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<th><strong>Title</strong></th>
<th>Synthesis of polymer-silica hybrid microparticles with defined geometry using surface initiated atom transfer radical polymerization</th>
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<td><strong>Author(s)</strong></td>
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<tr>
<td><strong>Publication Date</strong></td>
<td>2015</td>
</tr>
<tr>
<td><strong>Publisher</strong></td>
<td>Royal Society of Chemistry</td>
</tr>
<tr>
<td><strong>Link to publisher's version</strong></td>
<td><a href="http://dx.doi.org/10.1039/c5py00228a">http://dx.doi.org/10.1039/c5py00228a</a></td>
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<tr>
<td><strong>Item record</strong></td>
<td><a href="http://hdl.handle.net/10379/5510">http://hdl.handle.net/10379/5510</a></td>
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<td><strong>DOI</strong></td>
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Nanostructured polymer/silica hybrid materials have gained attention as they combine the advantageous properties of organic and inorganic materials in one entity. Herein, we report incorporation of chloromethyl moieties into a living diatom to prepare an alkyl-halide activated siliceous biotemplate. Subsequent, in situ polymerization via surface initiated atom transfer radical polymerization generated a polymer/silica microparticle with defined geometry.

The in situ growth of polymers from a surface, referred to as ‘grafting from’, may occur through a number of different chemical reactions including surface initiated atom transfer radical polymerizations (SI-ATRP). In SI-ATRP an initiator molecule is attached to a surface, thus enabling growth of a polymer from that surface. Advantages of SI-ATRP include the ability to control the thickness of the polymer coating on the surface,\(^1\) and the ability to design the polymer composition having properties such as sensitivity to pH or temperature.\(^2\)

Silica particles have been used as a substrate for growth of polymers from a surface via SI-ATRP,\(^3\) with both planar and colloidal substrates explored.\(^1\) However, the use of SI-ATRP for in situ polymerization on more intricate templates, including biotemplates such as diatoms, is underexplored.\(^5\)

We have previously reported on the preparation of polymer-silica composites employing the diatom Thalassiosira weissflogii as a substrate for ‘grafting from’ via deactivation enhanced-ATRP.\(^6\) This approach involved attachment of an initiator reactive centre onto the surface of a cleaned diatom prior to ‘grafting from’ the diatom. The introduction of the initiator onto a template post-synthesis can be affected by steric hindrance,\(^7\) thus incorporation of the initiator during template synthesis has been described.\(^8\) Herein, we report on the integration of an initiator into the diatom T. weissflogii during cultivation, and subsequent in situ polymer growth from the activated diatom surface via SI-ATRP. We have previously reported on the functionalization of the living diatom T. weissflogii via co-incubation with an alkoxysilane-organohaloalkoxysilane solution.\(^9\) A similar strategy was employed to incorporate an initiator reactive centre into the cell wall, termed the frustule, of T. weissflogii. SI-ATRP initiator sites are typically alkyl halide moieties and include alkyl-bromo groups\(^10\) and alkyl-chloro groups.\(^11\) (Chloromethyl)triethoxysilane was the organoalkoxysilane chosen for functionalization of the diatom with an alkyl-halide group as it provides the alkyl-chloro moiety required for SI-ATRP. Incorporation of chloromethyl moieties into the frustules of T. weissflogii was achieved by addition of a tetraethoxysilane-(chloromethyl)triethoxysilane (TEOS–CTEOS) solution to diatom cultures at 48 hour intervals. The hydrolysis of the TEOS–CTEOS (Scheme 1) in the culture media generates free silicic acid and hydrolyzed CTEOS, providing the building blocks for the siloxane backbone of the diatom frustules and the chloromethyl moiety respectively (Scheme 1).

