Surface-Initiated Polymerization within Mesoporous Silica Spheres for the Modular Design of Charge-Neutral Polymer Particles

Markus Müllner, Jiwei Cui, Ka Fung Noi, Sylvia T. Gunawan, and Frank Caruso*

Department of Chemical and Biomolecular Engineering, The University of Melbourne, Parkville 3010, Australia

KEYWORDS: *replica particles, nanoengineering, polymer particles, templated assembly, low-fouling, ATRP*

ABSTRACT: We report a template approach for the preparation of functional polymer replica particles via surface-initiated polymerization in mesoporous silica templates. Subsequent removal of the template resulted in discrete polymer particles. Furthermore, redox-responsive replicas could be engineered to disassemble in a reducing environment. Particles, made of poly(methacryloyloxyethyl phosphorylcholine) (PMPC) or poly[oligo(ethylene glycol) methyl ether methacrylate] (POEGMA), exhibited very low association to human cancer cells (below 5%), which renders the reported charge-neutral polymer particles a modular and versatile class of highly functional carriers with potential applications in drug delivery.

INTRODUCTION

Through combined efforts in nanotechnology and biomedicine, nanoengineered particles have emerged as potential candidates to enhance the diagnosis and treatment of various diseases.¹⁻⁷ Polymer carriers with tailor-made properties and functionalities have received growing interest in nanomedicine.^{8,9} Polymer micelles, polymersomes,¹⁰⁻¹² as well as dendritic polymers,^{13,14} and polymer capsules^{15,16} have demonstrated potential for biomedical applications, where delivery vehicles should preferentially be made of low-fouling materials. Materials made of zwitterionic- or poly(ethylene glycol) (PEG)-based polymers are known to exhibit low-fouling properties, and high resistance to nonspecific protein adsorption and cell association.¹⁷⁻²² These so called “stealth” materials allow for extended blood circulation times of drug carrier particles, which is in turn crucial, as this increases the bioavailability of the carriers to reach the site of action.

Recently, we demonstrated a facile approach for the preparation of polymer replica particles,

using mesoporous silica (MS) particles as sacrificial templates. The MS particles are infiltrated with preformed synthetic or bio-polymers, which are then cross-linked. Subsequent template removal produces polymer replica particles. Using this method, replica particles composed of various polymers, such as polyelectrolytes,²³⁻²⁵ polyesters,²⁶ polysaccharides,²⁷ polypeptides,^{28,29} and proteins,³⁰ have been reported. An advantage of this infiltration method is its applicability, where it becomes simple to produce materials with varying size, but also to tailor their physicochemical and mechanical properties.^{24,31} However, the infiltration method is mainly based on electrostatic interaction. Consequently, it becomes challenging to produce replica particles made of charge-neutral polymers, such as PEG,³² or zwitterionic polymers which may have little interaction with silica. Their infiltration into template particles (due to minimal interaction with silica), and also the synthesis of highly functional and cross-linkable PEGs or zwitterionic polymers, is challenging. Additionally, the functional groups are often used for cross-linking which, as a result, reduces

Scheme 1. Schematic illustration of the synthesis procedure: (A) An ATRP initiator-functionalized MS particle provides the template to produce (B) a polymer/silica hybrid after the surface-initiated polymerization of various comonomers, which becomes a (C) polymer replica particle after template removal.

Scheme 2. Reaction scheme for the ‘one pot’ infiltration of PMPC or POEGMA via SI-ATRP.

the overall number of reactive sites for further post-modification, such as attaching therapeutic molecules.

Besides the infiltration of preformed polymers, polymerization in and on MS particles has been used to obtain functional hybrid materials or to produce porous carbon materials after heat treatment.³³⁻³⁷ Several studies on controlled radical polymerization inside porous silica (e.g., SBA-15) have been reported.³⁸⁻⁴⁴ In a recent example, Mandal and co-workers used ATRP inside mesoporous silica nanospheres to prepare functional mesoporous polymer and carbon nanomaterials. Their templated poly(methyl methacrylate)-based particles showed potential in glucose sensing.⁴⁴ However, the synthesis of stealthy polymers in MS particles and the following formation of replica particles have not been reported.

Herein, we synthesized discrete polymer replica particles via surface-initiated atom transfer radical polymerization (SI-ATRP) in MS spheres and subsequent removal of the sacrificial templates (Scheme 1). Using this approach, it becomes feasible to produce replica particles com-

posed of charge-neutral polymers, such as poly(methacryloyloxyethyl phosphorylcholine) (PMPC) and poly[oligo(ethylene glycol) methyl ether methacrylate] (POEGMA). The replicas could be synthesized with various functionalities and exhibited promising behavior for biomedical applications. This approach offers a number of distinct advantages: i) it represents a highly modular approach for the fabrication of functional polymer replica particles, where the composition of the replica is predetermined by the co-monomer mixture; ii) the formation of polymer networks via copolymerization proceeds in a ‘one pot’ reaction, where cross-linking and incorporation of functional groups occur simultaneously; and iii) the copolymerization of responsive cross-linkers allows disassembly of the replica particles with specific triggers.

