinburgh Instruments) was used to investigate the calibration curve, which contained different concentrations of DXP-loaded PMOs. Confocal laser scanning (CLSM) microscopy was carried out with a Leica Microsystems (Heidelberg GmbH) (x 63 oil immersion objective was used). Cary 100 (Varian) double beam spectrophotometer was used for the determination of the adsorbed amount of PDL/PLL and serum protein on PMOs.

Table 1
The quantitative amount of DXP, PLL, PDL and serum proteins (μg) on ^{DXP}PMO-OH, ^{DXP}PMO-PLL and ^{DXP}PMO-PDL (1 mg).

	^{DXP}PMO-OH	^{DXP}PMO-PLL	^{DXP}PMO-PDL
DXP	1.1 ± 0.1	1.4 ± 0.1	1.3 ± 0.3
PLL	–	39 ± 2	–
PDL	–	–	37 ± 2
Serum proteins	686 ± 19	796 ± 19	932 ± 21

2.3. Synthesis of PMO-OH

484.5 mg of CTAB was dissolved in 90 mL of H_2O, 33 mL of ethanol, and a 28 wt% ammonia (0.075 g) solution. The reaction mixture was stirred at room temperature for 1 h before the addition of BTME (1.27 g). The above reaction mixture was continuously stirred for an additional 72 h at room temperature. The CTAB mesoporous template was removed by stirring the sample in ethanol (50 mL) with a 36 wt% aqueous solution of HCl (1.5 g) at 50 °C for 6 h. The resulting solid was recovered by centrifugation, washed with ethanol and acetone several times, and dried at 60 °C in vacuum.

2.4. DXP loading of PMO-OH

PMO-OH (100 mg) was suspended in 10 mL toluene and mixed with DXP (0.8 mg). This reaction mixture was refluxed at 120 °C for 2 days. The final product (^{DXP}PMO-OH) was obtained by centrifugation, washed with toluene x 2, ethanol x 2, and dried at room temperature.

2.5. Poly-L(D)-Lysine coating of ^{DXP}PMO-OH (^{DXP}PMO-PLL and ^{DXP}PMO-PDL)

^{DXP}PMO-OH (20 mg) was suspended in water (5 mL) and mixed with PLL or PDL (MW:30.000-70.000) (10 mg). The reaction mixture was stirred for 1 day at room temperature. The final product ^{DXP}PMO-PLL and ^{DXP}PMO-PDL was obtained by centrifugation of the resulting suspension, followed by washing of the residue with water two times and drying at room temperature. The amount of DXP in ^{DXP}PMO-OH, ^{DXP}PMO-PLL, and ^{DXP}PMO-PDL was determined by using a fluorescence intensity calibration curve of DXP ($y = 91204x + 17909$) (Table 1). The amount of adsorbed PLL and PDL was quantified using UV/Vis spectrophotometry (Table 1).

2.6. Protein adsorption experiments

The amount of serum proteins adsorbed on ^{DXP}PMO-OH, ^{DXP}PMO-PLL and ^{DXP}PMO-PDL was measured by using the BCA (bicinchoninic acid) protein assay kit according to the manual instruction (Table 1).

2.7. Cell experiments in serum media (+serum)

The two sets of fibroblast cells were suspended in their specific medium (RPMI 1640), seeded on glass cover slips in 24-well cell culture plates (in each set, 10.000 cells were seeded on three separate glass slips), and incubated for 1 day at 37 °C and 5% CO_2. ^{DXP}PMO-OH, ^{DXP}PMO-PLL and ^{DXP}PMO-PDL were suspended separately first in 10% fetal bovine serum (FBS) containing cell culture media (+serum) (1 mg PMOs/1 mL media) and mixed at room temperature for 1 day. Afterwards, the culture media in the 24-well plates were removed and 2 mL cell culture medium of ^{DXP}PMO-PLL, ^{DXP}PMO-PDL, and ^{DXP}PMO-OH (10 μg PMOs/1 mL media) was separately added on cell monolayers in 24-well plates. After incubation for 1 day the media were removed and the cell layers on glass cover slips were gently washed with phosphate-buffered saline (PBS) to

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