Fabrication of Nanopatterned Polymeric Microparticles using a Diatom as a Sacrificial Template

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Natural structures with complex hierarchical architecture are employed as biotemplates to fabricate constructs with both nano- and micro-scale features. Diatoms have served as templates for the preparation of inorganic constructs, but are under-explored for the preparation of organic constructs. Herein, we report a method to fabricate nanopatterned polymeric microparticles using the diatom as a sacrificial template.

Introduction

Material scientists have derived inspiration from nature in the design of hierarchical structures. Indeed, several distinct disciplines of bio-inspired research have emerged including biomimetics and bio-replication. The former exploits principles found in nature for the design of materials. The latter involves the use of a biological system as a template for replication. The diatom, a unicellular algae with a hallmark intricate siliceous cell wall, has provided motivation in both these themes of biomimetics and bio-disciplines of biomimetics and bio-replication research.

The sophisticated architecture of the cell walls, termed frustules, has drawn attention from many disciplines with a range of proposed applications including; catalysis, separation science, optics, and drug delivery. The identification of key proteins involved in the biomineralization processes underlying the formation of the elaborate diatom cell walls opened the door to exploration of biomimetic in vitro preparation of silica structures and more recently titania structures. Harnessing the full potential of the frustule architecture has led to exploration of replication strategies either through chemical conversion of the siliceous frustule into an alternative inorganic material, or through bio-replication to inorganic replicas using the core material as a template. Only a few studies have reported on the preparation of polymeric constructs employing the diatom as a biotemplate. Gaddis et al. described the generation of epoxy replicas of the pennate diatom Aulacoseira in order to retain the features in the replica at the mesoscale, dilute epoxy solutions were used in the solution coating process. The work of Holmes et al. yielded negative replicas of diatomaceous earth composed of carbon without retention of the gross morphological features that are the hallmark of the diatom. Attempts to replicate the structure employed immersion of the diatom in a polymer solution with subsequent polymerization or cross-linking and final dissolution of the silica core. Layer-by-layer (LbL) strategies to coat the diatom surface have been reported, but this approach has not been employed to create a free-standing structure. We report the preparation of a polyacrylic acid/polyallylamine hydrochloride (PAA/PAH) microparticle via LbL assembly using the diatom Thalassiosira weissflogii as a biotemplate. Figure 1 illustrates schematically the preparation of the PAA/PAH 3-bilayer system.

Fig. 1 – Amine groups are introduced on to the surface of the diatom via silanization with 3-aminopropyltriethoxysilane. Sequential deposition of polyacrylic acid and polyallylamine hydrochloride layers is performed to yield a three bilayer system. Hydrofluoric acid (HF) dissolution of the diatom core yields a free-standing nanopatterned polymeric microparticle.
Fabrication of PAA/PAH microparticle via layer-by-layer assembly on amine-functionalized *T. weissflogii*

Amine-functionalized *T. weissflogii* was washed three times with 0.7 M NaCl and re-suspended in PAA in 0.7 M NaCl at a weight ratio of 1:1. The solution was mixed for six hours at room temperature. Excess PAA was removed by three washes in 0.7 M NaCl. PAA-coated *T. weissflogii* was re-suspended in PAH (MW 17000) in 0.7 M NaCl at a weight ratio of 1:1. The solution was mixed for six hours at room temperature. Excess PAH was removed by three washes in 0.7 M NaCl. Cross-linking of the PAA/PAH bilayer was achieved by 12 hour incubation with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in de-ionised water at a weight ratio of 5:1. Excess EDC was removed by three washes in de-ionised water. This process was repeated to build up a three-bilayer system. PAA and PAH deposition was monitored by measuring zeta potential after the deposition of each layer. Dissolution of *T. weissflogii* was achieved by treatment with 1 M HF for 20 minutes. The pellet was washed six times with de-ionised water. Samples were examined both pre- and post-dissolution by energy-dispersive x-ray spectroscopy coupled to a scanning electron microscope (EDX-SEM). EDX-SEM analysis was performed using Hitachi S-4700 SEM with INCA® software. TEM images of frustules were collected using Hitachi H-7500 TEM with AMT image capture software. FTIR spectra were collected using a Shimadzu FTIR-8300 in transmittance mode at a resolution of 4cm⁻¹. Twenty scans were collected per sample and data processed using Shimadzu IR™ solution software. The zeta potential was collected from a minimum of ten runs per sample, suspended in water, using Malvern Instruments Zetasizer NanoZS90 (software ver.6.34).

