SUPPLEMENTARY INFORMATION

Eco-Friendly Photolithography Using Water-Developable Pure Silk Fibroin

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Methods

Silk fibroin solution preparation: Cocoons of the Bombyx mori silkworm were boiled for 30 min in a solution of 0.02 M Na₂CO₃ to remove the sericin protein. The extracted fibroin was rinsed with distilled water and then dried in air for 24 h. After drying, the fibroin was dissolved in a 9.3-M LiBr solution at 60 °C for 4 h, yielding a 20 wt% aqueous solution. The silk/LiBr solution was dialyzed against distilled water using a dialysis cassette (Slide-a-Lyzwe, Pierce, MWCO 3.5 K) at room temperature for two days until the solution reached a concentration of 7%. The obtained solution was purified using a centrifuge and a syringe filter with a 0.45-μm pore size.

ArF excimer lithography: Silk aqueous solution was dropped on a quartz substrate and spin-coated to yield a 200-nm-thick silk film. Crystallized silk films can be obtained by dipping them in methanol for 1 min. A laser beam from the ArF excimer laser (EXS-200-193nm-XS, Coherent Inc.) with a wavelength of 193 nm illuminated the silk film. The pulse width was 5 ns, and the repetition rate was 100 Hz. The stabilized pulse energy was 8 mJ. The dose can be controlled by the exposure time. After the exposure, the sample was developed in water for 1 min and blown by a nitrogen gun. In the water-injection case, after introducing a gap between the sample and the photomask, water was injected to one side of the rectangular sample using a syringe needle.

Dry etching and lift-off: An inductively coupled plasma (ICP) etcher (Multiplex ICP Etch, STS) was used to test the etching resistance of various resist materials. For SiO₂ etching, we used a pressure of 7 mTorr, an RF power of 100 W, and a mixed gas of O₂ and C₄F₈ with a 1:9 ratio. The etched samples were measured using AFM to determine the remaining thickness. For the fabrication of the Cr mask, a chrome layer with a thickness of 30 nm was deposited on the patterned silk layer using an electron beam evaporator (SEE-7, ULTECH).
Then, the metal-coated samples were immersed in NaCl solution to remove the remaining silk selectively.

_Biofunctionalization_: HRP enzyme (Sigma Aldrich) was mixed with aqueous silk solution to a final concentration of 20 units mL\(^{-1}\). The HRP-containing solution was spin-coated on a quartz substrate to yield a 200-nm-thick film, and then the film was crystallized using methanol. The resulting resist was exposed to different doses, and the unexposed and exposed areas were exposed to 3,3’,5,5’-tetramethylbenzidine (TMB) solution (Sigma Aldrich) to test the activity of the HRP stabilized within the silk resist.
**Adhesion tests of resists**

For adhesion tests, two commercial photoresists (DNR-300-30 and DPR-i7000) were used as comparisons. Hexamethyldisilazane (HMDS) adhesion promoter was spin-coated on the silicon and GaAs substrates before coating with the photoresists. In case of the silk resist, HMDS was not used. The resist-coated samples were immersed in isopropyl alcohol and then sonicated for 30 min. As shown in Figure 1S and 2S, two commercial resists were removed from the substrates even when an adhesion promoter was used, while the silk resist survived.

![Adhesion tests](image)

**Fig. S1** Adhesion tests. Spin-coated photoresists on silicon substrates (commercial photoresists in (a) and (c) and silk resist in (e)). (b,d,f) Photographs of the samples after sonication for 30 min. Only the silk resist survived under harsh sonication.
Fig. S2 Adhesion tests. Spin-coated photoresists on GaAs substrates (commercial photoresists in (a) and (c) and silk resist in (e)). (b,d,f) Photographs of the samples after sonication for 30 min. Only the silk resist survived under harsh sonication.
Removing silk resists and lift-off application

Fig. S3 (a-d) Removing silk resists using salt aqueous solutions. 20 wt% NaCl and LiBr solutions successfully removed the methanol-treated silk films. (e) Photograph of the chrome photomask generated using the silk photoresist. A lift-off process was applied.
In-situ patterning

**Fig. S4** AFM image of a periodic silk pattern and a cross section of the image along the line. Water was injected through the gap between the silk film and the photomask. Development and exposure were therefore performed simultaneously.