Bioactive “self-sensing” optical systems

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Free-standing silk films are useful materials to manufacture nanopatterned optical elements and to immobilize bio-dopants such as enzymes while maintaining their biological activity. These traits were combined by incorporating hemoglobin into free-standing silk diffraction gratings to fabricate chemically responsive optofluidic devices responsive to ambient gas conditions, constituting a simple oxygen sensor. This type of self-analyzing optical system is enabled by the unique ability to reproduce high-fidelity optical structures in silk while maintaining the activity of entrapped proteins such as hemoglobin. These bioactive optical devices offer a direct readout capability, adding utility into the bioresponsive material arena. © 2009 American Institute of Physics.

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Silk, the luxurious fiber¹ that drove ancient trade,² finds itself respun into highly transparent, strong optical materials.³ The constituent protein that provides the structural properties, silk fibroin,⁴ is isolated into aqueous solution⁵ that can be dried to film formats with thicknesses between a few nanometers to hundreds of micrometers. These films are optically flat and transparent across visible wavelengths into the mid-infrared.⁵ Through soft lithography^{6,7} these films can reproduce planar photonic structures with high fidelity, with resolution below the diffraction limit of visible light (fewer than 20 nm).⁵ Silk fibroin films also possess the ability to maintain the activity of embedded proteins that would otherwise degrade when in aqueous solution or in air.^{5,8} This combination of properties enables a class of active optical devices^{9,10} merging high quality photonic structures that respond through the embedded proteins to analytes in their surrounding environment.¹¹ We present the embodiment of such an active optical device: a hemoglobin-infused silk diffraction grating within a microfluidic device whose optical absorption changes in response to the oxygen content of water in a flow cell. Fabrication of optical structures using silk allows the introduction of any number of active proteins into a photonic structure to enable a broad range of sensing modalities.

The device presented here can be considered as a particular case of optofluidic devices, where the optical response is caused by the material constituent of the microfluidic device (and not strictly by the motion of fluids).

Optofluidics,^{9,10} was initially developed to unite into a single material platform the functionalities of microfluidics and photonics and offer novel optical modulation technologies.^{12,13} Traditional optofluidic devices, for the most part, have relied on inorganic constituents such as silica,¹⁴ silicon,¹⁵ polydimethylsiloxane (PDMS),⁶ polymethylmethacrylate and related polymers,¹⁶ which are commonly used materials in photonics or microfluidics. Such

constituents, in fact, possess suitable and well-characterized optical and device fabrication-relevant material properties but they are not inherently chemically sensitive or highly specific to analytes. To overcome this, it is possible to functionalize the surfaces of these materials with chemical reagents,¹⁷ however, a much broader range of sensitivities and specificities can be achieved if proteins or enzymes are used as the sensitizing agents. Binding or immobilizing proteins to inorganic or synthetic polymer surfaces while maintaining their function is complex and prone to loss of activity-if feasible at all.^{17,18} Ideally, a material that possesses excellent optical qualities that can be formed into a variety of geometries while maintaining the activity of embedded proteins would allow for the realization of a new class of bioactive optofluidic devices.

Pure silk films³ possess a combination of traits including optical transparency, ease of patterning, and the ability to entrap dopants and maintain their biochemical activity. This enables the marriage of active microfluidic structures with high quality photonic structures whose optical properties are dependent on the biochemical state of the embedded proteins and analytes in the surrounding fluid. Active silk fibroin optical devices exploit photonic structures to analyze changes in the base material to pave the way for compact, active, optical, protein based sensing device platforms. An embodiment of active silk optical devices is presented here with a silk diffraction grating doped with lysed red blood cells. The hemoglobin in the lysate remains active when mixed into the silk matrix and changes optical absorption depending upon the degree of oxygenation. The silk grating becomes “self-analyzing” providing both chemical and spectral analysis due to the activation of the hemoglobin-silk device materials.

The hemoglobin doped silk grating forms one side of a microfluidic flow cell. The remainder of the flow cell consists of PDMS faced with glass and filled with de-ionized water. Gas (pure N₂ or O₂) is bubbled through the water in the flow cell increasing and the effect on hemoglobin in the silk grating is tracked. Hemoglobin is responsible for oxygen transport in blood and coordinates naturally with O₂ molecules. As the concentration of N₂ in the flow cell increases the concentration of de-oxyhemoglobin in the silk film also

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FIG. 1. (Color online) Schematic of the experimental layout used to probe the activity of the hemoglobin doped silk grating (i.e., the “self-sensing” spectrometer). A recollimated white light source from a halogen lamp is directed onto the hemoglobin silk grating. The diffracted orders were detected using a linear CCD array in the dispersed direction of the spectrum in response to changes in oxygenation within the flow cell. The inset also shows an image of a free-standing silk-hemoglobin diffractive grating.

increases. Exposure to O_2 reverses this process. These exposures change the spectral absorption of the film that is analyzed with white light that is diffracted by the grating patterned on the silk film. Proteins such as hemoglobin degrade in solution over time.¹⁹ The capacity to store proteins embedded in the silk matrix for a prolonged period,⁸ a unique property of silk, enables the biochemical activity of the grating to be maintained under ambient conditions for months. Figure 1 shows the experimental layout used to build a simple spectrometer based on the hemoglobin doped silk grating. White light from a tungsten source illuminates the grating inside the flow cell. The grating diffracts the white light that is detected using a charge-coupled device (CCD) line camera. This simple spectrometer uses the silk grating as both the dispersive element and as the sample.

