

Pluronic polymer capped biocompatible mesoporous silica nanocarriers†

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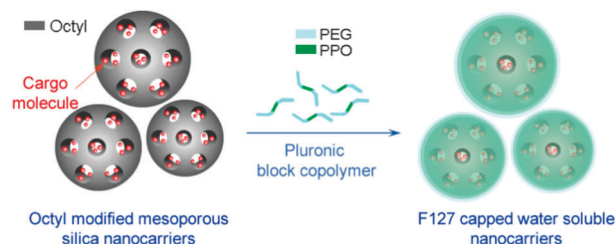
A facile self-assembly method is described to prepare PEGylated silica nanocarriers using hydrophobic mesoporous silica nanoparticles and a pluronic F127 polymer. Pluronic capped nanocarriers revealed excellent dispersibility in biological media with cyto- and blood compatibilities.

The high surface area and pore volume, good chemical stability and ease of surface functionalization of mesoporous silica nanoparticles (MSNs) make them promising materials for biological applications as drug carriers and theranostic agents.^{1,2} In addition silica based materials are generally accepted as biocompatible materials by the U.S. Food and Drug Administration (FDA). However, recent studies demonstrated their potential *in vitro* and *in vivo* toxicity, especially when their size is reduced to the nano scale.^{3,4} Although the toxicity of silica based nanomaterials depends on several factors including particle size, shape, surface chemistry and porosity,^{5–7} there is a general consensus that chemical structure of the surface is the predominant factor which determines the interactions with biological systems.⁸ The surface of bare silica is covered with negatively charged silanol groups, which can electrostatically interact with positively charged tetraalkylammonium moieties of the cell membrane and can lead to cytotoxicity by membranolysis or inhibition of cellular respiration.^{8,9} Also, rapid aggregation of silica based nanoparticles in biological media can result in mechanical obstruction in the capillary vessels of several vital organs, leading to organ failure and even death.^{10,11} Therefore, replacing the surface silanol groups with biocompatible molecules is essential to improve the biocompatibility of MSNs.

Among numerous polymeric or organosilane surface modification ligands, polyethylene glycol (PEG) is the mostly used one due to

its well established biocompatibility, hydrophilicity, and antifouling properties.¹² However, the PEGylation process has some limitations; (i) it mostly requires tedious organic synthesis and surface modification¹³ and (ii) pores of MSNs may be closed by the long PEG polymer chains, which can hinder the drug loading process. To overcome these limitations, here we report a facile self-assembly method using octyl modified hydrophobic MSNs and an amphiphilic block copolymer (F127). F127, a FDA approved biocompatible pluronic polymer, contains two hydrophilic PEG blocks and a hydrophobic polypropylene oxide (PPO) between the two PEG blocks.¹⁴ When the powder of hydrophobic MSNs is added into the F127 solution they are easily transferred into water by self-assembly of F127 molecules onto the MSN surface through the hydrophobic interaction between the PPO block of F127 and surface octyl groups of the MSNs (Scheme 1). In addition, cargo loaded and PEGylated MSNs can be simply prepared by loading the hydrophobic MSNs before the F127 capping process. The F127 capped particles are dispersible in both water and phosphate buffered saline (PBS), whereas uncapped MSNs are easily aggregated and precipitated. The improved biocompatibility of F127 capped MSNs was demonstrated using cytotoxicity, hemolytic activity and thrombogenicity assays. Lastly, cargo release kinetics of PEGylated MSNs was investigated.

Octyl modified MSNs (O-MSN) were prepared *via* respective condensation of tetraethyl orthosilicate (TEOS) and octyltriethoxy silane (OTS) monomers in a one-pot reaction.^{15–17} After polymerization of TEOS and formation of initial MSNs, OTS monomers were added in order to obtain hydrophobic coatings on MSNs. Also, for



Scheme 1 Schematic representation of F127 capped MSN preparation.

