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Biopatterning of Silk Proteins for Soft Micro-optics

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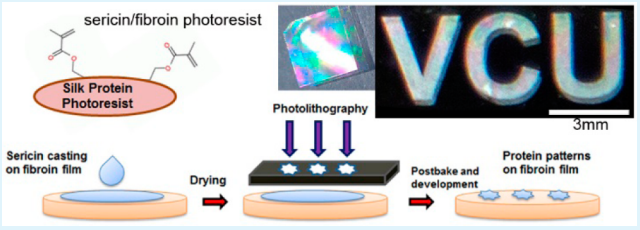
ABSTRACT: Silk proteins from spiders and silkworms have been proposed as outstanding candidates for soft micro-optic and photonic applications because of their optical transparency, unique biological properties, and mechanical robustness. Here, we present a method to form microstructures of the two constituent silk proteins, fibroin and sericin for use as an optical biomaterial. Using photolithography, chemically modified silk protein photoresists are patterned in 2D arrays of periodic patterns and Fresnel zone plates. Angle-dependent iridescent colors are produced in these periodic micropatterns because of the Bragg diffraction. Silk protein photolithography can used to form patterns on different substrates including flexible sheets with features of any shape with high fidelity and resolution over large areas. Finally, we show that these mechanically stable and transparent iridescent architectures are also completely biodegradable. This versatile and scalable technique can therefore be used to develop biocompatible, soft micro-optic devices that can be degraded in a controlled manner.



KEYWORDS: *silk protein, photolithography, iridescence, soft optics, microfabrication, Fresnel lens*

1. INTRODUCTION

Modern soft-micro optical devices are focused on new materials that can be easily processed and are robust and flexible. Currently, optical devices are largely fabricated from glass, semiconductors, metals, and synthetic polymers.[1−3] However, the intrinsic lack of biocompatibility and biodegradability of these materials typically render them unsuitable for applications including biophotonics and biointegrated systems.[4,5] The use of proteins in photonic and optoelectronic devices provides an excellent opportunity to take advantage of unique mechanical, chemical, and optical properties. Primary advantages include their relative abundance, low cost, and biodegradability of proteins and importantly, their biocompatibility and ability to be engineered for external stimuli. Natural and recombinant biopolymers such as silk,[6] collagen, chitin, chitosan,[7] and reflectin[8] have been proposed as alternatives for the design and fabrication of innovative optical devices. These include light guiding or concentrating devices for improved imaging, photodynamic treatments, color switching sensors, light propagation to relay physiological signals, and for reading and recording devices used in real time monitoring.[9,10]

Among the various biopolymers reported, silk proteins have stood out as particularly attractive building blocks for optical components at the micro and nanoscales.[11] This is because of their optical transparency, processability into a variety of architectures, and exceptional mechanical properties. The two constituent proteins of silk from silkworms, fibroin and sericin, are biocompatible when completely separated from each other, and have been shown to have excellent integration with biological systems.[12,13] A wide variety of 2D and 3D optical elements based on silk films, such as diffractive patterns, holograms, optical gratings, and waveguides have been demonstrated. Nonetheless, to develop biofriendly soft photonics, the challenge includes the coupling of silk proteins with accessible fabrication strategies, which is still an outstanding problem.[14] Various pioneering strategies from the Kaplan group have been reported to form photonic elements, including soft lithography,[15] inverse opals, and nanoimprinting.[16−19] These techniques are inexpensive, high-throughout fabrication methods for optical biomimetic materials with planar and relatively simple microstructures.[20] However, typically, these indirect methods require the formation of master patterns that can then be transferred to the biomaterial. Despite excellent resolution, limitations include the need to construct these masters and molds, loss of fidelity or incomplete molding during transfer steps, and often, high temperature and pressure requirements.[20,21] Direct printing techniques such as E-beam lithography, femtosecond laser machining, and two and three-photon interference lithography have been reported but are quite expensive.[22−26] Optically iridescent architectures have also been shown by patterning of silk films using a "breath figure approach".[27] Although this approach can be used to form cavities ranging from submicrometer to micrometer scales, the scalability and limitations on geometry and control of cavity size make these approaches limited.

