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<td>Author(s)</td>
<td>Lee, Elizabeth; Yong, Derrick; Yu, Xia; Li, Hao; Chan, Chi Chiu</td>
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In-fiber photo-immobilization of a bioactive surface

Elizabeth Lee
Derrick Yong
Xia Yu
Hao Li
Chi Chiu Chan
In-fiber photo-immobilization of a bioactive surface

Elizabeth Lee,a,b Derrick Yong,a,b Xia Yu,c,* Hao Li,a and Chi Chiu Chanb
aPrecision Measurements Group, Singapore Institute of Manufacturing Technology, 71 Nanyang Drive, Singapore 638075, Singapore
bNanyang Technological University, Division of Bioengineering, School of Chemical and Biomedical Engineering, 70 Nanyang Drive, Singapore 637457, Singapore

Abstract. We demonstrate the first in-fiber light-induced bioactive biotin-functionalization via photobleaching fluorophore-conjugated biotin. Photobleaching the fluorophores generated free radicals that bind to the albumin-passivated inner surface of pure silica photonic crystal fiber. The subsequent attachment of dye-conjugated streptavidin to the bound biotin qualified the photo-immobilization process and demonstrated a potential for the construction of in-fiber macromolecular assemblies or multiplexes. Compared with other in-fiber bioactive coating methods, the proposed light-induced technique requires only a low-power light source, without the need for additional preactivation steps or toxic chemical reagents. This method, hence, enables a simple and compact implementation for potential biomedical applications. © 2014 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.19.12.120502]

Keywords: photochemistry; fiber optics; photonic crystal fiber; biotin; streptavidin; fluorescence.

Paper 140605LR received Sep. 24, 2014; accepted for publication Nov. 12, 2014; published online Dec. 18, 2014.

Bioactive surfaces are synthetically engineered surfaces capable of biological interactions with a myriad of biomolecules. These surfaces have demonstrated the efficient capture and attachment of biomolecules, enabling applications like filtration, the directing of cell growth and movement, as well as improving the biocompatibility of implantable materials. Another notable application is surface-specific optical biosensing which exploits the biorecognition capability of the bioactive layer. It involves the coating of optical waveguide surfaces with desired bioactive elements for light–matter interactions localized to several hundred nanometers from its surface. Among the various optical waveguides, optical fibers clearly stand out with the advantages of miniaturization, long interaction lengths, and easy integration within systems. The conventional immobilization of bioactive layers on their outer surfaces, however, entails the incorporation of supplementary flow cells for sample introduction, overshadowing the miniaturization offered by optical fibers. On the contrary, microchannel-like air hole structures in microstructured optical fibers and photonic crystal fibers (PCFs) offer intrinsically self-contained systems. Although there have been successful demonstrations of coating the bioactive layer on their air hole surfaces, they were typically achieved via covalent or electrostatic layer-by-layer attachments. The former required the separate activation of both the surface and bioactive element, whereas the latter involved an elaborate successive layering process. Additionally, both methods employ harsh chemicals during the coating process. Laser-assisted protein adsorption by photobleaching (LAPAP)—a facile photo-immobilization of biomolecules (fluorophore-conjugated) on substrates (alummin-passivated)—presents itself as an attractive substitute. In brief, the method involves the photobleaching of fluorophores, leading to their radicalization and consequent binding to albumin. The immobilization process does not require any preactivation reactions, laborious procedures, or the use of toxic chemicals. Moreover, there have been recent demonstrations of using PCFs for photochemical reactions alluding to the feasibility of an in-fiber light-induced immobilization method. The added fact that the fluorophores to be photobleached have been shown to be easily conjugated to a wide range of nanoparticles and biomolecules, such as biotin, marked the versatility of this immobilization technique. Biotin is of particular interest as it is used extensively in biological studies due to its specificity and binding affinity with streptavidin and other avidin-like molecules. The ease of conjugating biotin or streptavidin with fluorophores, phospholipids, antibodies and antigens, nanoparticles, enzymes, and genetic sequences has led to a myriad of biomolecular applications. In addition, streptavidin has four binding sites to bind biotinylated molecules, further facilitating the formation of biomolecular assemblies or multiplexes for bioanalytical detection and separation.

We report the first experimental demonstration of an in-fiber light-induced bioactive coating using an LED light source. This process is further qualified by the subsequent optical detection of biotin-bound dye-conjugated streptavidin. We show that the method is simple yet effective in functionalizing in-fiber surfaces.

The PCF used was a defected-core PCF manufactured by Yangtze Optical Fibre & Cable Company Ltd. Each PCF was cleaved to a length of 105 mm and inserted into the optofluidic platform [described in our earlier work (Ref. 15)] at a bending radius of 12.5 mm, as shown in Fig. 1. Photo-immobilization within the aforementioned PCF was done via an adaptation of the LAPAP protocol. First, the PCF air holes were passivated with a base layer of bovine serum albumin (BSA) through a continuous infiltration with 0.5% BSA solution for 45 min, achieved with the aid of a modified syringe pump. Photo-immobilization was subsequently attained via a 10 min in-fiber excitation of infiltrated 77.6 µm biotin–4-fluorescein (B4F) solution, facilitated by the earlier mentioned optofluidic platform. A 10-min duration was chosen as no significant spectral changes were observed beyond that. The excitation source employed was a 2.3 mW, 490 nm fiber-coupled LED (Thorlabs M490F1), matching the absorption of B4F. Prolonged illumination of the fluorescein component in B4F hence led to its photobleaching, which resulted in the formation of BSA-binding radicals.