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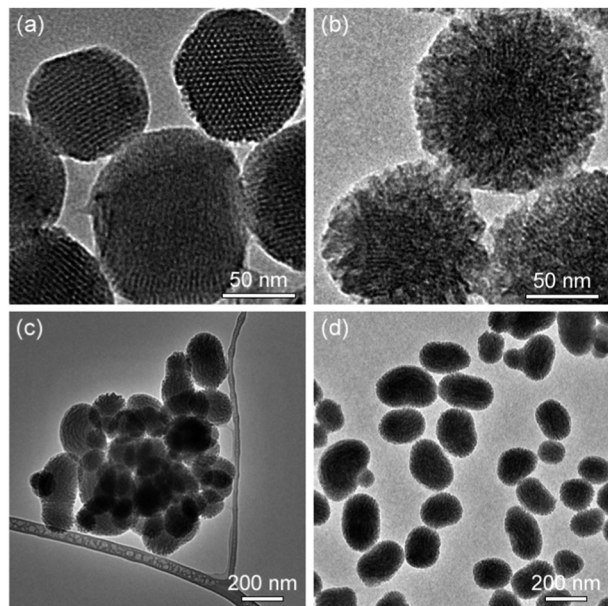


Fig. 1 TEM images of mesoporous silica nanoparticles. (a) Bare MSN, (b) and (c) O-MSN, and (d) F127-OMSN.

control experiments bare MSNs were synthesized without the addition of OTS (see ESI† for details). Fig. 1 shows TEM images of the prepared MSNs. The MCM-41 type ordered hexagonal porous structure was observed for bare MSN (Fig. 1a). Interestingly, O-MSN revealed bimodal pore structure; a randomly porous shell was observed over the MCM-41 type porous core (Fig. 1b). The shell thickness was calculated to be 12 ± 3 nm from the TEM images. Also, average sizes and size distributions of both particles were determined from TEM images and are given in Fig. S1 (ESI†). Surface area and pore volume of the particles were calculated from the adsorption-desorption data (Fig. S2, ESI†). O-MSN revealed slightly lower surface area ($961.2 \text{ m}^2 \text{ g}^{-1}$) and pore volume ($1.01 \text{ cm}^3 \text{ g}^{-1}$) compared to MSN ($1115.7 \text{ m}^2 \text{ g}^{-1}$ and $1.25 \text{ cm}^3 \text{ g}^{-1}$). The decrease in the surface area and pore volume is the result of pore narrowing after octyl modification.¹⁵ O-MSN formed aggregates while drying on the TEM grid, due to the hydrophobic interactions between particles (Fig. 1c). On the other hand, after coating with the pluronic polymer (F127-OMSN), particles are well dispersed on the grid (Fig. 1d) indicating the formation of the F127 coating on particles. The formation of a F127 layer on particles was further confirmed by TGA (Fig. S3, ESI†). For MSN, the weight loss at 800°C was only 8.3% which is due to residual surfactant molecules and dehydroxylation of the silica surface.¹⁸ For O-MSN, the weight loss reached 18.8% due to the decomposition of octyl groups. On the other hand, a large weight loss (47.1%) was observed for F127-OMSN. There are two sharp decreases in the TGA spectrum of F127-OMSN, which correspond to decomposition of F127 and octyl groups. In addition, FTIR spectra (Fig. S4, ESI†) of the particles clearly demonstrated the successful octyl modification and F127 capping (see ESI† for details) of MSNs.

