

Rapid Nanoimprinting of Silk Fibroin Films for Biophotonic Applications

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With soft micro- and nanopatterned materials becoming increasingly useful for various optical, mechanical, electronic, microfluidic, and optofluidic devices,^[1–4] the extension of this paradigm to a pure protein-based material substrate would provide entirely new options for such devices. Silk fibroin is an appealing biopolymer for forming such devices because of its optical properties,^[5] mechanical properties,^[6,7] all aqueous processing,^[8,9] relatively easy chemical and biological functionalization,^[5,10] and biocompatibility.^[11] Biologically functionalized silk fibroin films can be patterned on the micro and nanoscale using a soft lithography casting technique^[5,9] while maintaining the biological activity of the embedded proteins.^[12] The combination of these properties could enable a new class of active optofluidic devices^[13,14] that merge high-quality photonic structures whose very material constituent responds, through the embedded proteins, to analytes infused through integrated microfluidics.^[15] However, the silk fibroin casting process takes 12–36 h, hindering the ability to rapidly produce multiple devices and the resulting silk structures contain artifacts due to drying and liftoff. In this communication, we will show that silk has the properties of an ideal nanoimprint resist enabling rapid device fabrication, which in combination with its optical properties and biocompatibility make it a new technology platform that seamlessly combines nanophotonics, biopolymeric and biocompatible materials.

Optofluidics,^[13,14] though a relatively new field, is already undergoing evolution, finding applications to an ever-increasing range of problems, including varieties of biological sensing and detection. Initially optofluidics was developed as a fusion of microfluidics and photonics to enable compact, novel optical

modulation technologies.^[16,17] The union of optical and fluidic confining structures, however, led optofluidic devices to be applied to sensing problems^[18–20] especially looking toward highly parallel, sensitive and low analyte volume applications.^[21] A further development of the optofluidic paradigm, introduced here through the use of silk, is to “activate” the constituent material of the device to make it chemically sensitive to species flowed past it. Typically, optofluidic devices are fabricated from materials usually found in photonics or microfluidics such as silica,^[22] silicon,^[23] polydimethylsiloxane^[2] or polymethylmethacrylate and other polymers.^[24] These materials, while possessing suitable and well-characterized optical and material properties are not inherently chemically sensitive or specific. It is possible to functionalize the surfaces of these materials with chemical reagents,^[25] however, a much broader range of sensitivities and specificities can be achieved if proteins or enzymes are used as the sensitizing agents. The use of proteins presents an issue in itself. Binding proteins (or chemicals receptive to them) to inorganic or synthetic polymer surfaces is complex.^[26,27] Ideally, a material such as silk fibroin that possesses excellent optical and mechanical qualities can be formed into a variety of optofluidic geometries and maintains the activity of embedded proteins is needed for realizing active optofluidic devices. A proof of concept presented here is to build a self-sensing nanoscale imprinted optofluidic device based on imprinted silk doped with lysed red blood cells. The device can be thought of as “self-analyzing” in that the single optofluidic component provides both chemical and spectral analysis due to the activation of the constituent imprinted silk.

Nanoimprinting is a high-throughput lithography technique in which a mold is pressed onto a thermoplastic material heated above its glass-transition temperature. The softened material conforms to the mold due to applied pressure.^[1,4,28] Sub-100 nm structures by nanoimprint lithography were first demonstrated in polymethylmethacrylate (PMMA)^[29,30] and now structures as small as 10 nm are routinely achieved in PMMA.^[28,31] An ideal nanoimprint resist combines rapid imprinting times with low temperature and pressure as well as low surface energy to aid in mold removal. As such, the mold is often coated with a low surface energy surfactant.^[32]

Nanoimprinting of biopolymers presents additional challenges because of a restricted parameter space that limits the ranges of temperature and pressures usable. However, in this communication, we demonstrate that silk fibroin films exhibit many characteristics of an ideal nanoimprint resist, which in combination with its optical properties and biocompatibility make it a new technology platform that seamlessly combines

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nanophotonics, biopolymeric and biocompatible materials. We describe a process by which silk fibroin films can be easily nanopatterned in short times (seconds to minutes) with low pressure (50 psi), reproducing features down to 50 nm or less using either a hot (100 °C) or room-temperature embossing technique without any mold surface treatments. The nanoimprinting technique significantly increases quality and throughput when compared to previously demonstrated casting methods for silk.^[9] In addition, the speed and fidelity of production, as well as the mechanical and optical properties, are superior to other biopolymers and biocompatible polymers such as chitosan^[33] and PLA.^[34]