Recently, our group reported on the integration of high-resolution microfabrication platforms with silk proteins to form structures with desired spatial complexity and mechanical properties.[28,29] Using photoactive conjugates of the two

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constituent silk proteins, fibroin and sericin, we demonstrated extension of photolithography to natural biopolymers and use a process that inherently requires photoreactive precursors.[30] This opens up a new way of inexpensively fabricating photonic devices and architectures, using a rapid and scalable approach to form micro- and nanostructures over large (cm) areas. Using photolithography, it is possible to not only accurately control the nature of the patterns formed but also their aspect ratios and periodicity (or aperiodicity). Optically transparent or opaque microstructures can control and manipulate light through their matrices, and can be very easily patterned over macroscales in a benchtop environment. Here, we show the applicability of this silk protein photolithography (SPL) technique to form microstructures that can control and manipulate light through their matrices. Specifically, we demonstrate two kinds of optical components that can be easily fabricated—biomimetic, structurally iridescent surfaces that can alter the directional pattern of reflected light, and Fresnel zone plates for the focusing of light. Using photolithography, we can pattern such microstructures both on rigid glass and on flexible silk substrates that are completely biodegradable over a period of days. To demonstrate the versatility of this technique, we show architectures formed using both components of the silk cocoon, the water insoluble fibroin and the water-soluble sericin. In the case of the latter, these structures can be formed in completely aqueous, green process. Such protein-based photonic components can result in the formation of next-generation natural optical systems that are biofriendly and degradable and can open up new avenues in using the complete silk cocoon.

2. EXPERIMENTAL SECTION

2. 1. Synthesis and Purification of Photoactive Fibroin and Sericin. Fibroin protein was extracted and purified from silk cocoons (*B. mori*) following the protocol described elsewhere.[31] Sericin was obtained in pure form (Wako Chemicals, Richmond, VA). Photoreactive conjugates of fibroin and sericin were prepared as described earlier.[28,29] Briefly, these conjugates were prepared separately by first solubilizing the protein in 1 M LiCl/DMSO solution and reacting with a stoichiometric amount of 2-isocyanatoethyl methacrylate (IEM) at 60 °C and 5 h in a dry nitrogen environment. Following the reaction, the mixture was poured into excess cold ethanol to precipitate out the protein with conjugated methacrylate group. The conjugates were washed in a mixture of cold ethanol/acetone, centrifuged, and lyophilized for 48 h to obtain the final product.

2.2. Surface Functionalization. Protein micro- and nanopatterns were made on glass substrates to take advantage of optical transparency. To facilitate attachment of the features, we functionalized these substrates with acrylate moieties. The surfaces were initially cleaned using piranha (3:1 98%H_2SO_4:30%H_2O_2) solution for 30 min in ambient condition followed by functionalization with 3-(trichlorosily) propyl methacrylate (TPM). Chemical vapor deposition was used in a desiccator for 12 h at 0.4 bar. Treated surfaces were washed with hexane and water, and air-dried prior to use. To form patterns on silk sheets, we cast fibroin sheets as described earlier. No further modification was needed to facilitate attachment of the protein patterns.

2.3. Silk Protein Photolithography. Micropatterned holograms and Fresnel zone plates (FZPs) of silk proteins (fibroin and sericin) were fabricated on glass using contact photolithography. FPP and SPP were dissolved at 3.75% (w/v) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Sigma-Aldrich, St. Louis, MO) and 2,2,2-trifluoroethanol (TFE, Acros Organics, NJ), respectively. Photoinitiator (Irgacure 2959, BASF) 0.6% (w/v) was added to the silk photoresist solution and mixed. The solutions were cast on substrates functionalized with TPM. HFIP and TFE were allowed to evaporate for 5 and 10 min, respectively, followed with UV exposure (Lumen Dynamics OmniCure 1000 system) for 4.5 s at 2000 mW cm^{-2} using a 320–500 nm filter). The UV-exposed substrate was post baked for 15 min to completely cure the patterns. Patterns were developed in 1 M LiCl/DMSO solution to deionized water for 2 h followed by washing with a generous amount of deionized water and ethanol. To form dark binary phase Fresnel lenses, we added a dark carbon dye to the silk protein solutions. Micropatterned structures of the SPP were patterned on cast-films of fibroin prepared as described earlier.

