Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2015

Electronic Supplementary Information

Fabrication of stimulus-responsive diatom silica microcapsules for antibiotic drug delivery

Roshan B. Vasani[a], Dusan Losic[b], Alex Cavallaro Nicolas H. Voelcker*[a]

[a] ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Mawson Institute, University of South Australia, SA 5095, Australia

[b] School of Chemical Engineering, The University of Adelaide, SA 5000, Australia

E-mail: nico.voelcker@unisa.edu.au

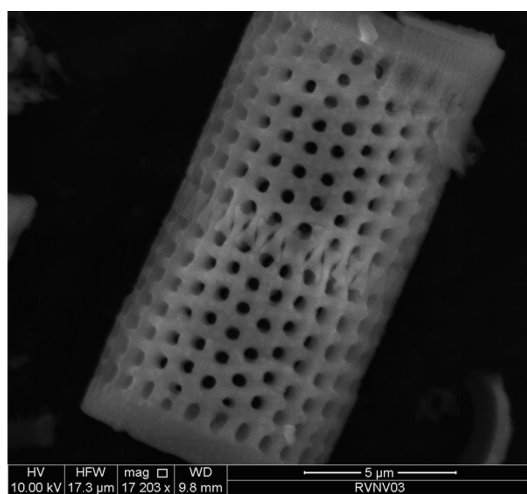

Fig. S1 SEM image of an *Aulacoseria sp*. diatom frustule.

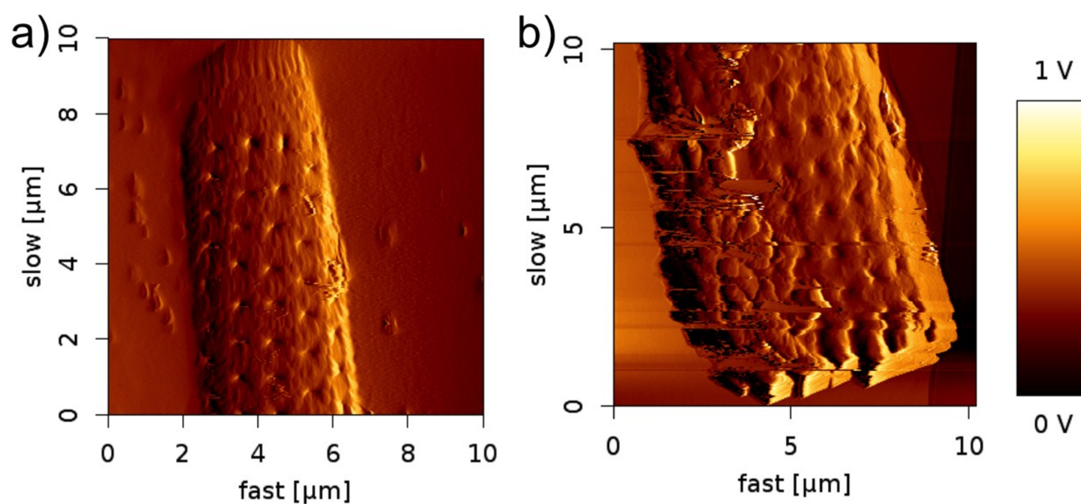

Fig. S2 AFM images (error signal) of the a) unmodified diatom microcapsules and b) the diatom microcapsules after P1 modification.

Experimental

Materials

The DE rocks composed of fossilised fresh water *Aulacoseria sp.* diatom microcapsules were obtained from Mount Sylvia Pty. Ltd. Sulfuric acid, hydrogen peroxide and THF were purchased from ChemSupply, triethyl amine (TEA) was purchased from Univar. All other chemicals used in this study were purchased from Sigma Aldrich.

Processing of fossilised diatom microcapsules

The fossilised diatomaceous earth was processed as previously to obtain individual unfractured microcapsules.[1] The DE rocks were broken down to the microcapsules using a series of steps such as, crushing the rocks with a pulveriser, removal of large aggregrates of diatoms, acid treatment (1 M sulfuric acid) for the removal of oxides and other impurities and finally, partical sizing by filtration and sedimentation.