Good dispersibility in biological media is vitally important for developing biocompatible and effective nanomaterials for biological applications, since aggregated particles can induce toxicity in several organs and result in poor pharmacokinetics.¹⁹ F127-OMSN showed

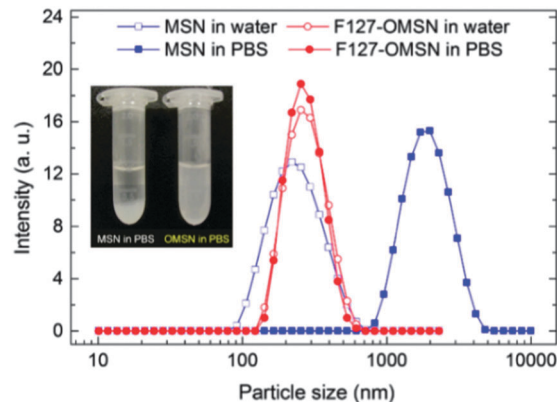


Fig. 2 Dispersibility of mesoporous silica nanoparticles in aqueous media. Size distributions of MSN and F127-OMSN in water and PBS. The inset shows the photograph of MSN (left) and F127-OMSN (right) dispersions in PBS showing the colloidal stability of PEGylated MSNs.

excellent dispersibility in both water and PBS ($\text{pH} = 7.4$) at a high concentration (1 mg mL^{-1}). The particle size distribution in both media was almost identical and the average particle size was around 280 nm (Fig. 2). The good aqueous dispersibility of F127-OMSN in highly salted media is attained by non-ionic and hydrophilic PEG blocks. On the other hand, MSN was fairly dispersible in water at 1 mg mL^{-1} , with slightly broader size distribution than F127-OMSN and the average particle size of around 235 nm (Fig. 2). However, MSN easily aggregated and precipitated in highly salted PBS media in a couple of minutes at the same concentration (Fig. 2 inset). Therefore, a more dilute solution (0.1 mg mL^{-1}) was used to retard the particle precipitation and particle size distribution of MSN in PBS was determined. Even at this low concentration MSN formed large aggregates and exhibited a broad particle size distribution with a large average particle size of $\sim 2 \mu\text{m}$ (Fig. 2). To test the stability of the F127 coating, we precipitated the particles, removed the supernatant and redispersed them in PBS; this cycle was repeated ten times. The average particle size almost remained intact after the washing cycles (Fig. S5, ESI†). The good stability of the F127 layer is due to the strong hydrophobic interaction between octyl groups of O-MSN and the hydrophobic PPO block of the F127, which is stronger than either hydrogen bonding or electrostatic interactions in highly salted solutions.¹⁰

In order to evaluate the *in vitro* cytocompatibility of the particles 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) cytotoxicity assay was used between particle concentrations of 0.1 and 1 mg mL^{-1} . Fig. 3a indicates that both MSN and F127-OMSN are highly compatible with MCF-7 (human breast cancer cell line) cells after incubation times of 24 or 48 h.

Blood compatibility of nanomaterials is a crucial issue when they are applied by intravenous injection, a commonly applied route for drug delivery.^{7,20} It is well known that interaction of MSNs with blood constituents may cause serious toxicity such as hemolysis of red blood cells (RBCs) and blood clot formation (thrombogenicity).^{6,21} Therefore, we investigated the hemolytic activity and thrombogenicity of MSNs and F127-OMSN using a hemolysis assay⁶ and measuring their activated partial thromboplastin time (aPTT) and prothrombin time (PT).²¹ Hemolytic activity of the particles was monitored by measuring light absorption