2.4. Proteolytic Degradation in Vitro and Imaging. To demonstrate biodegradability of the micropatterned features, we followed enzymatic degradation over time.[32] One hundred micrometer features (1.5 mg of protein cast/cm^2 of substrate) on glass substrate were incubated in 4 mL of protease (Protease XIV from *S. griseus*, ≥3.5U/mg, Sigma-Aldrich) solution (0.25U/ml of PBS buffer) at 37 °C. The enzyme solution was replaced every third day to preserve activity. As negative controls FPP and SPP patterns were incubated in PBS solution containing no enzyme. At different time intervals, samples were removed from solution, washed with deionized water, and imaged. The structures were studied using optical microscopy, scanning electron microscopy, and atomic force microscopy to observe changes in the surface morphology over time. Optical and scanning electron microscopy were performed using Nikon Eclipse and. JEOL JSM- 5610 LV instruments, respectively. FPP and SPP patterned substrates on glass were sputter-coated in 20 Å platinum (Denton Vacuum V cold sputtering system, Moorestown, NJ). For nanoscale surface morphology analysis, an MFD-3D atomic force microscope (Asylum Research, Santa Barbara) was used. Imaging was conducted using an AC 240TS (Olympus) cantilever with a nominal force constant of 2 nN/nm.

3. RESULTS AND DISCUSSION

Coupling optical behavior to biodegradable materials can provide the next generation of soft biophotonic devices.[33] For instance, periodically structured materials are commonly found in natural systems to form coloration and remarkable optical behavior that are purely physical in nature.[34] Such phenomena occur because of manipulation of light based on photonic crystals, diffraction of light, or multilayer interference and can therefore be adapted to flexible and soft substrates. Simultaneously, a big challenge in the fabrication of high-resolution soft micro-optical systems is the adaptation of fabrication strategies to form architectures of high complexity and spatial resolution, while being scalable and low cost. Photolithography is a widely used and well-developed tool in the semiconductor industry that is also scalable (micro- and nanostructures can be repeatably, rapidly, and reproducibly formed over large areas). The protocols are highly developed and a wide diversity of shapes and sizes can be patterned. However, photolithography inherently requires photoreactive precursors and has therefore not been applied to natural biopolymers.[30]

On the basis of earlier reported works, we have synthesized photoactive conjugates of the two silk proteins, fibroin and sericin, to form negative photoresist-like materials that can be cross-linked using light. The photopolymerizable conjugates are termed as silk protein photoresists (fibroin protein photoresist (FPP) and sericin protein photoresist (SPP)).[28,29] These proteins behave as negative photoresists that are cross-linked in the presence of UV-radiation through a photomask. This transforms the proteins into water-insoluble, mechanically robust patterns that are covalently attached to the underlying substrates. This is in contrast to the induced crystallization in silk due to e-beam lithography.[26] Following development (removal of un-cross-linked protein), structures of high resolution and fidelity can be patterned over large areas in a

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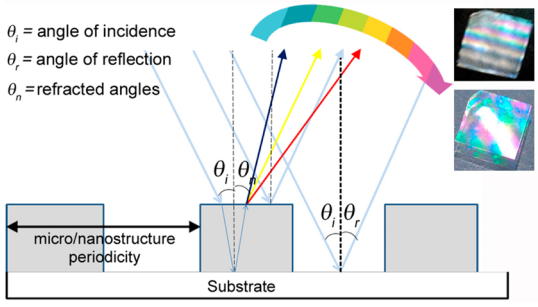
few seconds. Because the structural diversity of the patterns is only limited by the kind of mask used, it is possible to fashion virtually any design. Here we demonstrate the micropatterning of these silk proteins to demonstrate the formation of structurally induced iridescence and Fresnel zone plates that can be used for light focusing. Importantly, we can micropattern architectures on both surfaces such as rigid glass and also a flexible underlying substrate (silk sheet). We show how microfabrication tools such as benchtop photolithography can be used to form optical properties in a naturally derived biomaterial that is also completely biodegradable.