Fabrication of the hemoglobin-doped silk fibroin grating was performed using a simple modified-lithography casting technique. Lysed red blood cells were combined with an 8 w/w % solution of silk fibroin¹³ in a 1:1 ratio. This doped silk solution was cast on a holographic diffraction grating (600 lines/mm) and allowed to dry in ambient conditions for 16 h. After this time, successful replication of the diffraction grating is achieved in the fully dry, hemoglobin-doped silk film. To ensure water insolubility of the dried film, a beta sheet crystallization, or physical cross-linking, was enacted with exposure of the material to 100% methanol for 8 h. The device was allowed to dry in the open air for 2 h to evaporate the solvent and then was reimmersed in DI water. The soft gratings were cut into 1 cm squares using a scalpel and mounted vertically in a PDMS flow cell full of DI water. Either nitrogen or oxygen was then bubbled through the flow cell to affect the gas binding properties of the entrapped hemoglobin. The gas flow was regulated by mass flow controllers set to 0.3 SCCM with an input pressure of 7 psi.

The grating was probed using a tungsten white light source collimated by a $4\times$ microscope objective lens and spatially filtered with an iris. The beam passed through the silk-hemoglobin grating after which the first diffracted order was focused using a 10 cm focal length lens onto a CCD line camera. This arrangement formed a simple spectrometer based on the silk-hemoglobin diffraction grating. Calibration was obtained by using two band pass filters that had trans-

FIG. 2. (Color online) Experimental absorption spectra of the hemoglobin silk grating (spectrally resolved by the grating itself) when exposed to oxygen (solid) in comparison with tabulated absorption data for oxy-hemoglobin from the literature (dotted). Both traces are offset for clarity.

mission wavelengths of $\lambda_1=600$ nm and $\lambda_2=530$ nm with bandwidths of ± 5 nm. The resolving power of the silk spectrometer in this arrangement was calculated to be ~ 400 . The background spectral measurement was taken using an identically fabricated grating made of pure silk, without the hemoglobin. This measurement was used as the baseline to calculate the absorbance of the hemoglobin-silk gratings. The transmission spectrum of the hemoglobin doped silk film inside the water filled flow cell is shown in Fig. 2. Notably, this spectrum was taken *two months* after the fabrication of the silk grating during which time the material was stored in air at room temperature. The presence of the characteristic oxy-hemoglobin absorption peaks at 540 and 575 nm suggests persistence of the activity of hemoglobin within the free-standing silk matrix despite the fabrication process, storage in the laboratory and repeated experimentation.

The hemoglobin-silk grating was probed for activity in response to the surrounding environment by exposure to nitrogen and oxygen in the flow cell. The results are illustrated in Fig. 3. At first, nitrogen was flowed for 20 min into the cell and the spectral response of the system was probed. The hemoglobin spectral response was found to change through the suppression of the pair of spectral peaks at 540 and 575 nm suggesting an increase in the concentration of deoxyhemoglobin as N_2 is bubbled through the flow cell. Oxygen was then bubbled through the flow cell and absorbance measurements were recorded every 5 min. Over the course of 30 min, the characteristic oxy-hemoglobin absorbance peaks re-appeared indicating the maintained capacity of the hemoglobin contained within the silk grating to bind oxygen. The process described here was found to be repeatable and reversible by replicating the experiment multiple times on the same sample ($n=6$) and obtaining comparable results. No leaching of hemoglobin from the grating was observed by spectrally analyzing the water in the flow cell after each measurement. It is noteworthy that the hemoglobin in the silk film retained its activity after the two month storage time mentioned earlier and a further ~ 100 minutes of immersion in the flow cell despite the potential for protein degradation in solution.¹⁸

These results provide evidence of one of the unique properties of silk that enables this active optical device; the ability to embed proteins within its matrix and keep them chemically active. The mechanisms behind this stabilization

FIG. 3. (Color online) Spectral readings from the self-sensing hemoglobin spectrometer illustrating the change in device wavelength absorption as a function of decreasing oxygenation. The spectral features slowly changed and the characteristic absorption peaks for oxy-hemoglobin disappeared as the ambient gas concentration was changed and the nitrogen concentration increased: (a) trace after exposure to nitrogen for 20 min, [(b)–(d)] spectral responses of the grating after 10, 15, and 20 min of exposure to oxygen, respectively. The process is repeatable and reversible.

effect are being elucidated.⁸ Likely mechanisms include nanoscale structural features derived from the unique, highly hydrophobic, protein block copolymer features of the silk fibroin protein.^{20–22} These amphiphilic features result in nanoscale beta sheet crystals within small intervening hydrated regions with somewhat less structural organization and with an overall very low content of water. Essentially any biological molecule with an optical response could be incorporated within the silk material forming the basis for a multitude of optical sensors.

In summary, an active optical device was demonstrated where the material absorption features change in response to ambient dissolved gas concentration. This device is enabled by the unique properties of silk, its ability to house proteins and maintain their activity even when dried into film form, and the ability to form the protein into highly transparent, finely featured optical structures. Specifically, a hemoglobin doped silk diffraction grating that changes its absorption depending upon the ambient concentration of nitrogen gas was demonstrated. The film is self-analyzing by using the incor-

porated grating structure and constitutes a simple gas sensor. This device prototype constitutes a particular class of active optofluidic structures which are enabled by silk and offer a broad variety of device options dependent on the nature of the bioactive component embedded within the silk optical material used in device fabrication.

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