

## Morphosynthesis of complex inorganic forms using pollen grain templates

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**Porous micron-sized particles of silica, calcium carbonate or calcium phosphate are prepared with complex morphologies by template-directed synthesis employing intact pollen grains; the materials adsorb and release drug molecules and can be functionalized with metallic or magnetic nanoparticles.**

Biological structures are distinguished by the extremely high precision of their self-assembly, replication and functionality, and as such provide novel platforms and templates on which to construct and organize chemical processes. From a materials perspective, intact biosystems, such as bacterial<sup>1</sup> and fungal colonies,<sup>2</sup> viroid particles<sup>3</sup> and liquid crystals,<sup>4</sup> insect wings and plant leaves,<sup>5</sup> spider silk,<sup>6</sup> wood,<sup>7</sup> and silicified structures such as leaves of the plant *Equisetum arvense*<sup>8</sup> and diatoms<sup>9</sup> have been used for the biomimetic synthesis of a range of organized inorganic architectures with potential applications in catalysis, magnetism, separations science, electronics and photonics.

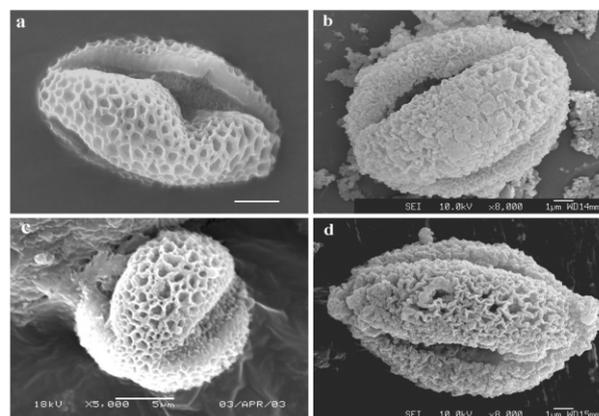
In this paper, we describe a facile method for replicating the complex surface morphology of flower and tree pollen grains, which in the case of silica produces complex colloidal materials with surface areas higher than 800 m<sup>2</sup> g<sup>-1</sup>. Pollen is a ubiquitous and inexpensive material with a high degree of species-specific morphological complexity. The tough outer shell (exine) of pollen grains is amenable to inorganic mineralization without consequent loss of fine structure, either as a result of geological processes,<sup>10</sup> or as described here through synthetic methods. We show that high-fidelity hollow inorganic replicas of pollen grains can be achieved with both amorphous (silica) or crystalline (calcium carbonate, calcium phosphate) minerals, and demonstrate their potential applicability by post-synthetic functionalization with magnetic or metallic nanoparticles. As proof of concept, we also show that the biocompatible replicas can be loaded with the anti-inflammatory drug, ibuprofen, and used as a potential drug delivery system with controlled release properties.

Inorganic replication of pollen grains from *Taraxacum*, *Trifolium*, *Papaver* and *Brassica* genera was achieved by soaking freeze-dried mixed-flower pollen (Pollen-Online, France) in various metastable solutions followed by thermal removal of the biological template to produce silica, calcium phosphate or calcium carbonate facsimiles.† Fig. 1 shows representative SEM images of native and mineralized pollen grains from *Brassica*. The native pollen grains were ca. 25 µm in length and exhibited a characteristic ellipsoidal morphology consisting of four longitudinal segments with foam-like surface structure. Remarkably, these features were also displayed in the hollow inorganic replicas, along with an additional macroporosity associated with removal of the biological template, and reduction in the size of the particles to 10–15 µm due to the thermal processing. Energy dispersive X-ray mapping of individual grains confirmed that the calcined replicas consisted of silica, calcium carbonate or calcium phosphate with negligible organic content. TGA studies indicated a typical organic weight loss during heating of ca. 70%. <sup>29</sup>Si MAS NMR spectra of the silica replicas showed Q<sup>4</sup>, Q<sup>3</sup> and Q<sup>2</sup> (Q<sup>n</sup> = Si(OSi)n(OH)<sub>4-n</sub>, n = 2–4) compositions of approximately 75%, 9% and 16% respectively, indicating that even after