3.1. Structure Induced Iridescence. Structural color can be produced by coherent or incoherent light scattering from the surface.[35] Coherent scattering occurs when the photonic structures have periodicity that is less than half of the wavelength of light. Scattered light produces precisely ordered constructive or destructive interference patterns, which results in forbidden wavelengths or photonic bandgaps, and only a few wavelengths of light can be visible.[36] In contrast, incoherent scattering occurs when the photonic structure periodicity is larger than the wavelength of light, where the phase relationship of scattered light is random. Structure-induced iridescence from two-dimensional (2D) periodic arrays of microstructures on a substrate may therefore result because of the incoherent scattering of light. The light reflected from the microstructures and diffracted light from the periodically arranged materials create an interference pattern (Scheme 1). At a particular viewing angle only a few particular wavelengths of light produce constructive interference so that those color lights are only visible and a change in hue is observed.[37]

Scheme 1. Nature of Structural Iridescence[a]



[a]The angle of viewing can change the colors observed. Here the image shows the micropatterned protein sheet (1 cm^2) with an almost metallic appearance in sunlight.

To demonstrate that silk protein microstructures can display structure-induced iridescent behavior, a variety of features were patterned as shown in Figure 1. Here we show structures ranging from 2 to 10 μm of both silk fibroin and sericin patterned on substrates. The column on the right shows the typical iridescent behavior observed in sunlight. As expected, the color changes with viewing angle and the films present a distinctly metallic appearance. In contrast, patterns of the same design but without constituent microstructures do not show any iridescence behavior. This confirms that the iridescence is caused by the diffraction of light from the microstructures and not by the interference of light from the thin film of the silk proteins. Simultaneously, it is important to note that larger microstructures (>10 μm) or structures that are further apart than 10 μm also do not show any iridescence showing that this

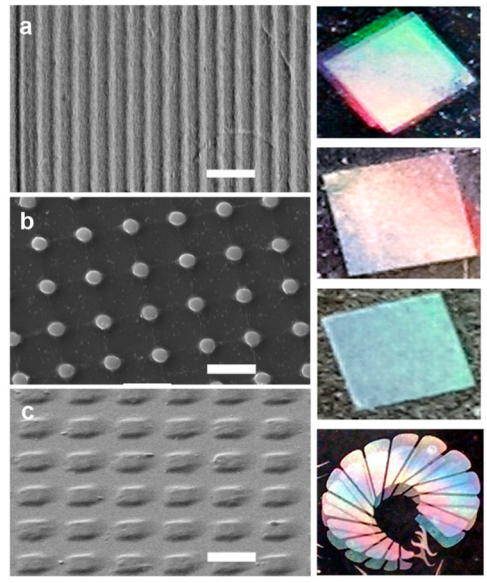


Figure 1. SEM images of diverse periodic silk microstructures that can display iridescence at the macroscale: (a) 2 μm lines, (b) 5 μm posts, (c) 10 μm squares (scale bars = 10 μm). Column on the right shows the iridescence of microstructured films (1−2 cm^2) in sunlight. The color varies with viewing angle caused by the diffraction of the arrays. The bottom shows how complex designs can be patterned over large areas with the same effect.

effect is indeed caused by the patterned microstructure. Figure 2 shows patterns patterned with and without constituent microarchitectures. Using photolithography, we can pattern any shape including lines, squares, circles, etc. In all these structures, the strength of the iridescent behavior is governed not as much by the shape of the microstructure but by their spacing. As the

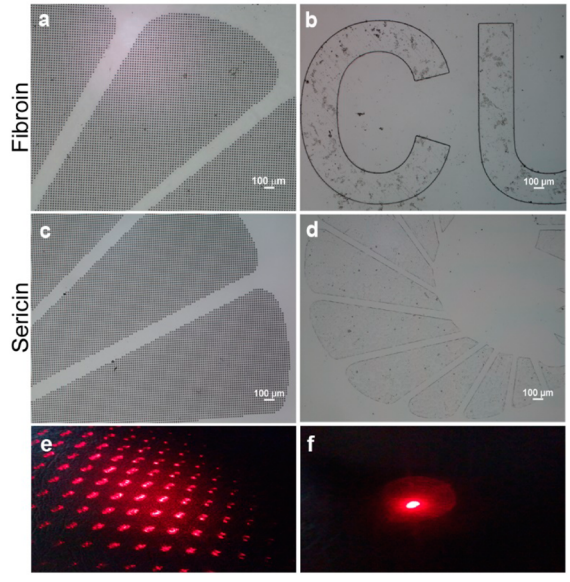


Figure 2. Optical micrographs of micropatterned fibroin (FPP) features on glass (a) with patterns, (b) without patterns and sericin (SPP) features on glass (c) with patterns, (d) without patterns. Features patterned in the absence of periodic microstructures showed no iridescent behavior. This is also (e) observed in the diffraction pattern and (f) absent in an identically formed film with no microstructures

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photonic structure periodicity is 2−10× larger than the wavelength of light, incoherent scattering causes a random phase relationship of scattered light.[38] This is further reflected in the pattern of scattered light from a microstructured film vs a nonmicrostructured film (Figure 2e, f). The structure-induced iridescence produced by the silk micropatterns strengthens the idea to use silk biomaterials and structures for soft optics and adds an important functionality to this versatile material.

