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Mesoporous core-shell silica nanoparticles with anti-fouling properties for ovarian cancer therapy

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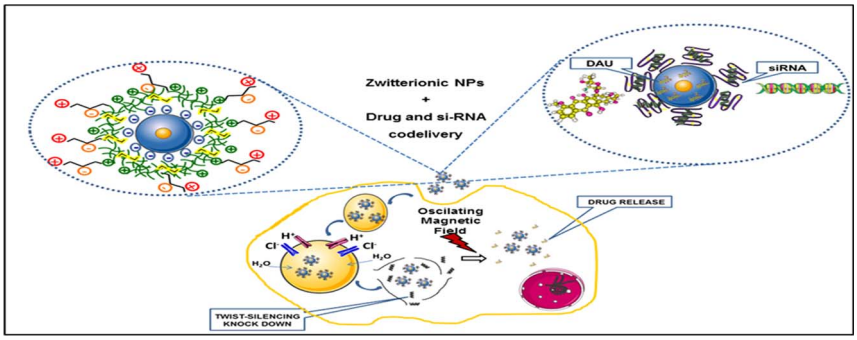
HIGHLIGHTS

- Multifunctional NPs able to avoid unspecific protein adhesion were prepared.
- NPs were crosslinked with GA and decorated with MPC for surface zwitterionization.
- Zwitterionic NPs co-delivered siRNA and Dau in response to an OMF has been described.
- This approach may provide a therapeutic strategy for the treatment of ovarian cancer.

GRAPHICAL ABSTRACT



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ABSTRACT

Mesoporous silica nanoparticles (MSNPs) have many potential applications in biomedical fields. However, when MSNPs are exposed to plasma, protein adsorption leads to opsonization and decreases blood circulation time. A new multifunctional nanodevice based on polyethylenimine (PEI) coated core-shell Fe_3O_4@SiO_2 MSNPs with a zwitterionic 2-methacryloyloxyethyl phosphorylcholine (MPC) surface was designed to minimize unspecific protein adhesion. Particle size measurements demonstrated an excellent non-fouling capacity in solutions containing Bovine Serum Albumin (BSA) and Fetal Bovine Serum (FBS) plasma proteins. The system was used in this study to co-deliver two different cargos: siRNA and daunorubicin. Anti-TWIST siRNA plays critical role in modulating knockdown of TWIST and sensitizing cells to chemotherapeutics such as daunorubicin for ovarian cancer therapy. The drug was released in response to externally controlled oscillating magnetic fields (OMF). siRNA (siGFP) silenced expression of green fluorescence protein (GFP) in Ovcar8 cancer cells, demonstrating the incorporation of core shell MSNPs into cells and siGFP delivery. The synergistic effect of the co-release of anti-TWIST-siRNA loaded in the PEI and daunorubicin loaded in NPs' pores caused increased cytotoxicity in Ovcar8 of up to 50% from both zwitteronic and non-zwitteronic NPs. The system is the first example of silencing by anti-

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TWITS-siRNA/daunorubicin co-delivered using zwitterionic core-shell nanoparticles with low-fouling adsorption. This engineered multifunctional approach may provide therapeutic potential for the treatment of currently incurable ovarian cancer.

1. Introduction

Silica based mesoporous materials and in particular mesoporosus silica nanoparticles (MSNPs) have attracted extensive attention due to their wide spread applications in biomedical fields including biosensing, drug delivery and diagnostic imaging. They are easily chemically modified with biomolecules and are used as theranostic agents for biomedical applications [1–5]. The principal requirements for such nanocarriers include safety (non-toxic) and absence of undesirable side-effects. When nanoparticles (NPs) enter a biological fluid (blood, plasma or interstitial fluid) they are coated with proteins, the “protein corona”, that may lead to the exposure of new epitopes, altered function and/or avidity effects [6]. The protein corona spontaneously forms upon exposure to proteins and may consist of multiple layers that have different affinities to the NPs surface, e.g. the soft corona consisting of the external layer of proteins weakly interacting with the NPs, and the hard corona that strongly adheres to the NPs surface [7]. Because the protein corona affects the properties of the NPs surface, it has significant impact on the interaction between the NPs and cell walls [8–10]. Many studies are described in the literature where NP cellular uptake involves the presence of a protein corona. A key factor affecting their final uptake rates is their adhesion to the cell membrane [11–14]. In order to be an effective delivery vehicle, the NPs must have prolonged blood circulation times and must escape the uptake by mononuclear phagocytes, macrophages and the reticuloendothelial systems [12]. The interaction between the positively charged polyethyleneimine (PEI) polymer and the negatively charged proteins might cause a protein corona effect in this type of nanocarriers. Hence, many PEIs have been chemically modified to decrease the side effect and improve their transfection efficiency [15,16]. An interesting approach to avoid this effect is the zwitterionization of MSNPs surface for imparting antifouling properties. Zwitterionic materials are a new class of antifouling surfaces that have demonstrated to be efficient due to the formation of a strong hydration layer through electrostatic interaction. Their weak interactions with serum proteins and their non-toxic nature make them ideal candidates for gene and drug delivery vectors [17–20].