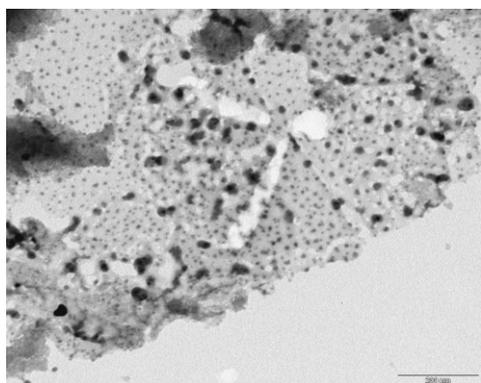
calcination at 600°C approximately 25% of the siloxane centres were not fully condensed and potentially reactive to further functionalization. PXRD indicated the formation of a highly crystalline calcite (CaCO<sub>3</sub>) replica, whereas the calcium phosphate replicas consisted of a mixture of poorly crystalline brushite (CaHPO<sub>4</sub>·2H<sub>2</sub>O) and monetite (CaHPO<sub>4</sub>).<sup>11</sup> Nitrogen adsorption-desorption measurements on the silica replicas showed a type IV isotherm with characteristic hysteresis due to mesoporosity (data not shown), and a relatively high BET surface area of 817 m<sup>2</sup> g<sup>-1</sup>. The data suggest that the high surface area and mesoporosity arise as a consequence of the complex foam-like surface morphology of the native pollen grains and outgassing of organic components during thermal degradation. Similar results were not obtained however for the calcium carbonate and calcium phosphate replicas, which showed only weak gas–solid interactions (type V isotherms) and surface areas of 2 and 10 m<sup>2</sup> g<sup>-1</sup> respectively.

The high surface area of the pollen-templated silica replicas suggests that these morphologically complex colloids could have important applications in chromatography, controlled storage–release processes and heterogeneous catalysis. As proof of concept we functionalized the calcined silica replicas by impregnation of the complex structures with silver<sup>12</sup> or magnetite (Fe<sub>3</sub>O<sub>4</sub>)<sup>13</sup> nanoparticles to produce metallic or magnetic derivatives, respectively. In the former, *in situ* photoreduction of adsorbed Ag(I) ions resulted in a homogeneous dispersion throughout the silica matrix of silver nanoparticles with mean size, 11 nm (SD = 5.83), as confirmed by TEM studies on fractured samples (Fig. 2). Similar data were obtained when the silica particles were immersed in a sol of magnetite nanoparticles, which resulted in the sequestration of nanoparticles with mean size of 7.6 nm (SD = 2.1), presumably by capillary uptake into the macro- and mesopores of the silica matrix.

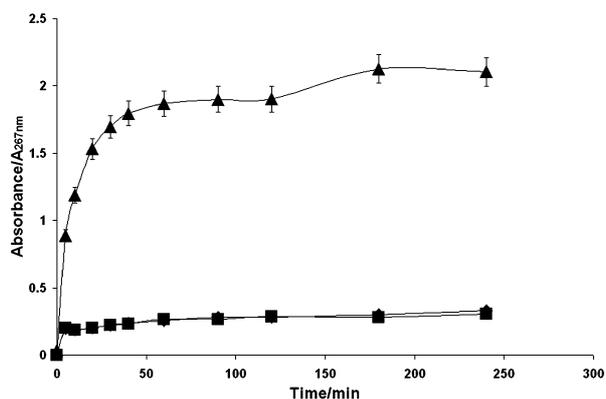
Finally, because silica, calcium carbonate and calcium phosphate are known biomaterials with potential bioactivity, we immersed replicas in aqueous solutions of the drug, ibuprofen,



**Fig. 1** SEM images of (a) Uncoated pollen grain; (b–d) inorganic replicas consisting of (b) calcium phosphate, (c) calcium carbonate and (d) silica. Scale bars; (a) and (c) 5 µm, (b) and (d) 1 µm.