3.2. Fabrication of Fresnel Zone Plates. Fresnel zone plates (FZP) utilize diffraction of light to focus light. A Fresnel zone plate consists of binary circular zones of opaque and transparent zones. Light passing through radially symmetric transparent zones gets diffracted. The zones are spaced in such a manner that constructive interference occurs at a desired focal distance to realize the image.[39] FZP lenses have been shown for concentrating lasers or X-rays as well as in imaging for environmental science and biomedical applications.[40−43] However, typically the fabrication of FZP lenses has been via techniques such as electroplating, dry etching, and atomic layer deposition.[42] Here, Fresnel plates consisting of 15 opaque and transparent zones were fabricated using silk proteins on glass via photolithography. Successive zone edges were calculated by utilizing the equation $r^2 = fn\lambda$, where r is radius of successive zones, n varying from 1 to 15, λ = wavelength of light taken as 600 nm, and f = focal length.[44] Three different arrays were fabricated with focal lengths from 1.35 to 13.5 cm. The radially symmetric rings, known as Fresnel zones, alternate between opaque and transparent. Here we show both alternating FZPs using a protein with a dark carbon dye (Figure 3a) as well as completely transparent rings without any dye (Figure 3b). SEM images showing close-ups of the lenses show a clear morphology and the ability to form arrays over large areas (Figure 3c, d). Finally, fabricated FZP lenses having focal length of 13.5 cm were tested under an optical microscope (5× magnification). The transmission image can be seen in Figure 3f. The focusing of the light can be seen at center of the Fresnel lens. It may be noted that better light concentration can be achieved if the opaque zones are perfectly dark and a monochromatic light source is used to perform the experiment. This has been a challenge in the fabrication of such lenses and only recently was the use of carbon nanotubes suggested as a possible solution.[45]

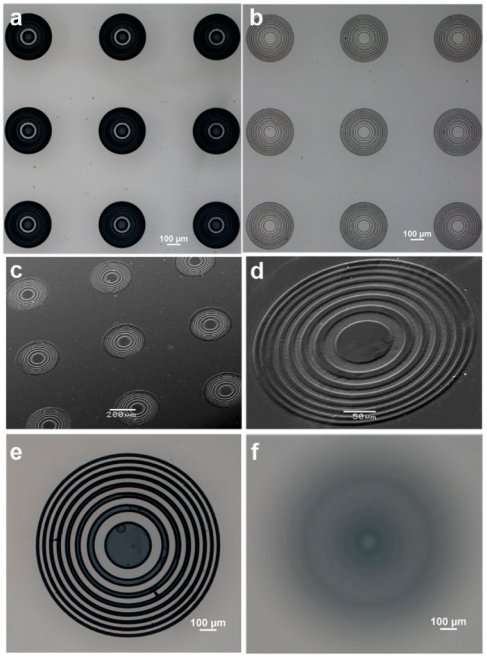

Figure 3. Optical image of (a, b) FZP arrays fabricated from sericin with and without carbon dye to form alternating circles. (c, d) SEM images of Fresnel lenses. (e) Optical image of single lens with a focal length 13.5 cm and (f) light focusing by the lens.

FZPs have many advantages over refractive lenses, specifically in that the amount of the material needed for focusing light is less. Second, large f-number Fresnel lenses can handle higher magnitude of ripples of membranes on which they are mounted without appreciable focal spot destruction. This latter advantage makes them particularly suitable for soft and flexible biomedical optics applications. However, typically the optical efficiency of the Fresnel zone plates is low as alternating opaque zones block approximately half of the incident radiation. To date, 12% optical efficiency has been achieved by utilizing vertically aligned carbon nanotube array as a material for opaque zones.[45] Using photolithography, FZPs can easily be patterned on flat surfaces instead of using complicated and expensive gray scale lithography techniques needed to form the structures of a Fresnel refractive lens.[46] The simplicity of fabrication by silk protein photolithography makes silk FZPs a suitable choice for application in implantable optical devices to manipulate light. It opens another avenue for research to be explored and further reinforces the idea of silk proteins to be used as optical materials for implantable sensors and devices.