Synchronized T. weissflogii cultures were inoculated in artificial seawater media enriched with F/2 Guillards media at 1 \(\times\) 10\(^5\) cells mL\(^{-1}\) in a final volume of 200 ml. Cultures received TEOS and CTEOS at a ratio of 3 TEOS : 1 CTEOS such that the silicic acid concentration in culture was 200 \(\mu\)M at each addition. Cultures received TEOS–CTEOS precursors at time of

\[ \text{Si-OH + H}_2\text{O} \rightarrow \text{Si-O-Si} + \text{H}_2\text{O} \]

**Scheme 1** Hydrolysis of TEOS and CTEOS generates ethanol.

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\(^4\)Electronic supplementary information (ESI) available: Protocols for; (i) incorporation of alkyl-halide initiator moiety into the frustule of T. weissflogii, (ii) characterization of TEOS/CTEOS T. weissflogii by EDX-SEM and TEM, (iii) investigating the effect of ethanol on the growth profile of T. weissflogii. See DOI: 10.1039/c3py00228a
inoculation and at 48 hour intervals until time of collection at 192 hours post inoculation.

Control cultures received Na$_2$SiO$_3$ at a final concentration of 200 µM at each dosing time-point. Cell density was determined using a haemocytometer. TEOS–CTEOS was shown to support diatom growth (Fig. 1a).

Increased growth rates compared to Na$_2$SiO$_3$ control cultures were observed between 96–192 hours post inoculation. This enhancement of growth rate in the presence of alkoxysilanes–organoaalkoxysilane solution has been demonstrated previously. Furthermore, it is possible that alcohol generated from the hydrolysis of TEOS–CTEOS (Scheme 1) may contribute to the increased growth. It has been reported previously that ethanol concentrations ranging from 34–51 mM stimulating the growth of *T. weissflogii* over a 96 hour period. The addition of ethanol to *T. weissflogii* cultures grown in the presence of Na$_2$SiO$_3$ brings about an increased growth compared to control cultures in the interval 144–192 hours post inoculation (Fig. S1f).

The hallmark feature of diatoms is their species specific architecture. There are estimated to be between 10 000–100 000 species of diatom, each with their unique intricate frustule. The complexity and the precision with which the frustule is synthesized, at both the micro- and nano-scale, has attracted attention from many disciplines interested in the production of nanostructured materials, with proposed applications in catalysis, separation science, filtration, nanotechnology, and drug delivery. The effect of incorporating the chloromethyl moiety into the frustule of *T. weissflogii* on the characteristic architecture of the diatom was evaluated to ensure that there was no adverse effect of chemical modification. Cleaned diatoms were characterized by energy dispersive X-ray spectroscopy coupled to scanning electron microscopy (EDX-SEM) performed using Hitachi S-4700 SEM with INCA® software, and transmission electron microscopy (TEM) using Hitachi H-7500 TEM with AMT image capture software. Architectural features related to the pores present on the frustules (perimeter, width, length and area) were quantified using ImageJ software. The mean pore parameters were calculated as follows; five sections per diatom were analyzed to calculate the mean pore parameters per diatom, three separate diatoms were analyzed to calculate the mean pore parameters per culture, three separate cultures were analyzed to calculate the mean pore parameters for both *T. weissflogii* and TEOS–CTEOS *T. weissflogii*. GraphPad Prism® software was used to perform statistical analysis.

The architecture of the diatom is unaltered following modification with TEOS–CTEOS (Fig. 1b). The native *T. weissflogii* frustule yielded mean values of 122 ± 8 nm, 32 ± 1 nm, 32 ± 1 nm, and 722 ± 22 nm$^2$ for perimeter, width, length and area respectively. These values were similar to those for TEOS–CTEOS *T. weissflogii*; 135 ± 1 nm, 33 ± 1 nm, 33 ± 1 nm, and 714 ± 65 nm$^2$ for perimeter, width, length and area respectively. Representative TEM micrographs of *T. weissflogii* and TEOS–CTEOS *T. weissflogii* are shown in Fig. 1c and d, illustrating the characteristic cob-web like feature on the face of the diatom with the underlying pores.