EXPERIMENTAL SECTION

Materials. High purity (Milli-Q) water with a resistivity $>18.2\text{ M}\Omega\cdot\text{cm}$ was obtained from an inline Millipore RiOs/Origin water purification system. Ammonia (28-30%), hydrofluoric acid (HF), trifluoroacetic acid (TFA, 99%), pyridine

(anhydrous, 99.8%), deuterium oxide (D_2O), copper(I) bromide (CuBr, 98%), L-glutathione (reduced GSH, 98%), methacryloyl chloride (97%), (3-aminopropyl)triethoxysilane (APTES, 98%), *tert*-butyl carbamate (BOC)-ethanolamine (98%), α -bromoisobutyryl bromide (98%), di(ethylene glycol) dimethacrylate (DEGDMA, 95%), *tert*-butyl methacrylate (*t*BMA, 98%), poly(ethylene glycol) diacrylate (PEGDA, average M_n 575 g·mol⁻¹), bis(2-methacryloyl)oxyethyl disulfide, glycidyl methacrylate (GMA, 97%), 2-hydroxyethyl methacrylate (HEMA, 97%), oligo(ethylene glycol) methyl ether methacrylate (OEGMA, average M_n = 300 g·mol⁻¹), and *N,N,N',N'',N'''*-pentamethyldiethylenetriamine, (PMDETA, 99%) were obtained from Sigma-Aldrich. 2-Methacryloyloxyethyl phosphoryl-choline (MPC) was purchased from HBCChem, Inc. Alexa Fluor 647 C2 maleimide (Mal-AF647) and Alexa Fluor 647 carboxylic acid succinimidyl ester (NHS-AF647) were purchased from Invitrogen (Australia). 2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carbox-anilide inner salt (XTT) was obtained from Promega. BOC-protected ethanolamine methacrylate was prepared according to literature.⁴⁵ All chemicals were used as received, except for CuBr, which was purified by washing with glacial acetic acid, followed by absolute ethanol and diethyl ether, and dried under vacuum. All liquid monomers were passed through a short silica column to remove the inhibitor, except for GMA, which was freshly distilled prior to use.

MS Preparation and Surface Modification. MS particles were synthesized according to a modified literature method.⁴⁶ To attach the ATRP initiators onto the surface of the MS particles, the particles were first amino-functionalized through reaction with APTES. In this process, the particles were dispersed in ethanol to a concentration of 30 mg·mL⁻¹ by sonication for 10 min, before ammonia and APTES were added to the suspension. The volume ratio of [ethanol]:[ammonia]:[APTES] was fixed at 20:1:1 and the suspension was stirred overnight. APTES-modified silica particles were washed with ethanol and Milli-Q water. In a second step, the amino-functionalized particles were

reacted with α -bromoisobutyryl bromide to attach the ATRP initiators via amide bond formation. The particles were washed twice with pyridine, and finally suspended into anhydrous pyridine. After the addition of α -bromoisobutyryl bromide, the particles were stirred overnight, before being washed twice with ethanol and three times with Milli-Q water. The particles were then stored in ethanol in the fridge (~8 °C).

SI-ATRP Polymerization. In a typical experiment, the molar ratio of the reactants was as follows: [initiator]:[monomer mixture]:[CuBr]:[PMDETA] = 1:1000:2:2. The monomer mixture itself had a ratio of different comonomers: [primary monomer]:[cross-linker monomer]:[functional monomer] = 87:10:3. The 'primary monomer', such as methacryloyloxyethyl phosphoryl-choline (MPC), endowed the replica particles with their main properties. The cross-linker monomer prevented the replica particle from disassembly after template removal, and the functional monomer added chemical moieties within the polymer replica particle for subsequent functionalization. The synthesis procedure was the same for all polymerizations, however for simplification, will only be explained for the example of PMPC-grafted MS particles. 1 mg of ATRP initiator-functionalized MS particles (100 μ L of 10 g·L⁻¹ ATRP-particles stock solution; initiator content: 0.26 mg, 0.91 μ mol) was added to a Schlenk flask containing a mixture of MPC (237 mg, 0.8 mmol), DEGDMA (22 mg, 0.09 mmol), HEMA (2.3 mg, 0.01 mmol) and PMDETA (0.31 mg, 1.82 μ mol) in 1.5 mL ethanol. Depending on the monomer solubility, other solvents such as DMSO or anisole were used for the polymerization. After three freeze-pump-thaw cycles, CuBr (0.26 mg, 1.82 μ mol) was introduced and the polymerization was left to stir for 15 h at 50 °C. After polymerization, the polymer-grafted hybrid particles were isolated by centrifugation at 2,000 *g* for 3 min and then thoroughly washed with methanol and acetone by repeated cycles of re-dispersion/centrifugation. A vortex mixer was used to aid redispersion. The isolated hybrids were stored in methanol. Different functional polymer replica particles were prepared via this

synthesis route (see Supporting Information S3).