3.3. Proteolytic Degradation of Micropatterns in Vitro. A significant challenge in the development of soft micro-optics is in the material of choice. As such, current devices are the purview of glass, metals and synthetic polymers. It is in this context that the search for optical biomimetic systems that also utilize natural biopolymers has been initiated.[6,8,18] As shown above, silk proteins can be used to make optical microarchitectures. One of the primary advantage of silk proteins is that they are proteolytically degradable in forms such as silk protein fibers, yarns, freestanding films, and scaffolds.[32,47,48] Mass loss; reduction of thickness, average molecular weight, and change in surface characteristics, mechanical properties or crystal structure have been studied in these bulk scale arrangements. However, in the formation of microarchitectures, their degradation behavior is largely unknown. When a silk film is studied for analyzing degradation behavior, usually it is open to proteolytic enzyme attack from all sides. Therefore, rate of degradation may be faster than if silk proteins patterns are anchored on a substrate. Here our objective is 2-fold- (a) demonstrate that the optical microarchitectures can also be completely degraded in vitro and, (b) investigate the degradation profiles of micropatterned structures of silk proteins (fibroin and sericin) on glass or silicon substrates. This may be useful in the design of patterns that may be controlled in terms of life span of silk-based optical devices subjected to a challenging or degradation environment.

Optical microscopy, AFM and SEM were used to characterize surface morphology and integrity of silk protein structures. As an example, the optical images of degradation behavior of fibroin patterns on a glass substrate have been shown in Figure 4. As the top surface was primarily available for the enzyme to act, the surface roughness of the features increased with time. The formed cavities due to degradation increase in size and number. After 5 weeks, the features were almost completely

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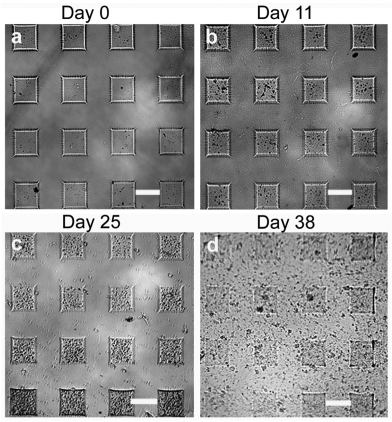

Figure 4. Optical imaging of FPP degradation study (A) day 0, (B) day 11, (C) day 25, (D) day 38 (scale bars = 100 μm).

degraded, after which the features delaminated from the surface of glass. The observed degradation behavior was not similar to previous studies. In contrast, for the water-soluble sericin, the photo-cross-linked microstructures were completely degraded in around 3 weeks (data not shown). In our earlier reports, free-standing films of fibroin and sericin were completely degraded in ~2 weeks, showing that the anchoring of the patterns on the substrate affects the kinetics of enzymatic action.[28,29]

To further explore the mechanism of progression of microstructure degradation at the surface, we conducted a shorter duration study, at which both micro- and nanoscale changes are observed. After 1 week, the disintegration can be observed by optical microscopy as discussed above. The SEM images of the microstructures at day 0; and at day 6 in degradation medium and in a phosphate buffer solution (control) have been shown in Figure 5 (top). The degradation of sericin patterns is higher than the fibroin patterns as evidenced by the higher growth of cavities. It may be noted that the controls in PBS also show a slight degradation, which is, however, much less compared to the degradation of patterns subjected to the enzyme solution. In contrast, the micropatterned films have maintained their structure for several months during storage in dry condition, showing their overall mechanical stability. Atomic force microscopy (AFM) was done to investigate the surface morphology of the silk protein structures (Figure 5, bottom). The results of AFM study are consistent with the results of SEM. At day 6, fibroin patterns subjected to protease solution shows several micrometer size cavities that were not evident on SEM images. The fibroin control samples increased in roughness, but no observable cavities developed on the surface. The root-mean-square surface roughness data as measured from AFM imaging is shown in Table 1. Several microstructures (n = 5) were imaged over 10 × 10 μm areas with replicates for this data.