The motivation for culturing *T. weissflogii* in the presence of TEOS–CTEOS was to introduce an alkyl-halide moiety into the frustules to serve as an initiator to allow growth of polymer from the surface of the diatom via SI-ATRP. Methylmethacrylate (MMA) and disulfide diacrylate (DS) were chosen as monomers in this study to fabricate a polymer with both lipophilicity and degradability respectively. Furthermore, the choice of monomers was based on the ability of MMA to undergo cross-linking reactions via the vinyl moieties and the introduction of a cleavable disulfide bond using DS. Methyl methacrylate, disulfide diacrylate, bis-[2-dimethylaminoethyl] methyamine, and copper(II) chloride were added to a round bottom flask at a mole ratio of 4 : 1 : 0.0125 : 0.025. Methyl ethyl ketone was added such that concentration of methyl methacrylate was approx 0.03 mg ml$^{-1}$. The solution was purged with argon gas for 20 minutes. TEOS–CTEOS *T. weissflogii* in a methyl ethyl ketone was added to the round-bottom flask such that the weight ratio of TEOS–CTEOS *T. weissflogii* : methyl methacrylate was 1 : 40. L-Ascorbic acid was prepared in de-ionized water and purged with argon for 20 minutes and added to the reaction vessel at a mole ratio of 0.0125 L-ascorbic acid : 4 methyl methacrylate. The reaction vessel was sealed.
and the reaction proceeded for 96 hours at 37 °C with constant rocking. A trial reaction conducted at 60 °C resulted in the formation of a gel in the solution within 3 hours. Thus, the reaction temperature was reduced to 37 °C and the reaction time increased to 96 hours.

SEM micrographs of TEOS–CTEOS T. weissflogii pre- and post-grafting are depicted in Fig. 2. The characteristic architectural features of the cleaned frustule are visible pre-coating (Fig. 2a and b). Following growth of MMA-co-DS polymer from the surface of the cleaned frustule there is a visible coating (Fig. 2c and d).

Growth of MMA-co-DS polymer from the surface of the diatom was monitored by thermogravimetric analysis (TGA) and Fourier transform infrared spectroscopy (FTIR). TGA was performed on samples dried at 60 °C for 48 hours using a Rheometric Scientific STA 625 thermal analyzer with Orches-trator software. Samples were heated from ambient temperature to 600 °C at a heating rate of 10 °C min⁻¹ under oxygen-free nitrogen at a flow rate of 50 ml min⁻¹. FTIR spectra were collected using a Shimadzu FTIR-8300 in transmittance mode at a resolution of 4 cm⁻¹ with Happ-genzel apodization. Twenty scans were collected per sample and data processed using Shimadzu IR™ solution software.

Grafting of MMA-co-DS from the diatom increased the organic content from 38% to 79% weight percentage (Fig. 3a). This result indicates that growth of the polymer from the surface occurred. FTIR spectra of the diatom post-coating revealed the emergence of additional peaks in the fingerprint region of the spectrum, representing various C–C and C=O bonds. In particular there is peak at 1720 cm⁻¹ that is characteristic of C=O bond emanating from both the MMA and DS components of the polymer (Fig. 3b). EDX-SEM spectra of the diatom pre- and post-coating support the TGA and FTIR findings (Fig. 3c). An increase in the carbon signal and reduction in the silica signal post-coating is seen. It is important to highlight that the success of the procedure was achieved only if the frustules were kept in solution at all times following collection and cleaning. This is in agreement with previous work demonstrating that drying of the silica particles following attachment of initiator led to difficulties in re-dispersing the particles in solvent.²⁰

Conclusions

We have presented the ability to tailor the chemistry of the diatom frustule during cultivation so as to introduce initiator sites for grafting polymers via SI-ATRP. The growth of MMA-co-DS polymer from the diatom surface is confirmed by TGA, FTIR and SEM-EDX analyses. The generation of such silica-polymer composites with defined geometry can be explored further using different diatom species. Investigations on the success of grafting from silica particles have revealed that properties such as the length of the carbonyl-spacer in the initiator,²¹ graft density, and molecular weight of the polymer will influence the conformation of the polymer coating. Further exploration and manipulation of the polymer coating can be achieved through modification of these parameters.

Acknowledgements

This material is based upon works supported by the Science Foundation Ireland under Grant no. [07/SRC/B1163]. The
authors acknowledge the facilities and scientific and technical assistance of the Centre for Microscopy and Imaging at the National University of Ireland Galway funded by NUIG and the Irish Government’s Programme for Research in Third Level Institutions, Cycles 4 and 5, National Development Plan 2007–2013. The authors also wish to thank Dr Oliver Carroll, Mr Dermot McGrath and Dr Hongliang Cao for technical assistance.

Notes and references