

Replication of biological organizations through a supercritical fluid route

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A novel and simple method to replicate biological organizations (cotton and pollen grains) with high precision was proposed, in which the precursor dissolved in supercritical CO₂ reacted with the surface active groups and adsorbed surface water on biological templates, followed by *in situ* SCF extraction of the byproducts and unreacted precursor, resulting in inorganic replicas faithfully copying both the macro- and microstructures of the biotemplates.

Nature provides many of her biological species with various morphological architectures from the nanometre scale upwards. Although it is found that full and exact imitation of the extremely complex structures of a biological species is difficult, or even impossible in the laboratory, scientists at least have succeeded in partially replicating some biological structures.¹ Pioneered by Davis *et al.* in synthesizing macrostructured silica and zeolite from bacterial thread templates,² most research on the replication of biological organizations adopts a sol-gel wet chemistry route. Yang and coworkers³ employed a sol-gel coating procedure to fabricate ordered macroporous TiO₂ networks using eggshell membrane as the biotemplate. More recently, by taking advantage of the surface sol-gel process, Huang and Kunitake⁴ replicated natural cellulosic substances with metal oxides and claimed nano-precision. The chemical vapor deposition (CVD) approach has also been developed to copy biological structures. For example, the controlled vapor-phase oxidation of silanes on the surface of fly wings and plant leaves produced an exact inorganic oxide replica of the natural form.¹ Despite the success of biological organization replication through the above mentioned methods, they have their inherent limitations. For the sol-gel process, which is carried out in liquid solvents, the high viscosity and surface tension of the liquid solution generally slow down the mass transfer and even prevent precursors from penetrating into narrow gaps due to the capillary effect. As a result, a uniform and faithful replication is often difficult to attain. As for the CVD route, it is feasible only for those precursors with high volatility and thermal stability, which limited its applications in many cases.

In recent years, supercritical fluids (SCFs) having features such as low viscosity, high diffusivity, zero surface tension, and tunable solvent power, have been demonstrated to be excellent solvents and carriers to distribute and/or impregnate precursors into porous substrates for preparation of composites and/or template synthesis of structural materials.⁵⁻⁹ Among SCFs, supercritical

CO₂ (SC CO₂) is the most popular because it has moderate critical parameters ($T_c = 31.1$ °C, $P_c = 7.38$ MPa), and is non-toxic, non-flammable, easy to obtain. Although usually high pressures are involved, CO₂ processes can be regarded as “soft” methods for many biological substances. For example, enzymes can keep their activities in a supercritical environment.^{10,11} SCF techniques, such as SCF deposition, have been applied to synthesize some new materials with special structures, which are difficult and/or impossible to prepare with conventional methods.

There is no doubt that exploring techniques to replicate biological organizations is an interesting topic of great importance. In the present work, we developed a new method to replicate biological organizations.† In this route, a precursor, such as titanium isopropoxide (TIP), was adsorbed on the surface of the cotton and pollen with the aid of SC CO₂. The precursor was hydrolyzed and condensed *via* the reactions with the adsorbed water molecules and surface hydroxyl groups on the biotemplates, resulting in the corresponding inorganic-organic composites. The composites were subsequently calcined in air to further condense the metal alkoxide and remove the biotemplates.

The products templated from cotton or pollen were first characterized by means of X-ray diffraction (XRD) on a D/MAX.RB diffractometer (Japan) with CuK α radiation ($\lambda = 0.154$ nm) at a generator voltage of 40 kV and a generator current of 100 mA, and the XRD patterns are shown in Fig. 1. Both of the XRD patterns can be indexed as the reflections of an anatase crystalline phase (SG: I41/amd; JCPDS No. 21-1272), which indicates that anatase has been produced. The average crystallite size of the replicas from cotton and pollen are 18 nm and 11 nm, respectively,