The two silk proteins, sericin and fibroin, have different molecular structure. Silk fibroin mostly comprises beta sheets of a highly repetitive sequence of Gly-Ala-Gly-Ala-Gly-Ser, which makes it highly crystalline and thus insoluble in water.[49] On the other hand, sericin is mostly a random coil structure that makes it amorphous and therefore soluble in water.[50] Protease enzymes are more active and tend to bind easily to amorphous regions, in comparison to crystalline regions and low-molecular-weight, noncompact proteins.[51,52] As most of the regions of sericin are amorphous random coils, the protease is

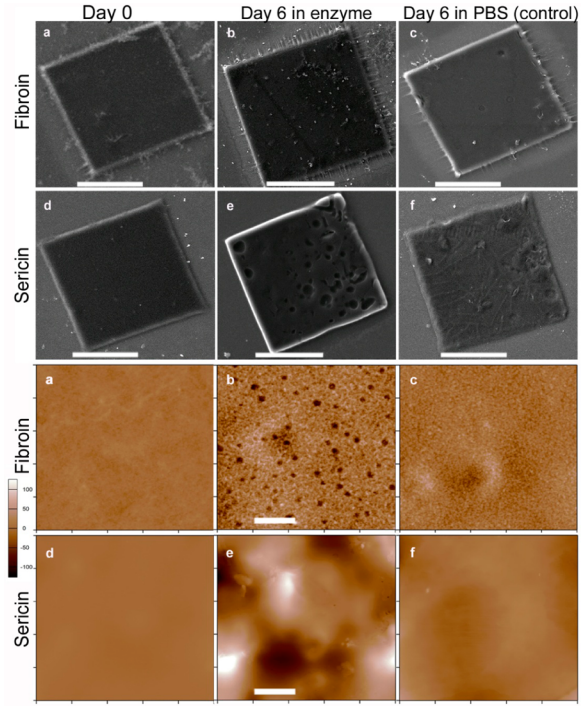

Figure 5. (top) SEM imaging of degradation study of micropatterned fibroin (a) day 0, (b) day 6, (c) day 6 (control); and sericin (d) day 0, (e) day 6, and (f) day 6 (control). Scale bar =50 μm. (bottom) Close-up using AFM images of the degradation study showing the surfaces of photopolymerized fibroin and sericin films. Fibroin: (a) day 0, (b) day 6 in enzyme, (c) day 6 in PBS buffer (control); and sericin: (D) day 0, (E) day 6 in enzyme, (F) day 6 in PBS buffer (control). Scale bar = 2 μm. z-scales are the same on all AFM images.

Table 1. RMS Surface Roughness (nm) of Fibroin Protein Photoresist (FPP) and Sericin Protein Photoresist (SPP) Microstructures during Enzymatic Degradation As Measured from AFM Imaging (values indicate mean ± s.d.)

		in enzyme solution	in PBS control
days	0	6	6
FPP	5.9 ± 0.3	25.5 ± 1.8	16.0 ± 1.2
SPP	4.1 ± 1.3	65.2 ± 28.7	15.9 ± 3.9

likely to attack and degrade the protein easily. On the other hand, fibroin with β-sheet crystalline domains is likely to be much more resistant to the protease attack. In our work, the overall degradation analysis also suggests that this is indeed the case, as the sericin degrades faster than the fibroin in the presence of protease, consistent with previous studies. The most probable mechanism of degradation of silk protein features is that the enzyme gets adsorbed in very small cavities from where it starts to degrade the protein patterns. These cavities then act as a nucleation sites for enzyme action. Therefore, over time, cavities increase in size and new cavities are created. The observed degradation behavior provides new insight on proteolytic degradation of proteins patterns attached to substrates. Finally, it is also important to note that the degradability of silk proteins can be controlled by changing the degree of cross-linking of silk photoresist.[28,29] This provides a route to form stable architectures that can be programmed to degrade over a specific time.

