

Localized biosensing with Topas microstructured polymer optical fiber

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We present what is believed to be the first microstructured polymer optical fiber (mPOF) fabricated from Topas cyclic olefin copolymer, which has attractive material and biochemical properties. This polymer allows for a novel type of fiber-optic biosensor, where *localized* sensor layers may be activated on the inner side of the air holes in a predetermined section of the mPOF. The concept is demonstrated using a fluorescence-based method for selective detection of fluorophore-labeled antibodies. © 2007 Optical Society of America
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Microstructured optical fibers (MOFs)¹ have a pattern of air holes that extend for the full length of the fiber, and the optical properties can be designed by adjusting the relative position, size, and shape of the air holes. Importantly, in our context, the holes can be used to hold a biological sample, which can be studied by evanescent-wave sensing.² The combination of these options offers an opportunity for a number of MOF-based sensor applications, especially within biosensing^{3–5} and gas sensing.^{6,7} MOF biosensors have now been used as a sensing element in biochips.⁸ In this family of fibers the microstructured polymer optical fibers (mPOFs)⁹ have received particular interest. The lower melting temperature of the polymer, the more flexible hole patterns made possible by drilling the preform, the easier manipulation, and improved biocompatibility are the main advantages against their silica-based counterparts. The biocompatibility is the key issue for development of mPOF biosensors, because it allows the use of simple immobilization procedures.⁴

The most commonly used material for fabricating mPOFs is polymethyl methacrylate (PMMA). However, monomer residues inside PMMA and its aptitude for water absorption often make the drawing of commercially available PMMA rods problematic due to bubble formation. These problems can be reduced by using other polymer materials, such as Topas cyclic olefin copolymers (Topas COCs).^{10,11} Topas cyclic olefin copolymers have no monomers, and their moisture absorption is 100 times lower than that of PMMA. In addition, they exhibit an improved melt viscosity¹² and high tear strength,¹³ allowing better conditions for drawing of optical fibers.

In this Letter we present what are believed to be the first mPOFs made from Topas. Furthermore, we present a novel concept for fiber-optic biosensing based on the biochemical properties of Topas, which allows one or more *localized* sensor layers to be defined in predetermined sections of the fiber. We show how a sensing layer can be activated locally inside the fiber by using ultraviolet (UV) light. This is demonstrated with immobilization of Cy3-labeled α -streptavidin antibodies. We also confirm the selective detection of labeled α -streptavidin and α -CRP antibodies with this Topas mPOF biosensor.

Figure 1 shows two types of Topas mPOFs fabricated from rods with a diameter of 2.5 cm and a length of 7 cm. The preforms were made from granules with the trade name Topas 8007. The structures of holes with 2 mm diameter were drilled into the preforms, and the fibers were then drawn in a 175 °C

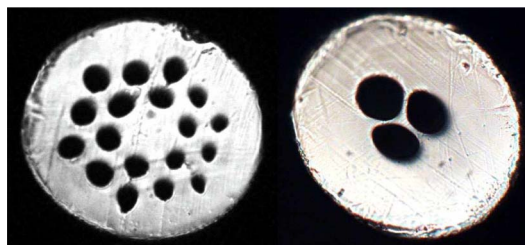


Fig. 1. (Color online) Microscope pictures of the end facets of Topas mPOFs fabricated at COM·DTU. Left: 18 hole mPOF with outer diameter 275 μm , hole diameter 32 μm , and core diameter 65 μm . Right: 3 hole mPOF with outer diameter 220 μm , hole diameter 50 μm , and core diameter 12 μm .

hot zone with a pressure of 1 mbar applied to the air holes. We have observed draw tension less than 1 g (sensitivity limit of our tension sensor), which is low compared with the usual 60–100 g for drawing of PMMA-based mPOFs.¹⁴ The low draw tension means that the optimum drawing conditions for Topas are different than from PMMA.¹⁵ The biosensor investigations presented here are done with the 3 hole mPOF, mainly because the larger holes give shorter filling time of the fiber.¹⁶ The poor guiding properties of the 3 hole Topas mPOF are not an issue for this first proof-of-principle of localized biosensing.