Nanoimprinting of silk fibroin films is performed via two processes which depend on adjusting the silk fibroin film glass-transition temperature. The glass-transition temperature of silk fibroin films depends on the absorbed moisture.^[35] For films prepared at ambient humidity (~35%) the glass transition temperature is ~100 °C while for water saturated films the glass-transition temperature is room temperature. To nanoimprint films at ambient humidity, a hot embossing process is employed whereby a silk film is pressed (~50 psi) on to a heated (100 °C) master pattern for 5 s (Fig. 1a). After imprinting, the master pattern adheres to the silk film and is then easily removed by levering off with a thin razor blade after cooling slightly (60 s). Alternatively, to capitalize on the variable glass-transition temperature of silk and achieve room-temperature nanoimprinting, a small amount of water (~1 μL) is deposited on the film to locally decrease the glass-transition temperature before pressing on the master pattern (Fig. 1b). The master pattern can be easily removed after the film has returned to ambient humidity and the excess water has evaporated (~10 min). After imprinting, the films can be annealed by exposure to methanol to eliminate water solubility.^[36–38] Upon exposure to methanol, the secondary structure of the silk fibroin protein changes from primarily random coil to primarily β-sheet and the glass temperature increases.^[39] After annealing, the imprinted films are quite stable and last years.

In order to demonstrate the feasibility of the proposed nanoimprinting approaches, we used several different master

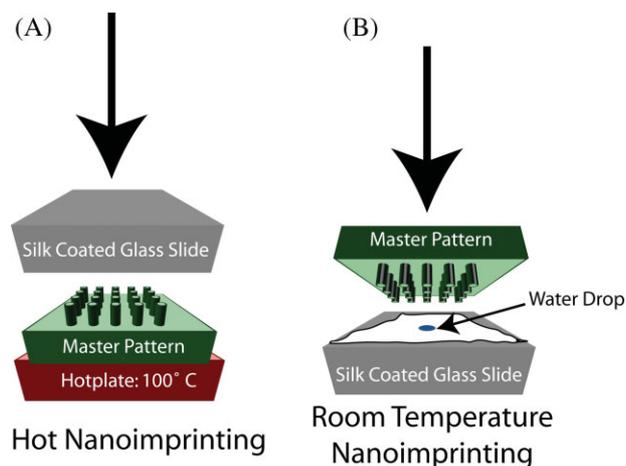


Figure 1. A schematic of the two nanoimprinting processes presented: a) hot embossing and b) room-temperature embossing.

patterns for silk imprinting. In particular, we have utilized a 3600 grooves/mm holographic diffraction grating (Edmund Optics, Inc.) and periodic chromium nanoparticle (200 nm in diameter and 30 nm in height) arrays fabricated with electron-beam lithography on silicon substrates with varying particle separations between 700 and 250 nm.^[40,41] Additional imprinted patterns such as 3D diffractive optics and multiply imprinted gratings appear in the supporting information (SI). The areas of the masks range from 0.5–1 cm². Characterization of the nanoimprinted silk films was accomplished with scanning electron microscopy (SEM) and atomic force microscopy (AFM).

Figures 2a and 2b shows an AFM image and cross section of a silk film nanoimprinted at 100 °C with a periodic pattern of 200 nm diameter chromium nanoparticles separated by 700 nm. Figure 2c shows an SEM image of a silk film imprinted with similar 200 nm diameter chromium nanoparticles but this time separated by only 250 nm. These images indicate that the smallest transverse features that can be imprinted in silk are 50 nm or smaller. The casting process described previously using similar e-beam fabricated master patterns, while effective, introduces artifacts such as uneven edges from mechanical liftoff and extra depth in the reproduced features caused by the drying process.^[9] In contrast, these artifacts are eliminated in the nanoimprinting process described here.

Figure 3a displays an AFM image of a 3600 grooves/mm grating imprinted at room temperature on a cast silk film. Figure 3b shows a cross section of the image indicating the grating period of 277 nm and a feature depth of 75 nm. Figure 3c