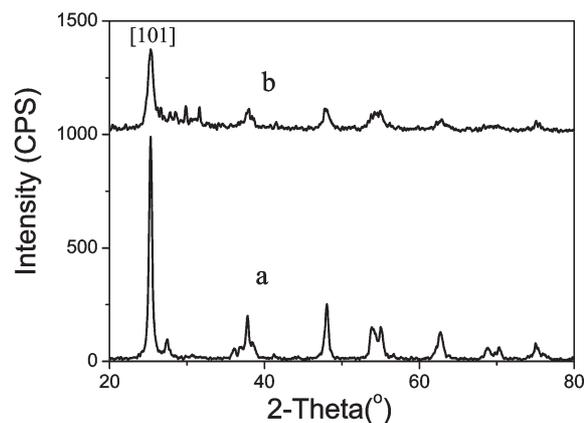


Fig. 1 XRD patterns of replicas from cotton (a) and pollen (b).

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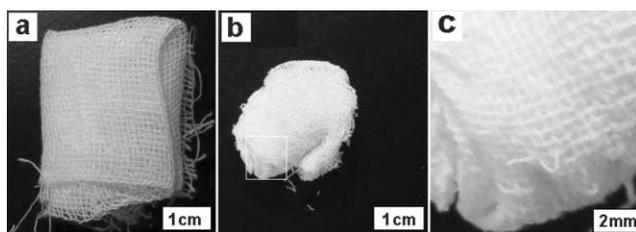


Fig. 2 Photographs of (a) the original fabric template and (b) the inorganic replica after removal of the template, (c) enlargement of the boxed area in (b).

as estimated from the anatase (101) diffraction peak based on the Scherrer formula.

Fig. 2 shows photographs of the original cotton fabric and the calcined inorganic replica, taken with an Olympus C-4000 Zoom digital camera. Apparently, except for the size shrinkage, the inorganic replica also presents as a network structure consisting of crossed longitudinal and latitudinal fibers, just like its mother template.

The morphology and structure of the templates, composites, and the as-prepared replica were also examined by scanning electron microscopy (SEM) on a Hitachi-S4300 electron microscope operated at 15 keV and transmission electron microscopy (TEM) on a JEOL 2010 electron microscope operated at 200 keV. Not only the macroscopic architecture but also the microscopic structure of the template is faithfully reproduced. Under SEM observation, we can see that the macroscopic fibers of the template fabric (Fig. 3 a) are actually bundles of twisted and flat cross-sectioned microfibers with diameters in the range of 15 to 20 μm . These fiber bundles were preserved after the SC CO_2 -aided deposition and template (Fig. 3 b) calcination process (Fig. 3 c), and the twisted and flat cross-sectioned morphology of the template was inherited by the titania fibers in the replica, but the diameter of the titania fibers was smaller and calculated to be to 2–5 μm . Furthermore, with the aid of closer SEM and TEM observation, we find that the titania replicas have a hollow interior (Fig. 3d and 3e), *i.e.*, the obtained inorganic fabric is composed of

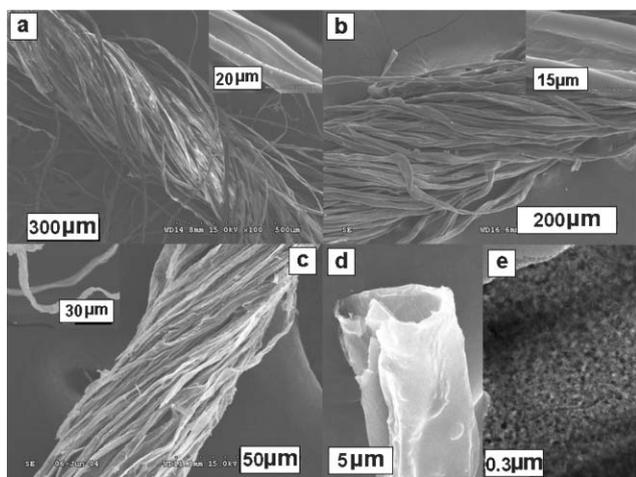


Fig. 3 SEM images of an individual fiber bundle of (a) the original cotton, (b) the coated cotton, and (c) the titania replica. Insets are enlarged images. (d) Closer SEM image and (e) TEM image of a titania microtube.