Labeling and Template Removal. To produce fluorescently-labeled replica particles, hybrid particles bearing -OH groups were reacted with NHS-activated dyes. The hybrid particles were dispersed into anhydrous DMSO and slightly vortexed after the addition of 3 μL of NHS-dye (in anhydrous DMSO, 1 $\text{g}\cdot\text{L}^{-1}$). After shaking for 12 h, the particles were washed with methanol several times before redispersion into Milli-Q water. The polymer hybrids could be readily labeled after polymerization and purification. When protected monomers were used, a deprotection step was required before further modification (refer to Table S3 in the Supporting Information). To remove the silica template, buffered HF solution (pH 5, 200 μL of a 5 M HF solution and 400 μL of a 13 M NH_4F solution) was added and left to incubate for 5 min. *Caution! HF is highly toxic. Care should be taken when handling HF solution and only small quantities should be prepared.* The resultant polymer particles were centrifuged (6 min, 3800 g) and washed three times with Milli-Q water.

Characterization Methods. ζ -potential measurements were carried out in Milli-Q water adjusted to pH 7.4 containing 10 mM NaCl using a Zetasizer Nano ZS (Malvern). Thermogravimetric analysis (TGA) was conducted on a PerkinElmer Diamond instrument in an air atmosphere. The measurements were scanned in the temperature range 50 – 700 $^{\circ}\text{C}$ at a heating rate of 10 $\text{K}\cdot\text{min}^{-1}$. The surface areas and porosities of the MS particles were measured by a Micromeritics Tristar surface area and porosity analyzer at 77 K, using nitrogen (N_2) as the adsorption gas. The pore size distributions were derived from the desorption curve using the Barrett-Joyner-Halenda (BJH) method. Nuclear magnetic resonance (^1H NMR) spectra were recorded in D_2O using a 400 MHz Varian INOVA system at 25 $^{\circ}\text{C}$. Polymer replica particles were imaged on an Olympus IX71 inverted fluorescence microscope (100 $\times$ 1.40 oil objective, Olympus). Transmission electron microscopy experiments (TEM, Philips CM120 BioTWIN, operated at 120 kV) were performed by placing samples dispersed in water onto Formvar-coated copper

grids and allowed to air-dry. Scanning electron microscopy (SEM, Philips XL30, operated at 10 kV) was used to examine the silica particle morphology. SEM samples were prepared on silicon wafers and allowed to air-dry prior to gold sputter-coating. Non-contact mode atomic force microscopy (AFM) imaging was performed in air using a JPK Nanowizard II (JPK Instruments AG, Berlin, Germany) with tapping-mode cantilevers (40 $\text{N}\cdot\text{m}^{-1}$, Tap300-G, Budget Sensors, Bulgaria). Images were post-treated using JPK image processing software and algorithms. Prior to AFM measurement, glass substrates were immersed in isopropanol and treated with ultrasound for 15 min. A dispersion of replica particles was then placed onto the glass slide and allowed to dry under a nitrogen stream. To determine the particle concentration of the replica particle dispersions, flow cytometry analysis was performed on an Apogee A50-Micro Flow System. Cellular interactions were studied using a DeltaVision (Applied Precision, USA) microscope with a 60 $\times$ 1.52 NA oil objective for deconvolution fluorescence microscopy with a standard FITC/TRITC/CY5 filter set. Images were processed with Imaris (Bitplane) using the maximum intensity projection. Furthermore, the interaction between polymer particles and HeLa cells was studied using flow cytometry. More than 10^4 cells were counted for each experiment.

Cell Culture. HeLa cells were cultured in regular growth medium consisting of high glucose DMEM supplemented with 10% fetal bovine serum (FBS) and 1% Glutamax at 37 $^{\circ}\text{C}$, in a 5% CO_2 humidified incubator. The cells were routinely passaged until 80 – 90% confluence. Cells were trypsinized and plated into 24-well plates (6×10^4 cells per well). The cells were allowed to adhere overnight. Subsequently, the cells were incubated with replica particles for up to 24 h. After incubation, the cells were gently washed twice with DPBS before being trypsinized and investigated by flow cytometry.

Cell Fixation and Staining. HeLa cells were plated at 3×10^3 cells per well into 8-well Lab-Tek I chambered coverglass slides (Thermo Fisher Scientific, Rochester) and allowed to adhere overnight. Cells were then incubated with

Figure 1. TEM images of (A,C) ATRP initiator-functionalized MS template spheres and (B,D) polymer-grafted hybrids. (E) shows the resulting PMPC polymer replica particles after template removal.

polymer replica particles at a cell-to-particle ratio of 1:100 for 5 h (37 °C, 5% CO₂). Subsequently, cells were gently washed with PBS three times and fixed with 3% paraformaldehyde (PFA) for 10 min at 37 °C. The cell membrane was stained with Alexa Fluor 488 wheat germ agglutinin (WGA) (3 μL, 2.5 mg·mL⁻¹) incubated at room temperature for 30 min.