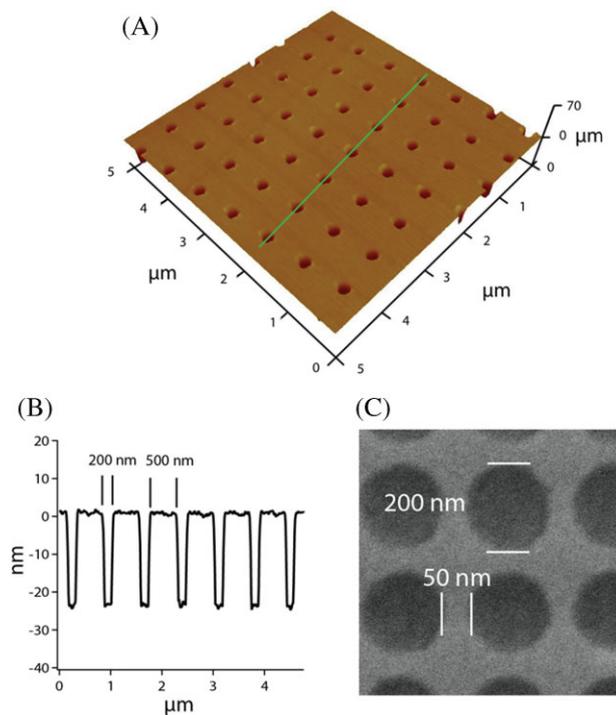


Figure 2. a) AFM image of a periodic pattern imprinted in silk with 200 nm diameter holes separated by 700 nm. b) Cross section of (a) measured along the line illustrated in (a). c) SEM image of a silk film imprinted with a periodic array of 200 nm diameter 30 nm height chromium nanoparticles separated by 250 nm.

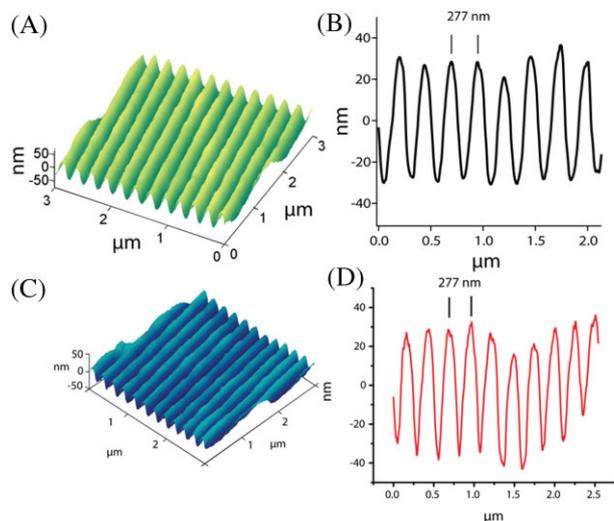


Figure 3. Room-temperature embossing. a) AFM image of a 3600 groove/mm grating imprinted on a silk film at room temperature and b) related cross section measurement of (a). c) AFM of 3600 groove/mm grating master pattern and d) related cross section measurement of (c).

and 3d show the corresponding AFM image and cross section of the master grating showing the accuracy of reproduction in imprinted silk.

The sub-50-nm dimensions reproducible in silk using the nanoimprinting technique described are superior to other biopolymers. For example, chitosan reproduces features of ~ 150 nm when using a comparable process at a similar temperature, but does not have the optical clarity of silk fibroin and the nanoimprinting process takes far longer (30 min versus a few seconds).^[33] Furthermore, silk offers superior mechanical properties and is not subject to solubilization under acidic conditions. Poly(lactic acid), an optically clear biocompatible polymer reproduces features down to hundreds of nanometers, requires solvents in its preparation and surface treatment of the mold to prevent sticking.^[34] Silk does not require solvents in preparation or mold surface treatments. Other room-temperature nanoimprinting methods and resists exist, but require orders of magnitude more pressure than room-temperature nanoimprinting of silk.^[42,43] The nanoimprinting process also compares favorably with the silk soft lithography casting process described previously,^[9] reproducing similar feature sizes but without the drying artifacts. Most importantly, the approaches presented here require several orders of magnitude less time when compared to casting, which will enable significantly larger yields in the production of nanopatterned silk devices. In addition, the ability to nanoimprint at room temperature by locally reducing the glass-transition temperature is ideally complementary to the unique feature of silk fibroin films of being able to maintain biological activity of dopants in the silk films^[12] further enabling facile production of bioactive nanoscale devices.

To demonstrate the utility of silk nanoimprinting for use in biophotonic sensing applications, we construct a self-sensing optofluidic device. To make such a device, we first dope the silk solution with lysed red blood cells and cast a film on a glass slide. Then using the room-temperature nanoimprinting method described above, we imprint a 600 grooves/mm grating in the

hemoglobin doped silk film and anneal with methanol to preserve the imprinted grating and eliminate water solubility. The doped imprinted silk grating then forms one side of a microfluidic flow cell. The remainder of the flow cell consists of polydimethylsiloxane (PDMS) faced with a glass cover slip filled with de-ionized water. The flow cell is kept open at the top to allow for easy addition of water and a small opening is made on the bottom to allow gas to be passed into the cell. A tungsten light source is collimated with a $10\times$ microscope objective and directed through the imprinted grating to a Thorlabs, Inc. LC1 charge-coupled device (CCD) line camera calibrated with spectral notch filters for spectral analysis. A similarly prepared silk grating without lysed blood cells is used as a reference.