3.4. Flexible Soft Micro-optics. Soft optical systems, which can be folded or stretched repetitively without any

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significant loss to their mechanical or optical properties, have applications as adaptive microlenses, paperlike displays, and importantly, biointegrated photonic micro/nanodevices.[53] Further, because of their biocompatibility they can be integrated into microfabricated implanted devices as integrated biosensors or as ocular systems.[54,55] Herein, we demonstrate that the silk photolithography technique can be used to fabricate microstructures on a biodegradable elastic sheet to form flexible optical systems. As discussed above, rigid substrates such as silicon and glass can be used to form both periodic structures and FZP lenses. Using a silk fibroin sheet as the underlying substrate, we are able to form an integrated optical device completely out of silk proteins. Both periodic microstructures of SPP and Fresnel lenses were patterned as shown in Figure 6. These sheets with lenses and structures can

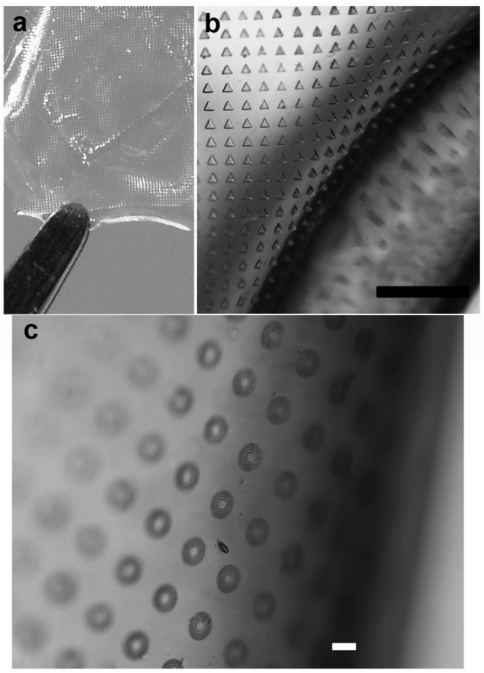

Figure 6. Large-scale flexible micropatterns formed by photolithography. (a) Flexible fibroin sheet with periodic microstructures on the centimeter scale, (b) bending of the films (scale bar = 500 μm), (c) Fresnel lenses patterned on a flexible sheet (scale bar = 100 μm).

be patterned over large (cm) scales and can be repeatedly twisted or folded as shown in Figure 6b, c. Similar to the patterns on glass, the sheets are mechanically robust and can be stored for several weeks without any degradation. In solutions of protease, the films disintegrate over a period of around 4 weeks. By controlling the degree of cross-linking of the films (MeOH activation of the fibroin sheets, higher cross-linking of the protein photoresist), it will be possible to increase the life of these soft optics for various implantable systems.

3.5. Note on Biocompatibility. These results show how the facile microfabrication of periodic microstructures using the two silk proteins—fibroin and sericin can be used to form optical devices that may be considered for biological and implantable applications. To date, fibroin has comprised the bulk of research because of its processability, high mechanical strength, and biocompatibility. In contrast, the sericin protein, typically discarded during the fibroin purification process has received considerably less attention.[56] Our group and others have noted that sericin is indeed biocompatible,[13] whereas the perceived inflammatory behavior of silk sericin is often when it is present in conjunction with fibroin (or that of fibroin when it coexists with sericin).[57] Both proteins in the photoresist form are easily and cheaply synthesized, and via photolithography can be used in complementary ways for engineered micro- and nanostructured biophotonic elements.

4. CONCLUSIONS

We have shown that the silk proteins fibroin and sericin can be photopatterned into high-precision microscale features of various shapes and sizes ranging from 1 to 100 μm using photolithography. The processing conditions of silk photolithography are relatively benign in comparison to other micro/nanopatterning techniques reported, and can be conducted rapidly and inexpensively on the benchtop. This method is versatile and can be formed into diverse shapes that can be patterned at the macroscale (several cm) on both rigid substrates and flexible sheets. The 2D array of periodic micropatterns produce angle-dependent structure-induced iridescence, while Fresnel zone plates can be used for the focusing of light. In addition, these soft optical systems are mechanically stable in air over several months, and stable in liquids for weeks. On the other hand, they degrade completely in a few weeks because of proteolytic degradation, which can be controlled by controlling the degree of cross-linking of the proteins. Therefore, the silk protein lithography route may be a potential route to make precise biofriendly and biodegradable features rapidly without any limitations on shape and size. The characteristics reported here can lead to further studies where silk can be studied as a potential optical biomaterial.

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Notes

The authors declare no competing financial interest.

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