The consequential photo-immobilized B4F, forming the bioactive surface as depicted in Fig. 2, was qualified with the use of a dye-conjugated streptavidin—Atto488-conjugated
streptavidin (Atto488Strep) with a concentration of 1 mg/ml—
that was allowed to incubate for 5 min upon infiltration. It
should be noted that after each layer, the PCF was flushed
with distilled ultra-filtrated water to remove excess reagents
and its corresponding output spectrum was recorded with an
Ocean Optics Maya2000 spectrometer using the same LED
excitation. To ensure repeatability and to mitigate intensity fluc-
tuations when comparing triplicates, a dimensionless ratiometric
relationship between the intensities at Atto488’s peak emission
and absorption wavelengths was used.

$$\frac{I(\lambda_{\text{ems}})}{I(\lambda_{\text{abs}})}$$

Certain values are shown in Table 1. Figure 3 shows the collected output spectra, normalized to
their respective peak intensities. Upon Atto488Strep binding
(BSA-pbB4F-Atto488Strep), a significant dip and hump was observed around the 500 and 523 nm regions, respectively,
as shown in Fig. 3. These corresponded to Atto488’s peak
absorption and emission and thus were indicative of the presence of biotin-bound Atto488Strep, implying a successful
photo-immobilization of B4F.

Another repetition made without photobleaching the B4F, by
essentially incubating the B4F for the same amount of time
without illumination, exhibited residual amounts of Atto488
absorption and emission. However, it should be noted that
this corresponded to the presence of only minute quantities
of biotin-bound Atto488Strep. This could be attributed to
small amounts of photobleaching due to the LED exposure dur-
ing measurements and ambient light; and/or the nonspecific
binding of Atto488Strep directly on the PCF inner surface.
To assess the contribution from the nonspecific binding, a rep-
etition of the process excluding B4F altogether with no incuba-
tion of Atto488Strep was done. It showed that any introduction
of Atto488Strep caused the spectrum to deviate from the BSA
only plot as shown from the ratios obtained in Table 1. However,
this corresponded with only a diminutive presence of

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<th>PCF coating</th>
<th>$\frac{I(\lambda_{\text{ems}})}{I(\lambda_{\text{abs}})}$</th>
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<tr>
<td>BSA-pbB4F-Atto488Strep</td>
<td>1.2454 ± 0.2539</td>
</tr>
<tr>
<td>w/o photobleaching</td>
<td>0.6512 ± 0.07203</td>
</tr>
<tr>
<td>w/o B4F</td>
<td>0.3671 ± 0.04727</td>
</tr>
<tr>
<td>BSA only</td>
<td>0.2078 ± 0.01522</td>
</tr>
<tr>
<td>BSA-pbB4F</td>
<td>0.3193 ± 0.02660</td>
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Atto488Strep which further substantiated the successful photo-immobilization of B4F above.

To further eliminate spectral contributions due to BSA or pbB4F, plots representing the BSA only layer and the subsequent layer of pbB4F (BSA-pbB4F) were likewise acquired as shown in Fig. 3 (inset). As expected, the UV-absorbing BSA coat did not present any fluorescence emission. Likewise, the subsequent photo-immobilization of B4F did not contribute to the distinct dip and hump observed in the BSA-pbB4F-Atto488Strep plot. The relatively Gaussian-like pbB4F spectrum points to the absence of fluorescing B4F, which absorbs at $\lambda_{abs} = 494$ nm and emits at $\lambda_{ems} = 523$ nm. These observations further corroborate that the BSA-pbB4F-Atto488Strep spectrum seen is certainly due to the presence of biotin-bound Atto488Strep.

It was observed that the $I(\lambda_{ems})/I(\lambda_{abs})$ ratios obtained could be categorized into three distinct groups: (1) definite presence of Atto488 (BSA-pbB4F-Atto488Strep); (2) minute presence of Atto488 (w/o photobleaching); and (3) no presence of Atto488 (BSA, BSA-pbB4F, and w/o B4F). These ratios give an indication of the concentration of Atto488, correlating with the extent of biotin photo-immobilization for the plots in Fig. 3 and further exemplifying that all three spectra were distinct from each other.

In summary, we demonstrated an in-fiber bioactive biotin-functionalization via a simplification of the LAPAP protocol. The consequent detection of biotin-bound Atto488Strep alluded to the successful photo-immobilization of B4F. The biotin bioactive layer would serve as a foundation for the subsequent construction of biomolecular assemblies or multiplexes in-fiber. In addition to conventional applications such as bioanalytical detection and separation, in-fiber light guidance opens up new avenues for photochemistry and catalysis using these constructs. To sum it up, this simple method for in-fiber photo-immobilization of a bioactive surface without the use of toxic reagents would offer great convenience and compactness for potential biomedical applications.

Acknowledgments

We wish to acknowledge the funding for this project from the Singapore National Research Foundation, the A*STAR SERC Advanced Optics Engineering TSRP under grant 1223600011.

References