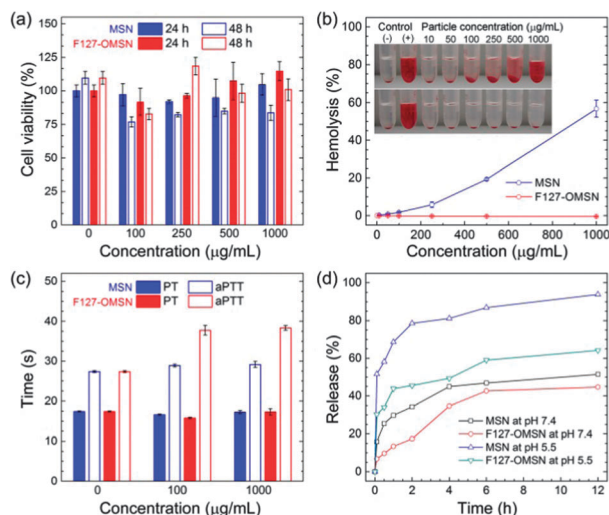


Fig. 3 (a) Cell viability results of MSN and F127-OMSN after 24 and 48 h. (b) Hemolysis results of MSN and F127-OMSN. The inset shows photographs of RBCs treated with MSN (up) and F127-OMSN (down) at different concentrations. (c) PT and aPTT values of particles. (d) DOX release profiles MSN and F127-OMSN at different pH values.

(at 570 nm) of released hemoglobin from the lysed RBCs. MSN revealed significant hemolytic activities of 5.8% at 0.25 mg mL⁻¹ and 56.8% at 1 mg mL⁻¹ (Fig. 3b). Strong electrostatic interaction between negatively charged silanol groups and positively charged trimethyl-ammonium head groups of the membrane lipids can cause membranolysis of RBCs.²⁰ Capping the MSNs with uncharged F127 molecules completely prevented the hemolytic activity even at high particle concentration (1 mg mL⁻¹) by blocking the electrostatic interaction of silanol groups with the RBC membrane (Fig. 3b). Also, the photograph of the MSN treated RBCs shows the hemoglobin (red colour) released to the supernatant, whereas no red colour was observed for F127-OMSN treated cells (Fig. 3b, inset). PT and aPTT tests are applied to investigate extrinsic and intrinsic blood coagulation pathways, respectively. The PT and aPTT results of the particles at low (0.1 mg mL⁻¹) and high (1 mg mL⁻¹) concentrations revealed that all the values are in their normal range (Fig. 3c).²¹ It is well known that some dry and porous silica materials can activate the coagulation cascade due to their high absorption capacities.²¹ On the other hand, the pores of the MSN and F127-OMSN are already filled with PBS, which significantly decreases the absorption capacity of particles and results in the observed anticoagulant behaviour of the particles.^{20,21}

Cargo loading and release studies were performed using a cancer drug, doxorubicin (DOX). We calculated a DOX loading capacity of 15.3 μg mg⁻¹ for MSN. For O-MSN, we observed a slightly lower loading efficiency (11.5 μg mg⁻¹) under the same conditions which is most likely due to the lower surface area and pore volume of octyl modified particles. The powder of DOX loaded O-MSNs was directly capped with F127 in PBS (pH 7.4) and used in drug release studies. We observed slower release profiles for F127-OMSN under both acidic (pH 5.5) and neutral (pH 7.4) conditions compared to MSN (Fig. 3d). For instance, at pH 5.5 MSN released 93.9% of its load in 12 hours, on the other hand, 64.2% DOX release was observed for F127-OMSN under the same conditions. The F127 polymer

layer around the particles slows down the DOX release rate by acting as a diffusion barrier against cargo release from the pores. Also, at pH 5.5 faster DOX release was observed for both particles compared to that at pH 7.4, which is in good accordance with the previous reports.²² The slow rate of DOX release from F127-OMSN can provide more effective therapy by preserving desired drug concentration in the body for longer times.

In conclusion, we have developed a facile method to prepare cargo loaded and PEGylated MSNs using a pluronic F127 block copolymer and hydrophobic MSNs. F127 molecules self-assembled onto the surface of hydrophobic MSNs. The resulting structures revealed good dispersibility and stability in highly salted media, in which uncoated MSNs were easily aggregated and precipitated. F127 capped MSNs demonstrated good cytocompatibility with MCF-7 cells and RBCs. Also, we did not observe any abnormality in aPTT and PT values of F127-OMSN treated blood samples. Furthermore, we demonstrated the preparation of cargo loaded and PEGylated MSNs using the pluronic capping approach. We believe that the simple PEGylation method demonstrated in this study is an excellent candidate for developing biocompatible silica based nanocarriers and theranostic agents.

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