Cell Viability Assays. XTT assays were conducted to examine the cytotoxicity of the polymer replica particles. 1×10^4 cells per well were plated into 96-well plates. HeLa cells were then incubated with replica particles at various particle-to-cell ratios for 48 h (37 °C, 5% CO₂). The cell-to-particle ratios used were 1:10, 1:100, and 1:500. Experiments were performed in triplicates. No significant changes in cell proliferation were observed. Normalizing the viability of untreated cells as 100% indicates that the polymer

replica particles (PMPC, POEGMA or PMAA) exhibit negligible cytotoxicity.

RESULTS AND DISCUSSION

We synthesized MS particles with a diameter of ~1.1 μm and bimodal pore sizes (smaller mesopores in the 2-4 nm range and larger mesopores between 15-40 nm). Scanning electron microscopy (SEM) and N₂-physisorption measurements highlighted the porous morphology of the silica spheres (Supporting Information S1).⁴⁶ Subsequent modification with APTES yielded aminated silica spheres, which could in turn be reacted with α-bromoisobutyryl bromide to covalently attach an ATRP initiator. Thermogravimetric analysis (TGA) of the amine- and ATRP initiator-functionalized particles revealed a mass increase after each subsequent modification step (see Supporting Information S2). The

Figure 2. (A,B) AFM height and (C) phase images of the air-dried PMPC replica particles on glass slides. (D) Height profile of the replica particles along the dashed black line in (A). Z-values are 100 nm (A) and 90 nm (B).

ATRP initiator-functionalized spheres provided a homogeneous template for the modular preparation of various polymer replica particles. The polymerizations were performed as a ‘one pot reaction’ in polar or nonpolar solvents, such as ethanol, DMSO or anisole, depending on the solubility of the monomer mixture. Various monomer mixtures were polymerized to demonstrate the universal applicability of this method, resulting in polymer replica particles with different properties and functionalities (see Supporting Information S3) after template removal. However, in this study, we focused on the fabrication of replica particles that mainly consist of charge-neutral polymers, such as PMPC or POEGMA. Scheme 2 illustrates the infiltration of charge-neutral copolymers into pores via SI-ATRP. The successful ‘one pot’ polymerization can be observed in TEM images in Figure 1A and C. Before polymerization, the template spheres appeared porous, which can be seen as inhomogeneous shades of grey. After polymerization, the resulting hybrid spheres appeared homogeneously darker, indicating that the pores were filled with polymer (Figure 1B and D). Subsequent treatment of the hybrids with HF removed the silica templates, yielding replica polymer particles. The HF conditions used in our study are sufficient for effective removal of silica.²⁶ The replicas appeared much smoother after template removal (Figure 1E). TGA of the PMPC/silica hybrid particles revealed an organic material content of about 52%, including the ATRP initiator (see Supporting Information S2).

Atomic force microscopy measurements (AFM) in tapping mode were performed to study the morphology of the PMPC replica par-

ticles in the ‘dry state’ (Figure 2A). AFM highlighted the homogeneity in size of the produced polymer particles, and that the particles collapse upon drying after template removal. Figure 2B and 2C reveal the porosity in the particles, which was retained from the initial silica template, confirming that the particles are replicas. These replicas have different morphologies to hollow polymer particles, such as polymer capsules prepared using layer-by-layer template assembly. Polymer capsules exhibit creases and folds in their membrane when air-dried, indicating their hollow core.²⁴ However, this phenomenon is not observed here, which suggests that the interior is also filled with polymer, as opposed to polymer that has only been grown on the outer surface of the template spheres. However, the replica particles do spread on the glass substrate upon drying. Initially polymerized into a 1.1 μm template with a low cross-linker content of 10 mol%, the diameter of the replicas expands to about 3 μm . The height, as measured by AFM (dashed line in Figure 2A and resulting height profile 2D), drops over 90% to approximately 100 nm. This is further evidence that the silica template has been removed.

The modularity of the presented method to obtain uniform and functional polymer particles is demonstrated by post-labeling the particles with dyes after polymerization. The fluorescent tags can hereby either be seen as a model compound for drugs or targeting molecules, or simply demonstrate the accessibility of the functional moieties. Figure 3 shows fluorescence microscopy images of NHS-AF647-labeled PMPC replica particles. The copolymer used here is depicted in Scheme 2. Briefly, HEMA (3 mol%) and MPC

Figure 3. (A) Fluorescence and (B) DIC microscopy images of PMPC replica particles labeled with NHS-AF647, dispersed in Milli-Q water.

(87 mol%) were copolymerized in the presence of 10 mol% PEG₉-diacrylate as a cross-linker to produce cross-linked PMPC replica particles with pendant hydroxyl groups for further functionalization. Subsequently, the OH groups were reacted with *N*-hydroxysuccinimide (NHS)-activated molecules to form an ester bond, in this case with an AF647 dye. The DIC image (Figure 3B) shows that replica particles keep their integrity in an aqueous suspension. The size of the particles increased only marginally to about 1.2 μm , indicating the effective cross-linking during polymerization of the monomer mixture.