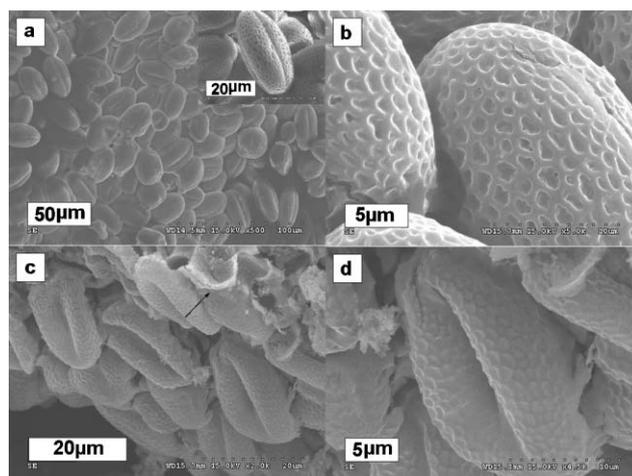


Fig. 4 SEM images of (a) original cole pollen grains (inset is the enlarged image), (b) the coated pollen grains, (c) and (d) titania replicas of pollen grains.

titania microtubes. This result is consistent with some other reports in which inorganic replicas consisted of micro- or nano-tubes, or hollow fibers were fabricated using electrospun polymer fibres or cellulose acetate membrane as templates by a sol-gel replication process.^{12,13}

As for the pollen grains as templates, the hollow interior of the replicas is more evident. Fig. 4 gives the SEM images of the original pollen grains and their titania replicas. The native pollen grains exhibit an ellipsoid-like morphology with longitudinal axes of about 30 μm and latitudinal axes of about 10 μm , and there are many irregular, shallow dents with diameters of about 1–2 micrometres on the pollen surface. The titania replicas of the pollen grains are hollow shells with lengths of about 15 μm . The characteristic dents in the pollen templates are preserved on the surface of the replicas, although about 50% reduction in size is observed. The hollow interior of the replicas is also clearly demonstrated by those broken shells, marked with an arrow in Fig. 4 (c).

It was reported that inorganic facsimiles of pollen grains could also be fabricated through a wet chemistry route by soaking pollen in metastable solutions followed by thermal removal of the biological template.¹⁴ Comparing the replicas of our work to those obtained *via* a wet chemistry route,¹⁴ we found that the SCF replication route in this work clearly has a higher replication precision. The wet chemistry route is capable of reproducing the macroscopic architecture of the templates, however, it is unable to faithfully copy their microscopic structures. Moreover, replicas through the wet chemistry route have a rough surface with many irregular aggregates of particles, and these particles coat the biotemplate surfaces in an uneven way, severely masking the fine surface structure of the template. In contrast, the surface of the replicas through the SCF route is much smoother, and the surface structures are faithfully replicated. This is attributed to the special properties of SC CO_2 , such as low viscosity, high diffusivity, and zero surface tension, which can help the precursor molecules enter every fine void of the template, and react with the surface active groups, *i.e.*, $-\text{OH}$ and the adsorbed water molecules. The reaction of $-\text{OH}$ or water with TIP has been discussed by other authors, and it was confirmed that sol-gel reaction could also occur in SC

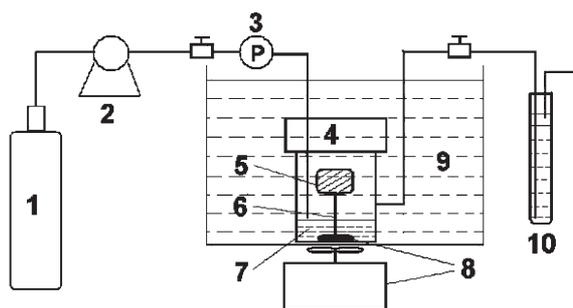


Fig. 5 Schematic diagram of the experimental apparatus: (1) CO₂ cylinder; (2) syringe pump; (3) pressure gauge; (4) stainless steel autoclave; (5) template to be replicated; (6) stainless steel support; (7) precursor; (8) magnetic stirrer; (9) constant temperature water bath; (10) solvent trap.