Topas is a chemically inert material, and direct binding of biomolecules on its surface is difficult. However, commercially available Antraquinon (AQ) linker molecules can attach to the Topas surface when activated by UV light¹⁷ and can subsequently accept sensor layers, as illustrated in Fig. 2(a). In contrast, PMMA will always accept sensor layers. As a consequence of the need for UV activation of the AQ molecules for binding of these to the Topas surface, a UV mask can be used to define localized sensor layers inside a Topas mPOF.

Figure 2(a) illustrates the concept of capture of labeled antibodies by a sensing layer defined on the Topas surface using UV-light-activated AQ molecules. A 30 cm long piece of the 3 hole mPOF shown in Fig. 1 is filled with AQ linker solution with a concentration of 1.6 mg/l. After an incubation period of 1 h, half of the fiber is illuminated with a hand-held UV lamp with an intensity of 90 mW/cm² for 10 min by moving the lamp back and forth. The peak emission of the lamp is close to the maximum absorption wavelength of the AQ biomolecules, which is at 325 nm. After this procedure the fiber is washed with distilled water. Now half of the mPOF is ready to accept the sensor layer.

To confirm the *localized* binding of the AQ molecules to the surface and hence the localized defini-

tion of a sensing layer in only one end of the fiber, we use a well-known antigen-antibody binding detection procedure,⁴ where the sensing layer is built from streptavidin molecules. A 0.5 mg/ml streptavidin solution in a 0.1 M Na carbonate buffer is incubated for 1 h before the air holes are flushed for 3 min with a phosphate buffered saline (PBS) solution. To eliminate the possibility of the AQ molecules' reacting at a later stage with nonspecific nucleophiles, a quenching procedure is applied by injecting 10 mM ethanolamine in a 100 mM sodium carbonate, pH 9.6 buffer for 1 h, after which a PBS wash is used again. As a consequence, we obtain a Topas mPOF biosensor that has half of the length prepared with a sensing layer for the selective capture of α -streptavidin antibodies.

We now expose the biosensor to a 0.05 mg/ml aqueous solution of Cy3-labeled α -streptavidin for 1 h. The fiber is finally washed with PBS and emptied with nitrogen. The result of the capture process is investigated under an epifluorescence microscope. Figure 2(b) shows an image of a 2 mm section of the fiber end with a UV-activated sensing layer, and Fig. 2(c) shows the other end with no UV activation. An excellent differentiation between the two parts is observed, proving that the sensor layer is present only where the AQ molecules have been activated by UV light. Hence we have shown that sensor layers can be defined locally in a Topas mPOF.

The main novelty of this Letter is the possibility of fabricating a robust fiber-optic biosensor with a locally defined sensing layer. However, the Topas polymer and the AQ linker are novel materials in the context of fiber-optic biosensors. Therefore we need to verify that selective detection of antibodies can indeed be obtained with this biosensor configuration. To confirm that the selective sensing scheme can be applied to detect α -streptavidin molecules, a sensing layer consisting of streptavidin molecules is bound to the immobilized AQ molecules. This sensing layer is then probed by aqueous solutions of Cy3-labeled target molecules (α -streptavidin-Cy3) or Cy3-labeled nontarget molecules (α -CRP-Cy3).

Selective detection of α -CRP antibodies is also demonstrated in a similar way. However, as in the case of PMMA-based fiber-optic biosensors for the detection of α -CRP antibodies,⁴ the definition of a CRP sensing layer in Topas mPOFs requires two steps, as CRP molecules do not bind efficiently to the AQ linker molecules. Therefore, a sandwich structure is created from α -CRP and CRP. The α -CRP sensing layer is also probed by Cy3-labeled target molecules (α -CRP-Cy3 in this case) or Cy3-labeled nontarget molecules (α -streptavidin-Cy3). In both selective detection experiments (α -streptavidin and α -CRP) the concentration of the Cy3-labeled antibody solutions is 0.05 mg/ml and the incubation time is 1 h. As in the demonstration of localized biosensing described previously, 30 cm sections of the 3 hole Topas mPOF were used. An easy-to-use fluorescence setup, where the mPOF is exposed from the side with a line-shaped laser beam,³ is used for optical characterization. The results from the fluorescence measure-