By choosing the appropriate monomer mixture, functional polymer particles with different charges and properties were produced (see Supporting Information S3). It was possible to introduce additional functionalities, such as amines and epoxy groups for post-functionalization. Various (meth-) acrylate-based cross-linkers could be used to stabilize the replica particles after template removal. However, the use of a disulfide-containing cross-linker allowed disassembly of the particles in a reducing environment (Supporting Information S4). PMPC particles cross-linked with only 3 mol% of bis(2-methacryloyl)oxyethyl disulfide completely disassembled in PBS buffer containing 10 mM GSH within three hours. It was further possible to vary the template size to obtain different-sized replica particles (Supporting Infor-

the relevant signals for PMPC, it does not resolve the presence of the cross-linker or OH functionality. The amount of DEGDMA (10 mol%) and HEMA (3 mol%) repeating units is presumably not high enough to be distinctively seen in NMR. The PEG-based cross-linker overlaps with the PMPC spectra at 3.6 ppm.

A straightforward modification and labeling method was of importance, as we intended to study the interaction of these particles with biological systems. Therefore, we produced three different types of replica particles, namely (A) PMPC, (B) POEGMA, and (C) poly(methacrylic acid) (PMAA), and labeled them with AF647 dyes. Their chemical structures are shown in Figure 5. (Their composition is as described in the Experimental Section). The PMAA particles were used as a control, as it is known that charged materials are readily internalized and interact with the cells *in vitro*.⁴⁷ ζ -potential measurements at pH 7.4 indicated neutral charges for both PMPC (0.2 ± 3.5 mV) and POEGMA (1.4 ± 3.4 mV) particles. In contrast, PMAA particles exhibited a negative ζ -potential (-28.3 ± 3.8 mV) at pH 7.4. The cytotoxicity of these three types of particles in human cervical cancer cells (HeLa) was investigated using a XTT cell viability assay. The results indicated that all replica particles have a negligible effect on cell viability, even at a high particle-to-cell ratio (500:1).

We further incubated all three types of the replica particles (separately) with HeLa cells at a cell-to-particle ratio of 1:100 to study their interaction with cells. Cellular internalization and association were investigated using deconvolution microscopy and flow cytometry. Whereas PMAA replicas (control) were internalized relatively quickly, the POEGMA and PMPC replicas showed very low signs of internalization. Deconvolution microscopy revealed that the cells treated with PMPC particles appeared pristine after incubation for about 5 h (Figure 5A) at 37 °C. Similar to PMPC, POEGMA particles (Figure 5B) were internalized to a lesser extent than PMAA particles (Figure 5C) after 5 h at 37 °C. The white arrows in Figure 5 indicate the respective fluorescently-labeled (red) polymer particles. Cell membranes were fixed and stained

Figure 4. ¹H NMR of the PMPC replica particle dispersion in D₂O.

mation S5).

A ¹H NMR of non-labeled PMPC replicas in D₂O is shown in Figure 4. While the NMR shows

Figure 5. (A-C) Representative deconvolution microscopy images of HeLa cells treated with AF647 fluorescently-labeled replica particles (A) PMPC, (B) POEGMA, or (C) PMAA at cell-to-particle ratios of 1:100 for 5 h. Cell membranes were stained using WGA488. (D-F) Quantitative flow cytometry analysis (number of cells versus fluorescence intensity (logarithmic scale)) of cells incubated with (a) PMPC, (b) POEGMA, or (c) PMAA replica particles, after 24 h incubation at 37 °C at cell-to-particle ratios of 1:100. At least 1×10^4 cells were counted per measurement. (G-I) Representative copolymer structures used (OH group was reacted with NHS-AF647 prior to incubation with cells).

(green) prior to the measurements. Additional microscopy images are given in Supporting Information S6.

Flow cytometry experiments underlined the observations made with deconvolution microscopy, and was subsequently used to quantify HeLa cell association of the replica particles (Figure 5D-F). More than 1×10^4 cells (performed in triplicates) were counted after being incubated with AF647-labeled polymer particles at a cell-to-particle ratio of 1:100 for 24 h at 37 °C. Compared to the PMAA particles (control, Figure 5F), POEGMA (Figure 5E) and PMPC (Figure 5D) particles showed about 15 times lower association to cancer cells. The association with HeLa cells was 25 times lower compared to previously reported replica particles made from PMAA-derived polymers, such as PMA_{SH}, which showed 95% cell association after 24h (at comparable reaction conditions).⁴⁸ The remarkably low interaction of charge-neutral polymer replica particles with cancer cells render them promising candidates for targeted drug delivery ap-

plications, where a low background interaction of the pristine carrier systems is desired.

CONCLUSIONS

Different copolymer replica particles were prepared utilizing surface-initiated ATRP in MS templates followed by subsequent template removal. The modular approach describes a rational and versatile method for the fabrication of hydrophilic polymer particles with control over physicochemical properties, size, and functionality. Utilizing redox-responsive crosslinkers allowed the disassembly of particles in a reducing environment. The low association of the charge-neutral replica particles to cancer cells makes these replica particles promising candidates in drug delivery applications. Future studies will include the utilization of these particles in drug delivery, using cargoes and targeting molecules to investigate their potential in biomedical applications.