Synthesis of ATRP initiator

The silane-based initiator of ATRP polymerisation, 3-(2-bromoisobutyramido)propyl(triethoxy)silane (BiB-APTES), was synthesised using a previously published protocol.[2] Briefly, 3-aminopropyltriethoxysilane (APTES) (20 mmol, 4.7 mL) was added to a mixture of tetrahydrofuran (25 mL) and triethylamine (4 mL) and cooled to 0 °C in an ice bath with constant stirring. α-bromoisobutyryl bromide (25 mmol, 3.15 mL) was then added drop wise to the reaction mixture. The reaction was allowed to warm to room temperature and stirred overnight. The product was filtered twice to remove the TEA-Br salt produced and the THF and TEA were evapourated under vacuum. No further purification was carried out. 1HNMR (300MHz, CDCl3, δ): 0.65 (t, 2H), 1.21 (t, H), 1.64 (quin, 2H), 1.93 (s, 6H), 3.26 (q, 2H), 3.79 (q, 6H), 6.93 (s, 1H). 13CNMR (75MHz, CDCl3, δ): 7.59, 18.31, 22.71, 32.59, 42.58, 58.45, 62.99, 171.85.

Silanisation of diatom microcapsules

Prior to silanisation, the diatom microcapsules were immersed in a Piranha solution (3:1 H_2SO_4:H_2O_2) for 1 hour in order to generate hydroxyl groups on the silica surface as well as to clean off any residual organic matter or other impurities. The microcapsules were removed from the Piranha solution via centrifugation (3000 rpm for 1 min), washed with MQ (18 MΩ) water 3 times and collected by centrifugation. A small portion of the diatoms was separated and dried in air for SEM and FTIR characterisation. Following this, an aqueous silanisation procedure was performed using a previously reported protocol.[3] Briefly, the diatoms were immersed in 5 mL of water acidified using 10 µL of glacial acetic acid. To this mixture, 500 µL of BiB-APTES was added and the entire solution was sonicated for 2 min to facilitate dispersion. The reaction was allowed to proceed for 48 hours under constant agitation

on an orbital shaker. Once complete, the silanised diatoms were collected out of the reaction mixture by centrifugation and washed 3 times with ethanol to remove any unbound BiB-APTES and dried at room temperature overnight.

SI-ATRP procedure

Activators regenerated by electron transfer based atom transfer radical polymerisation (ARGET-ATRP) was used to generate copolymers of $O(EG)_2MA$ and $O(EG)_{4-5}MA$ in solution. For this reaction, a mixture of water and methanol was used as solvent, with a $CuBr_2$/bipyridyl catalyst system and ascorbic acid as reducing agent. 10 uL of ethylene bromoisobutyrate (EBiB) and was taken in a glass vial 5 mL of 25% MEOH in MQ (18 MΩ) water was added to it. Following this, $O(EG)_2MA$ or $O(EG)_{4-5}MA$ monomers were added to the solution either separately or at a molar ratio of 1:0.16 respectively, to obtain a 0.5 M monomer solution. The molar ratios of monomer to catalyst to reducing agent used were 100/0.01/0.20. Polymerisation reactions were carried out for 1 hour at 25 °C under constant Ar gas bubbling. Once the reaction was complete, the reaction mix was mixed with 10 mL of THF and passed through a column of neutral alumina to remove the catalyst. The polymer chains were then precipitated in diethyl ether.

The polymers were grafted from the diatom microcapsules using a similar procedure. 20 mg of the initiator-modified microcapsules were placed in a vial and 20 mL of the above reaction mixture. The polymerisation was allowed to proceed for 1 hour at 25 °C. Following polymerisation, the microcapsules were centrifuged for separation from the reaction mix and washed 3 times with ethanol. They were dried at room temperature overnight.

Levofloxacin (LVX) loading into microcapsules

LVX was loaded into the diatom microcapsules using a process known as vacuum infiltration. For this method, a known weight of diatom microcapsules immersed in a 100 mg/mL solution of LVX in DMF in a sealed vial, the solution was then frozen in liquid nitrogen and the vial was evacuated by using a pump for 10 minutes. Following this, the solution was allowed to warm back to room temperature. Once at room temperature, the vial was quickly brought back to atmospheric pressure. The microcapsules were collected by centrifugation, transferred to a vial filled with Milli-Q water and incubated for 30 min. They were then separated from the water by centrifugation, rinsed quickly with acetone and dried at 60 °C prior to use. Total loading was found to be 1.53 μg/mg of microcapsules in the case of the samples and approximately 14.76 μg/mg in the case of the controls.