CO₂.^{15,16} During the reaction process, TIP molecules were first anchored to the OH-containing template surfaces *via* a ligand exchange reaction of the surface hydroxyl groups with alkoxide ligands. Then dehydrative polycondensation and dealcoholic polycondensation occurred.¹⁷ At the same time, the byproducts of the reaction were dissolved in the SC CO₂ phase, and removed together with the unreacted precursor in the following extraction process. All of these features in our method are favorable to forming smooth and well-copied replica surfaces.

In addition, we also succeeded in fabricating a silica replica of cotton using tetraethyl orthosilicate (TEOS) as an alternative precursor through the SCF replication method. Similarly, both the macroscopic and microscopic structures of the template are inherited in the silica replica, although the size was reduced 70%. Furthermore, we selected other materials including cellulose acetate membrane and filter paper as templates to investigate the generality of the SCF replication method. In all cases, we got inorganic replicas, which inherited the macro-architecture and micro-structure of their mother templates, indicating that the SCF replication route is an effective and versatile method to copy materials, especially biological organizations with fine structures.

While this paper emphasizes replication of biological organizations, it can be viewed as a coating or deposition route if the calcination step was not performed. In this process, precursor dissolved in SC CO₂ reacts with the surface water and active groups to form an inorganic thin layer. Carefully examining the composites (before calcination) and biotemplates with the naked eye or with the aid of an electron microscope (see Fig. 3a and 3b for the case of cotton and Fig. 4a and 4b for the case of pollen), we can hardly find any notable difference in shape, size and micro-structure between them, except that the composite is a little harder and there is a weight increase of about 10%–20% after the deposition. This method gives a uniform, compact and smooth coating. Therefore, it is a potential method to give a protective inorganic coating to delicate biological bodies and cultural relics to prevent them from environmental erosion.

In summary, anatase TiO₂ replicas of biological organizations including cotton and pollen have been fabricated through a SCF route. Both macro- and micro-scopic structures of the biotemplates have been copied, although the size of the replicas is smaller. Compared with the wet chemistry and CVD methods, the new route has some unique features, such as having higher replication precision, avoiding high temperature, *etc.* The SCF

replication process is a promising route not only for the synthesis of structured materials using biological templates, but also for the coating or deposition of delicate biological organizations and cultural relics for the purpose of fixing or conservation. This SCF replication process can also be extended to the synthesis of some other inorganic materials.

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Notes and references

† Titanium isopropoxide (TIP) was purchased from Acros. Defatted cotton fabrics were cut from a face mask, and *Brassica oleracea* pollen grains were obtained from Beijing Dongfang Yiyuan Bee Products Co, Ltd, and contained 5 wt% water as determined by TG analysis. Carbon dioxide with a purity of 99.95% was provided by Beijing Analytical Instrument Factory. All other reagents (analytical grade) were purchased from Beijing Chemical Plant, and used without further purification. The schematic diagram of the apparatus used in this work is shown in Fig. 5. It consisted mainly of a stainless steel high-pressure autoclave of 22 ml with an inlet and an outlet, a constant temperature water bath, a pressure gauge, a magnetic stirrer, and a trapper. In a typical experiment, TIP (2.0 g) was first charged into the autoclave. The biotemplate (2.0–3.0 g) was then placed onto a stainless steel support fixed in the autoclave to prevent direct contact with the liquid precursor. The autoclave was put into a water bath at 50.0 °C. The air in the autoclave was replaced with CO₂, and more CO₂ was charged up to 12.0 MPa using a DB-80 high-pressure syringe pump after starting the magnetic stirrer. The system was maintained under these conditions for about 12 h. Then the byproducts and unreacted precursor were extracted *in situ* from the autoclave using SC CO₂ at 50.0 °C and 12.0 MPa for about three hours with a flow rate of 20 g CO₂ per hour. After the pressure in the autoclave was slowly released, the sample was taken out of the autoclave and transferred into a muffle burner and heated to 600 °C for 6 hours to burn off the biotemplate and condense the precursor.

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