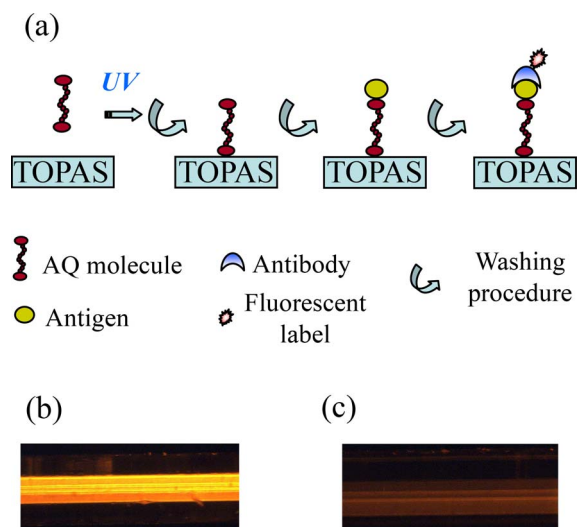


Fig. 2. (Color online) (a) Concept of immobilization of antibodies with UV light on the Topas surface. (b), (c) Epifluorescence microscope pictures of 2 mm sections of the ends of a Topas mPOF biosensor (b) with and (c) without a UV-activated sensor layer. Fluorescence is observed from only the activated fiber end in (b).

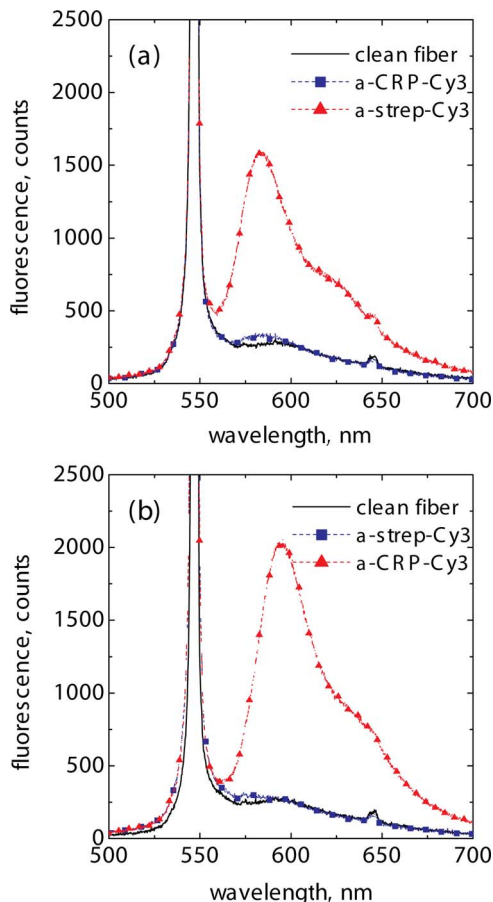


Fig. 3. (Color online) Fluorescence measurements from the 3 hole Topas mPOF biosensor (see Fig. 1) showing selective detection of (a) α -streptavidin and (b) α -CRP.

ments are presented in Fig. 3. They show an excellent selective detection of α -streptavidin and α -CRP using the two Topas mPOF biosensors. The fluorescence in the case of mismatch is close to the fluorescence from the fiber material itself, which presents the noise limit of the biosensor.

In conclusion, we have presented what is to our knowledge the first mPOF fabricated from Topas cyclic olefin copolymer. This polymer material has attractive material properties (no monomers and very low moisture absorption) and biochemical properties (it is chemically inert) compared with PMMA, the most widely used material for mPOF fabrication today. In this paper, we primarily utilize the biochemical properties of Topas and demonstrate *localized* selective detection of fluorescently labelled α -streptavidin and α -CRP antibodies from aqueous

solutions. The possibility of defining the sensing layer locally in a section of the fiber introduces the possibility of upconcentrating the target molecules in a confined region, which is advantageous in, for example, grating-based sensor configurations for label-free detection of biomolecules.¹⁸

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