Figure 4 shows the absorption spectrum of the doped, imprinted silk grating in the presence of oxygen or nitrogen. First, nitrogen is bubbled through the flow cell to completely deoxygenate the hemoglobin. Upon oxygen flow, peaks appear at 540 and 575 nm indicating the binding of oxygen to the hemoglobin. The process is reversible by switching to nitrogen gas and repeatable after storage of the imprinted film for several months (data not shown). These results demonstrate the persistent activation of the hemoglobin protein inside the silk matrix despite being subjected to the fabrication process, storage in the laboratory and repeated experimentation. The entire operation of the device is enabled by the unique longevity and activation of protein embedded in silk.

Nanoimprinting photonic components in silk films is a fast, inexpensive, and high-throughput method of producing optically relevant micro- and nanoscale features. Either a hot embossing technique or a room-temperature embossing technique can be used by locally varying the glass transition of the film. This technique, combined with the favourable optical properties of silk such as high transparency in the visible range, high mechanical stability, and all aqueous processing opens avenues for fabrication of all-organic biophotonic components on the nanoscale that can be readily functionalized and employed as a new material

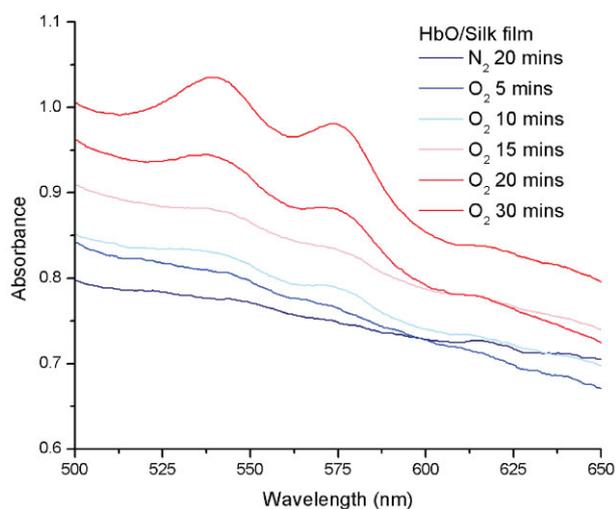


Figure 4. Spectral response of the silk doped with lysed red blood cells. First, nitrogen is bubbled through the flow cell to completely deoxygenate the hemoglobin. Upon oxygen flow, peaks grow in at 540 and 575 nm indicating the binding of oxygen to the hemoglobin.

platform. The self-sensing optofluidic device presented here is the first example of this new technology. This technology seamlessly combines nanophotonics, biopolymeric and biocompatible materials, adding a novel dimension to biomedical optical devices.

Experimental

Silk Processing: The fabrication of the nanoimprinted silk films starts with purified silk fibroin solution. Production of the silk fibroin solution has been previously described in the literature [8,9]. Briefly, the purification of silk fibroin from *Bombyx mori* cocoons initially involves the removal of sericin, a water-soluble glycoprotein which binds fibroin filaments, by boiling the cocoons in a 0.02 M aqueous solution of sodium carbonate for 60 min. Upon completion of this step, the remaining fibroin bundle is rinsed thoroughly in Milli-Q water and allowed to dry overnight. The dry fibroin bundle is then dissolved in a 9.3 M aqueous solution of lithium bromide at 60 °C for four hours. The lithium bromide salt is then extracted through a water-based dialysis process. The resulting solution is extracted from the dialysis cassette (Slide-a-Lyzer, Pierce, MWCO 3.5K) and remaining particulates are removed through centrifugation and syringe based micro-filtration (5 μm pore size, Millipore Inc., Bedford, MA). This process enables the production of 8–10% w/v silk fibroin solution with minimal contaminants and reduced scattering for optical applications.

After purification of silk fibroin, 1 mL of the solution is cast on a glass microscope slide (1 in. × 1.5 in.) and allowed to crystallize in air overnight. The resulting film adheres to the glass slide and is approximately 10–15 μm thick depending on the concentration of the silk solution used [5]. Alternatively, one can spin coat the silk solution to produce films from 30–2000 nm thick [7] or cast films on hydrophobic surfaces to make free standing films for imprinting [5]. These films have excellent surface quality and optical transparency.

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