Cloud point measurements of the polymers

Cloud point measurements were performed using an Agilent technologies 8452 UV-vis Chemstation. Spectra were measured at temperature changes of 0.5 °C for both the heating and cooling cycles. The cloud point curves were plotted using the intensity of the spectra at 600 nm wavelength vs. temperature.

Fourier Transform Infrared measurements

The IR spectra of the microcapsules were characterised after each functionalisation step using a Nicolet iN10 (Thermo Scientific) coupled to a liquid nitrogen cooled mercury-cadmium-telluride (MCT) detector in transmission mode. Spectra were measured in the range of 600 – 4000 cm^{-1} and a total of 64 scans were averaged at a resolution of 4 nm.

Scanning electron microscopy (SEM) analysis

SEM analysis was performed on a Quanta 450 FEG Environmental SEM (FEI, Netherlands) fitted with solid state detector. The microcapsules were coated with a thin (5 nm) layer of platinum to prevent charging. A spot size of 3 mm and an accelerating voltage of 10 kV were used to obtain the images.

Atomic force microscopy (AFM) analysis

AFM imaging was performed on a JPK NanoWizard III (JPK Instruments, Berlin, Germany). Images were recorded in intermittent contact mode using an App Nano ACT probe cantilever with a frequency of 200 – 400 KHz and spring constant 25 – 75 N/m. A two-component epoxy glue was used to affix the diatom microcapsules onto glass coverslips and the imaging was performed in air.

Size exclusion chromatography (SEC)

Measurements of the real molecular weights of polymers synthesised in solution were performed on a Viscotek GPCmax VE2001 GPC coupled to a Viscotek TDA 305 triple detector array system (Malvern Instruments Ltd, UK) with refractive index, viscometry and light scattering detectors. The detectors were calibrated using a 70 kDa polystyrene standard with tetrahydrofuran (THF) as the eluent. Two Agilent PLgel 5 μm mixed C columns were used for the analysis.

Drug release studies

The release of LVX from the microcapsules was measured by immersing a known mass (0.5 – 1 mg) of loaded particles into phosphate buffered saline (PBS) buffer (pH 7.4; 137 mM NaCl, 2.7 mM KCl and 10 mM phosphate) at either 25 °C or 45 °C and measuring the fluorescence signal of LVX in the solution. Fluorescence measurements were performed on an Agilent Technologies Cary Eclipse fluorescence spectrometer fitted with a Peltier temperature control system using an excitation wavelength of 292 nm and an emission wavelength of 454 nm.

Zone of Inhibition studies (Kirby-Bauer test)

Drug release measurements were performed by incubating 1 mg of loaded P1-modified diatoms in 1 mL of PBS at 25 °C or 45 °C. At the desired time point, the solutions were centrifuged to remove the diatoms and then stored in the dark at 4 °C. *S. aureus* ATCC 4330 and *P. aeruginosa* ATCC 27853 isolates from human wounds were used for these experiments. The bacteria were inoculated onto agar plates and incubated overnight at 37 °C. Single colonies from the plates were then transferred into 5 mL of Tryptic Soya Broth (TSB) and left in the incubator overnight at 37 °C. Following this, 2 mL of 1 x 10^{-7} cfu/mL solutions of the bacteria were prepared and 100 µL of the solution was transferred to agar plates and spread uniformly. Five Kirby-Bauer discs (6 mm, Whatman®) were placed on each plate and 50 µL of the release solution was pipetted onto each of the discs and the plates were incubated overnight. The tests were repeated 3 times for each specimen.

References

1. Y. Yu, J. Addai-Mensah and D. Losic, *Langmuir*, 2010, **26**, 14068-14072.
2. R. B. Vasani, S. J. P. McInnes, M. A. Cole, A. M. M. Jani, A. V. Ellis and N. H. Voelcker, *Langmuir*, 2011, **27**, 7843-7853.
3. N. L. Rosi, C. S. Thaxton and C. A. Mirkin, *Angew. Chem. Int. Ed.*, 2004, **43**, 5500-5503.