Ovarian carcinoma is a deadly disease because many patients are diagnosed in the metastatic stage of the disease and drug resistance is a major problem [21]. Chemotherapy and surgery are the initial treatment options, but the development of drug resistance is almost universal. For this reason, much effort has been spent on developing alternative therapeutic strategies based on targeted gene therapy in the attempt to exert a cytotoxic action both on the primary tumor and on the peripheral metastases. In recent years, co-delivery of chemotherapy drugs and siRNA knockdown target genes to promote anti-tumor therapy has provided a new approach for cancer treatment and has generated widespread attention [22]. Huang et al. reported that administration of polymeric nanoparticles able to deliver diphtheria toxin suicide protein encoding DNA, combined with transcriptional regulation to target gene expression suppresses ovarian tumor growth and reduces tumor burden [20]. Bai et al. designed cationic heparin-polyethyleneimine nanogels able to inhibit cell viability by apoptosis induction achieving a tumor weight reduction of ~58.55%. [23]. More recently, Roberts et al. [24] used the protein TWIST as a therapeutic target. TWIST is a developmental transcription factor reactivated in cancers and linked to angiogenesis, metastasis, cancer stem cell phenotype, and drug resistance. They successfully delivered an anti-TWIST siRNA in combination with cisplatin from PAMAM dendrimers and PEI coated silica NPs. Their results revealed a significant impediment of metastatic growth and significant reduction of the tumor size in animal models of a metastatic and chemoresistant phenotype.

Another key element in addition to delivering siRNA is the ability to release anticancer drugs on demand in the desired location after reduction of drug resistance [25,26]. External control of the release can be achieved by using iron oxide superparamagnetic nanoparticles that generate heat upon exposure to an oscillating magnetic field (OMF) application and uncap pores in MSNPs to release the anticancer agent at a specific rate and site, overcoming the problems of conventional techniques for diagnosis and therapy [27]. The core-shell NPs respond to oscillating magnetic fields by generating local nanoparticle heating but the bulk heating effect is negligible, allowing controlled drug delivery strategies [28]. In this work, we present a proof of concept of a new smart multifunctional nanocarrier consisting of core shell silica mesoporous nanoparticles able to avoid unspecific protein adhesion and release two different cargos, anti-TWIST siRNA and daunorubicin, in response to an external oscillating magnetic field.

2. Experimental section

2.1. Synthesis of core-shell Fe_3O_4@SiO_2 mesoporous nanoparticles and PEI coating

2.1.1. Synthesis shell Fe_3O_4@SiO_2 and functionalization of the NPs surface with DPTES

Core-shell Fe_3O_4@SiO_2 mesoporous nanoparticles were obtained through a [29]. Briefly, 1.25 mg/L of a chloroform dispersion of superparamagnetic iron oxide nanoparticles (SPION, Ocean Nanotech, San Diego, CA) was added to a 22 mL water solution containing 80 mg of CTAB. The mixture was then sonicated for 10 min to allow an homogeneous dispersion of the organic solvent in the water phase after which the resulting solution was stirred at 85 °C to allow the chloroform to evaporate during 10 min. Once the solution became clear the flask was sonicated for 2 min to ensure a good dispersion of the SPION After another 10 min at 85 °C, 25 mg of arginine was added and finally 200 µL of TEOS were added drop by drop. In order to obtain functionalised core–shell nanoparticles, 20 µL (2-diethylphosphatoethyl)triethoxysilane (DPTES) (95 wt%, Gelest Inc.) was added to the mixture after 3 h leading to nanoparticles with phosphonate group on the surface. The surfactant was removed by ionic exchange soaking nanoparticles in 200 mL of a NH_4NO_3 solution (6 g/L) in ethanol at 80 °C two times during 2 h under magnetic stirring. NPs were collected by centrifugation, washed three times with ethanol.

2.1.2. Zwitterionization of the NPs-PEI surface with 2-methacryloyloxyethyl phosphorylcholine

The addition of the cationic PEI coating (1.8 kDa branched polymer, Gelest) to the MSNPs has previously been described [30]. In short, homogeneously suspension of MSNPs was dispersed in PEI ethanolic solution (2.5 mg/mL) which was then sonicated and stirred 30 min (repeated twice). The NPs were then washed in ethanol to remove unbound PEI leading to NPs-PEI. 1 mg/mL of NPs-PEI was mixed in water with 1.2 µL de GA/mg NP glutaraldehyde (GA, 50 wt%, Sigma-Aldrich, USA). After 30 min. a solution of MPC were added and mixed during 24 h at different MPC/DPTES ratios (x:1), where x = 0.25, 0.50, 0.75 and 1, giving rise to 25-MPC, 50-MPC, 75-MPC and 100-MPC zwitterionic NPs, respectively. Finally, the samples were washed three times with water giving rise to MPC-25, MPC-50, MPC-75- and MPC-100.