**Fig. 2** TEM image of fractured silver-doped silica replica. Scale bar, 200 nm.



**Fig. 3** UV-vis spectra showing release of ibuprofen from (▲) silica, (◆) calcium carbonate and (■) calcium phosphate replicas.

and monitored the corresponding release profiles in simulated body fluid (SBF).<sup>14</sup> The high surface area silica replicas adsorbed significantly increased levels of the drug compared with the calcium-containing minerals. However, in each case, the pollen-templated replicas released *ca.* 50% of the entrapped drug within the first 10 minutes of immersion, after which the rate of release decreased significantly, such that 70 to 80% of ibuprofen was transferred into the SBF after 3 hours (Fig. 3), suggesting that the materials could be useful in controlled release applications.

## Notes and references

† Silica replication was achieved by soaking *ca.* 0.5 g of pollen for 18 h in a solution of silicic acid ( $\text{Si}(\text{OH})_4$ , 5 mL, pH = 7). The silicic acid was prepared by passing a sodium silicate solution (27% wt  $\text{SiO}_2$ ) through an acidified cation-exchange column that was charged by flushing with hot distilled  $\text{H}_2\text{O}$ , 2M HCl (aq.) and then cold distilled  $\text{H}_2\text{O}$ , followed by addition to the eluate of a few drops of the silicate stock solution to raise the pH to 7. The soaked pollen was centrifuged (Eppendorf 5415D centrifuge, 5 min, 13.5 k rpm), and excess silicate removed, and the silicate-coated grains resuspended in 10 mL of ethanol for 30 min to induce silica

precipitation. The resulting silica-coated pollen grains were filtered, washed with distilled deionized water, and dried under ambient conditions. Hollow silica replicas of individual pollen grains were obtained by heating mineralized samples from ambient temperature to 600 °C at a rate of 1 °C  $\text{min}^{-1}$  overnight.

Calcium carbonate and calcium phosphate replicas were produced by soaking pollen grains (0.5 g) in calcium chloride (5 mL, 1 M) for 18 h, followed by centrifugation and removal of excess aqueous  $\text{CaCl}_2$ , and re-suspension in  $\text{Na}_2\text{CO}_3$  (3 h, 5 mL, 1M, pH 12) or  $\text{Na}_2\text{HPO}_4$  (18 h, 5 mL, 1 M, pH = 9.04), respectively. The coated pollen grains were filtered, washed with distilled deionized water, and calcined at 400 °C overnight to produce hollow inorganic replicas.

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- PXRD results (*d* spacings (Å) and *{hkl}* values): Calcite; 3.841 (102), 3.028 (104), 2.839 (006), 2.651 (105), 2.516 (110), 2.805 (113), 2.090 (202), 1.924 (204), 1.873 (116), 1.602 (212). Brushite; 3.050 (111), 2.920 (221), 2.688 (220). Monetite; 3.399 (020), 3.330 (220), 2.754 (230), 2.533 (122), 2.156 (013), 2.221 (112).
- Silver-containing silica replicas were prepared by soaking the calcined particles in aqueous  $\text{AgNO}_3$  (10 mL, 4 M) overnight at room temperature in the dark. Samples were filtered and allowed to photoreduce in natural light for 24 hrs. For TEM studies, samples were ground and redispersed in ethanol, and air-dried onto TEM grids.
- Magnetic silica replicas were prepared by incubation of calcined particles with a magnetite ( $\text{Fe}_3\text{O}_4$ ) sol, synthesized according to previous methods. [ R. Massart, *IEEE Trans. Magnetics*, 1981, **17**, 1247] The sol consisted of crystalline nanoparticles with roughly spherical morphology and mean size of 10 nm.
- For drug release studies, 0.5 g of the silica, calcium carbonate or calcium phosphate pollen grain replicas were immersed in an ibuprofen solution (10 mL, 4.5 M) for 3 days. The suspension was then filtered and washed, and subsequently immersed in an aqueous solution with ionic composition corresponding to a simulated body fluid ( T. Kokubo *et al.*, *J. Mater. Sci. Mater. Med.*, 1992, **3**, 79). Aliquots of the solution were taken at regular intervals and the amount of release ibuprofen determined by UV-vis spectroscopy. Percentage data are based on 100% being the absorbance of the ibuprofen solution without pollen present.