ASSOCIATED CONTENT

Supporting Information. Additional experimental data containing a SEM image and pore size distribution of MS template particles, TGA analysis of amine- and ATRP-modified MS particles, as well as PMPC-grafted hybrid particles. Furthermore, an overview of various copolymer structures of copolymer replica particles, including a degradation profile of redox-responsive replica PMPC particles measured via flow cytometry, a fluorescence microscopy image of 500 nm-sized PMPC replica particles, and additional deconvolution microscopy images of the cell studies. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

AUTHOR INFORMATION

Corresponding Author

* Fax: +61 3 8344 4153; Tel: +61 3 8344 3461; E-mail: fcaruso@unimelb.edu.au.

Author Contributions

All authors have given approval to the final version of the manuscript.

Funding Sources

This work was supported by the Australian Research Council under the Australian Laureate Fellowship (F.C., 120100030) and the Super Science Fellowship (F.C., FS10200025) schemes.

ACKNOWLEDGMENT

M.M. acknowledges The University of Melbourne for a McKenzie Fellowship. J.C. is the recipient of an Australian Research Council Super Science Fellowship (FS10200025).

REFERENCES

- (1) Petros, R. A.; DeSimone, J. M. Strategies in the Design of Nanoparticles for Therapeutic Applications. *Nat. Rev. Drug. Discov.* **2010**, *9*, 615-627.
- (2) LaVan, D. A.; McGuire, T.; Langer, R. Small-Scale Systems for In vivo Drug Delivery. *Nat. Biotechnol.* **2003**, *21*, 1184-1191.
- (3) Farokhzad, O. C.; Langer, R. Impact of Nanotechnology on Drug Delivery. *ACS Nano* **2009**, *3*, 16-20.
- (4) Wang, A. Z.; Langer, R.; Farokhzad, O. C. Nanoparticle Delivery of Cancer Drugs. *Annu. Rev. Med.* **2012**, *63*, 185-198.
- (5) Khemtong, C.; Kessinger, C. W.; Gao, J. Polymeric Nanomedicine for Cancer MR Imaging and Drug Delivery. *Chem. Commun.* **2009**, 3497-3510.
- (6) Ariga, K.; Lvov, Y. M.; Kawakami, K.; Ji, Q.; Hill, J. P. Layer-by-Layer Self-Assembled Shells for Drug Delivery. *Adv. Drug Deliv. Rev.* **2011**, *63*, 762-771.
- (7) Ruiz-Hitzky, E.; Darder, M.; Aranda, P.; Ariga, K. Advances in Biomimetic and Nanostructured Biohybrid Materials. *Adv. Mater.* **2010**, *22*, 323-336.
- (8) Euliss, L. E.; DuPont, J. A.; Gratton, S.; DeSimone, J. Imparting Size, Shape, and Composition Control of Materials for Nanomedicine. *Chem. Soc. Rev.* **2006**, *35*, 1095-1104.
- (9) Wang, Y.; Byrne, J. D.; Napier, M. E.; DeSimone, J. M. Engineering Nanomedicines Using Stimuli-Responsive Biomaterials. *Adv. Drug Deliv. Rev.* **2012**, *64*, 1021-1030.
- (10) Sutton, D.; Nasongkla, N.; Blanco, E.; Gao, J. Micellar Systems for Cancer Targeted Drug Delivery. *Pharm. Res.* **2007**, *24*, 1029-1046.
- (11) De Oliveira, H.; Thevenot, J.; Lecommandoux, S. Smart Polymersomes for Therapy and Diagnosis: Fast Progress toward Multifunctional Biomimetic Nanomedicines. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2012**, *4*, 525-546.
- (12) Bae, Y.; Kataoka, K. Intelligent Polymeric Micelles from Functional Poly(ethylene glycol)-Poly(amino acid) Block Copolymers. *Adv. Drug Deliv. Rev.* **2009**, *61*, 768-784.
- (13) Astruc, D.; Boisselier, E.; Ornelas, C. Dendrimers Designed for Functions: From Physical, Photophysical, and Supramolecular Properties to Applications in Sensing, Catalysis, Molecular Electronics, Photonics, and Nanomedicine. *Chem. Rev.* **2010**, *110*, 1857-1959.
- (14) Cheng, Y.; Zhao, L.; Li, Y.; Xu, T. Design of Biocompatible Dendrimers for Cancer Diagnosis and Therapy: Current Status and Future Perspectives. *Chem. Soc. Rev.* **2011**, *40*, 2673-2703.
- (15) Städler, B.; Price, A. D.; Zelikin, A. N. Critical Look at Multilayered Polymer Capsules in Biomedicine: Drug Carriers, Artificial Organelles, and Cell Mimics. *Adv. Funct. Mater.* **2011**, *21*, 14-28.
- (16) Delcea, M.; Möhwald, H.; Skirtach, A. G. Stimuli-Responsive LbL Capsules and Nanoshells for Drug Delivery. *Adv. Drug Deliv. Rev.* **2011**, *63*, 730-747.
- (17) Zhang, Z.; Chao, T.; Chen, S.; Jiang, S. Superlow Fouling Sulfobetaine and Carboxybetaine Polymers on Glass Slides. *Langmuir* **2006**, *22*, 10072-10077.
- (18) Zhang, Z.; Finlay, J. A.; Wang, L.; Gao, Y.; Callow, J. A.; Callow, M. E.; Jiang, S. Polysulfobetaine-Grafted Surfaces as Environmentally Benign Ultralow Fouling Marine Coatings. *Langmuir* **2009**, *25*, 13516-13521.
- (19) Lutz, J.-F. Polymerization of Oligo(ethylene glycol) (meth)acrylates: Toward New Generations of Smart Biocompatible Materials. *J. Polym. Sci. Part A: Polym. Chem.* **2008**, *46*, 3459-3470.
- (20) Ladd, J.; Zhang, Z.; Chen, S.; Hower, J. C.; Jiang, S. Zwitterionic Polymers Exhibiting High Resistance to Nonspecific Protein Adsorption from Human Serum and Plasma. *Biomacromolecules* **2008**, *9*, 1357-1361.
- (21) Cao, Z.; Jiang, S. Super-Hydrophilic Zwitterionic Poly(carboxybetaine) and Amphiphilic Non-ionic Poly(ethylene glycol) for Stealth Nanoparticles. *Nano Today* **2012**, *7*, 404-413.
- (22) Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S. Poly(ethylene glycol) in Drug Delivery: Pros and Cons as Well as Potential Alternatives. *Angew. Chem. Int. Ed.* **2010**, *49*, 6288-6308.
- (23) Wang, Y.; Yu, A.; Caruso, F. Nanoporous Polyelectrolyte Spheres Prepared by Sequentially Coating Sacrificial Mesoporous Silica Spheres. *Angew. Chem. Int. Ed.* **2005**, *44*, 2888-2892.
- (24) Best, J. P.; Cui, J.; Müllner, M.; Caruso, F. Tuning the Mechanical Properties of Nanoporous Hydrogel Particles via Polymer Cross-Linking. *Langmuir* **2013**, *29*, 9824-9831.
- (25) Cui, J.; Yan, Y.; Wang, Y.; Caruso, F. Templated Assembly of pH-Labile Polymer-Drug Particles for Intracellular Drug Delivery. *Adv. Funct. Mater.* **2012**, *22*, 4718-4723.
- (26) Cui, J.; Wang, Y.; Hao, J.; Caruso, F. Mesoporous Silica-Templated Assembly of Luminescent Polyester Particles. *Chem. Mater.* **2009**, *21*, 4310-4315.