Results and Discussion

A critical step for the success of LbL assembly is the deposition of the first PAA layer, which in turn is dependent on the success of cleaning the *T. weissflogii* frustule for subsequent amine functionalization. Contaminants such as extraneous proteins or polysaccharides on the frustule compromise successful coating of the surface and ultimately affect the fidelity of retention of the characteristic architectural features. The organic casing of the diatom was removed from the frustule by successive washes in HCl (50 % v/v in de-ionized water), de-ionized water, and methanol. Residual organic matter was enzymatically degraded by treatment with chitinase. SEM images of chitinase-treated cleaned *T. weissflogii* reveal the characteristic protrusions, known as fultoportulae, on the valve face (Fig. 2a). The external valve face is also decorated with openings and rib-like structures. The cleaned frustule is composed of SiO₂ that is dissolved by HF treatment to reveal fibres that are embedded in the frustule (Fig.2b). These fibres were considered to be chitin, as the chitin synthase gene has been identified in *T. weissflogii* and it is a known to produce chitin²³.
Irrespective of the chemical composition of the fibres embedded within the frustule, it was important to recognise the presence of such structures so as to avoid confusing these fibres with polymeric material following dissolution of the diatom core.

Surface modification of cleaned frustules was achieved by silanization with APTES. This approach has been reported previously for the modification of cleaned diatoms\textsuperscript{26,30,31}, and is a strategy routinely employed to functionalise silica particles for assembly of a polyelectrolyte bilayer\textsuperscript{32–34}. Surface Si–OH groups present in T. weissflogii show an FTIR absorption peak between 3400–3200 cm\(^{-1}\) (Fig. 3a). Primary amines have two characteristic FTIR bands between 3600–3100 cm\(^{-1}\) as observed in amine-functionalized T. weissflogii. Silanization of T. weissflogii with APTES was also confirmed by a net change of 10.2 ± 0.9 mV in zeta potential (Fig. 3b).

Sequential deposition of three PAA/PAH bilayers was monitored on the surface of T. weissflogii. (Fig. 3b). Deposition of the initial PAA layer generated a net change in zeta potential of -7.8 ± 0.6 mV. Deposition of PAH led to a net change of 64.7 ± 0.6 mV. Subsequent layers showed a similar profile of a more negative charge following PAA and less negative following PAH. LbL build-up has typically been reported on planar and spherical systems. Surfaces with hierarchical features pose a challenge to the success of individual layer coating. The absence of complete charge reversal following deposition of layer five and layer six in this study, is indicative that complete surface coating was not achieved, or that preferential coating of certain regions on the frustule surface may have occurred. The deposition of three PAA/PAH bilayers resulted in a coating on the valve face visible by SEM analysis (Fig. 4a). The characteristic fultoportulae were visible at the centre of the valve, as were the rib-like features. The valleys interspersed between the ribs were coated concealing the pore openings on the valve face. Alteration to the morphology of a particle surface following deposition of polyelectrolyte layers has been observed previously and has been attributed to the stacking of the oppositely charged components\textsuperscript{34}. It is possible that during the deposition of the layers the pores became filled with the polyelectrolyte and were concealed. The flattened appearance of the ribs on the surface of the frustule following deposition of the three PAA/PAH bilayers is suggestive of the polyelectrolytes coating the surface. However, a comparison of the rib width pre- and post-layer build up did not show statistical significance (Fig. 4b). It is possible that the thickness of the coating is less than that which is quantifiable by analysis of SEM micrographs.

![Fig. 3](image1.png)

**Fig. 3** - (a) FTIR spectra of T. weissflogii and amine-functionalized T. weissflogii. (b) Δ Zeta potential following amine-functionalization of T. weissflogii and sequential deposition of alternating PAA and PAH layers on the surface of amine-functionalized T. weissflogii.

EDX analysis confirmed a silica signal in the amine-functionalized T. weissflogii (Fig. 5a). HF treatment resulted in the complete removal of the T. weissflogii core confirmed by the absence of silica signal in the EDX-SEM spectrum (Fig. 5b). The characteristic rib-like features on the valve face (Fig. 5c) and the protrusions on the periphery of the valve face (Fig. 5d) are retained. The polymeric microparticle is a hollow structure as revealed by both SEM and TEM analysis where the internal void space is visible (Fig. 5e-f). Interestingly, fibres akin to those embedded in the frustule were present post HF treatment,
confirming dissolution of siliceous frustule (Fig. 5e).

Conclusions

A free-standing polyelectrolyte microstructure that retains the characteristic nanoscale architectural features was successfully fabricated using a siliceous diatom as a core material. The broad range of both PEs and diatoms available allows for tailoring of both the chemistry and architecture of such microstructures. These microstructures have potential uses in the field of drug delivery and biosensing.

Fig. 5 - EDX spectra of (a) amine functionalized T. weissflogii (b) HF treated PAA/PAH bilayer coated T. weissflogii revealing the absence of a silica signal post-HF treatment. (c-e) SEM micrographs of PAA/PAH bilayer coated T. weissflogii post HF treatment illustrating the rib-like features on the valve face, and the protrusions on the periphery of the valve. (f) TEM micrograph of PAA/PAH bilayer coated T. weissflogii post HF treatment

Notes

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