- (27) Mertz, D.; Tan, P.; Wang, Y.; Goh, T. K.; Blencowe, A.; Caruso, F. Bromoisobutyramide as an Intermolecular Surface Binder for the Preparation of Free-standing Biopolymer Assemblies. *Adv. Mater.* **2011**, *23*, 5668-5673.
- (28) Zhang, X.; Oulad-Abdelghani, M.; Zelkin, A. N.; Wang, Y.; Haikel, Y.; Mainard, D.; Voegel, J.-C.; Caruso, F.; Benkirane-Jessel, N. Poly(l-lysine) Nanostructured Particles for Gene Delivery and Hormone Stimulation. *Biomaterials* **2010**, *31*, 1699-1706.
- (29) Tan, J.; Wang, Y.; Yip, X.; Glynn, F.; Shepherd, R. K.; Caruso, F. Nanoporous Peptide Particles for Encapsulating and Releasing Neurotrophic Factors in an Animal Model of Neurodegeneration. *Adv. Mater.* **2012**, *24*, 3362-3366.
- (30) Wang, Y.; Caruso, F. Nanoporous Protein Particles through Templating Mesoporous Silica Spheres. *Adv. Mater.* **2006**, *18*, 795-800.
- (31) Best, J. P.; Neubauer, M. P.; Javed, S.; Dam, H. H.; Fery, A.; Caruso, F. Mechanics of pH-Responsive Hydrogel Capsules. *Langmuir* **2013**, *29*, 9814-9823.
- (32) Yap, H. P.; Johnston, A. P. R.; Such, G. K.; Yan, Y.; Caruso, F. Click-Engineered, Bioresponsive, Drug-Loaded PEG Spheres. *Adv. Mater.* **2009**, *21*, 4348-4352.
- (33) Jang, J.; Lim, B.; Choi, M. A Simple Synthesis of Mesoporous Carbons with Tunable Mesopores Using a Colloidal Template-Mediated Vapor Deposition Polymerization. *Chem. Commun.* **2005**, 4214-4216.
- (34) Ji, X.; Hampsey, J. E.; Hu, Q.; He, J.; Yang, Z.; Lu, Y. Mesoporous Silica-Reinforced Polymer Nanocomposites. *Chem. Mater.* **2003**, *15*, 3656-3662.
- (35) Yang, J.; Shen, D.; Zhou, L.; Li, W.; Li, X.; Yao, C.; Wang, R.; El-Toni, A. M.; Zhang, F.; Zhao, D. Spatially Confined Fabrication of Core-Shell Gold Nanocages@Mesoporous Silica for Near-Infrared Controlled Photothermal Drug Release. *Chem. Mater.* **2013**, *25*, 3030-3037.
- (36) Chung, P.-W.; Kumar, R.; Pruski, M.; Lin, V. S. Y. Temperature Responsive Solution Partition of Organic-Inorganic Hybrid Poly(*N*-isopropylacrylamide)-Coated Mesoporous Silica Nanospheres. *Adv. Funct. Mater.* **2008**, *18*, 1390-1398.
- (37) Choi, M.; Kleitz, F.; Liu, D.; Lee, H. Y.; Ahn, W.-S.; Ryoo, R. Controlled Polymerization in Mesoporous Silica toward the Design of Organic-Inorganic Composite Nanoporous Materials. *J. Am. Chem. Soc.* **2005**, *127*, 1924-1932.
- (38) Blas, H.; Save, M.; Boissière, C.; Sanchez, C.; Charleux, B. Surface-Initiated Nitroxide-Mediated Polymerization from Ordered Mesoporous Silica. *Macromolecules* **2011**, *44*, 2577-2588.
- (39) Save, M.; Granvorka, G.; Bernard, J.; Charleux, B.; Boissière, C.; Grosso, D.; Sanchez, C. Atom Transfer Radical Polymerization of Styrene and Methyl Methacrylate from Mesoporous Ordered Silica Particles. *Macromol. Rapid Commun.* **2006**, *27*, 393-398.
- (40) Zhou, Z.; Zhu, S.; Zhang, D. Grafting of Thermo-Responsive Polymer inside Mesoporous Silica with Large Pore Size Using ATRP and Investigation of its Use in Drug Release. *J. Mater. Chem.* **2007**, *17*, 2428-2433.
- (41) Kruk, M.; Dufour, B.; Celer, E. B.; Kowalewski, T.; Jaroniec, M.; Matyjaszewski, K. Synthesis of Mesoporous Carbons Using Ordered and Disordered Mesoporous Silica Templates and Polyacrylonitrile as Carbon Precursor. *J. Phys. Chem. B* **2005**, *109*, 9216-9225.
- (42) Pasetto, P.; Blas, H.; Audouin, F.; Boissière, C.; Sanchez, C.; Save, M.; Charleux, B. Mechanistic Insight into Surface-Initiated Polymerization of Methyl Methacrylate and Styrene via ATRP from Ordered Mesoporous Silica Particles. *Macromolecules* **2009**, *42*, 5983-5995.
- (43) Audouin, F.; Blas, H.; Pasetto, P.; Beaunier, P.; Boissière, C.; Sanchez, C.; Save, M.; Charleux, B. Structured Hybrid Nanoparticles via Surface-Initiated ATRP of Methyl Methacrylate from Ordered Mesoporous Silica. *Macromol. Rapid Commun.* **2008**, *29*, 914-921.
- (44) Banerjee, S.; Paira, T. K.; Kotal, A.; Mandal, T. K. Surface-Confined Atom Transfer Radical Polymerization from Sacrificial Mesoporous Silica Nanospheres for Preparing Mesoporous Polymer/Carbon Nanospheres with Faithful Shape Replication: Functional Mesoporous Materials. *Adv. Funct. Mater.* **2012**, *22*, 4751-4762.
- (45) Kuroda, K.; DeGrado, W. F. Amphiphilic Polymethacrylate Derivatives as Antimicrobial Agents. *J. Am. Chem. Soc.* **2005**, *127*, 4128-4129.
- (46) Wang, J.-G.; Zhou, H.-J.; Sun, P.-C.; Ding, D.-T.; Chen, T.-H. Hollow Carved Single-Crystal Mesoporous Silica Templated by Mesomorphous Polyelectrolyte-Surfactant Complexes. *Chem. Mater.* **2010**, *22*, 3829-3831.
- (47) Yan, Y.; Wang, Y.; Heath, J. K.; Nice, E. C.; Caruso, F. Cellular Association and Cargo Release of Redox-Responsive Polymer Capsules Mediated by Exofacial Thiols. *Adv. Mater.* **2011**, *23*, 3916-3921.
- (48) Yan, Y.; Lai, Z. W.; Goode, R. J. A.; Cui, J.; Bacic, T.; Kamphuis, M. M. J.; Nice, E. C.; Caruso, F. Particles on the Move: Intracellular Trafficking and Asymmetric Mitotic Partitioning of Nanoporous Polymer Particles. *ACS Nano* **2013**, *7*, 5558-5567.

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Muellner, M;Cui, J;Noi, KF;Gunawan, ST;Caruso, F

Title:

Surface-Initiated Polymerization within Mesoporous Silica Spheres for the Modular Design of Charge-Neutral Polymer Particles

Date:

2014-06-03

Citation:

Muellner, M., Cui, J., Noi, K. F., Gunawan, S. T. & Caruso, F. (2014). Surface-Initiated Polymerization within Mesoporous Silica Spheres for the Modular Design of Charge-Neutral Polymer Particles. *LANGMUIR*, 30 (21), pp.6286-6293. <https://doi.org/10.1021/la501324r>.

Persistent Link:

<http://hdl.handle.net/11343/123262>