**Molecularly Imprinted Polymers Used as Optical Waveguides
for the Detection of Fluorescent Analytes**

Jennifer J. Brazier, Mingdi Yan, Scott Prahl, and Yin-Chu Chen
Chemistry Department, Portland State University,
Portland, OR 97207- 0751, U.S.A.

**ABSTRACT**

This article demonstrates the novel approach of fabricating molecularly imprinted polymers (MIPs) as fiber optic waveguides for the detection of fluorescent analytes. Combining a polyurethane system and the soft lithography technique of micromolding in capillaries (MIMIC), polymer waveguides of 50 μm and 100 μm dimensions were patterned onto a silicon substrate. Laser coupling into small waveguide segments has been verified visually. Binding experiments using the waveguides are currently being explored. Some preliminary binding studies have been performed, however, for smaller, freestanding filaments of sizes consistent with conventionally prepared MIP particles. Using fluorimetry measurements, templated fibers of 20 μm dimension preferentially bound the analyte molecules by a factor of 1.5 as compared to control polymers.

**INTRODUCTION**

Molecularly imprinted polymers are biomimetic materials used for the sensitive and selective detection of small organic molecules. Through host-guest interactions, imprinted polymers often display recognition capabilities comparable to those of antibody-antigen systems.[1] The imprinted polymers, however, are much more stable to organic solvents, pH, and temperature than their biological counterparts. With such benefits, this technology has found a niche in various separation techniques such as HPLC, CEC, TLC,[2-4] and in many sensor applications.[5-6]

Traditionally, molecularly imprinted polymers are synthesized in bulk and then subjected to a grinding and sieving process that results in particles of 25 μm or larger dimension. This process, however, is tedious and often creates particles of irregular shape and size. Furthermore, many of the imprinted sites are destroyed during the grinding procedure. Because of these drawbacks, various other synthetic techniques have been employed to create polymers ranging from thin films to small beads.[7-8] This article demonstrates the novel approach of fabricating imprinted polymers in the form of fiber optic filaments for the detection of fluorescent polyaromatic hydrocarbons.

**EXPERIMENTAL DETAILS**

<u>Materials</u>

PDMS (Sylgard 184 elastomer, Dow Corning) and its corresponding curing agent were purchased from K.R. Anderson, Inc. (Kent, WA). A silicon master was patterned via conventional photolithography using SU-8 photoresist (Microchem Corporation, Newton, MA). Bisphenol A, phlorglucinol, and anthracene were purchased from Aldrich and were used as
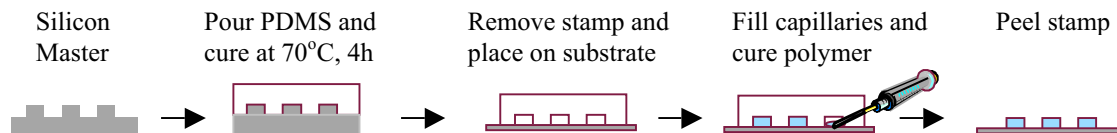
| Silicon Master | Pour PDMS and cure at 70°C, 4h | Remove stamp and place on substrate | Fill capillaries and cure polymer | Peel stamp |

**Figure 1.** The process of micromolding in capillaries.

received. A mixture of *p,p'*-diisocyanatodiphenylmethane and 30% *p,o,p'*-triisocyanatodiphenylmethane were purchased from Merck-Schuchardt (Hohenbrunn, Germany) and stored under nitrogen after use. Dimethylformamide, was distilled over $MgSO_4$ under reduced pressure and was stored over molecular sieves.

## Fabrication of MIP waveguides

Fabrication of polymer waveguides utilized the soft lithography technique of micromolding in capillaries, MIMIC,[9] shown in Figure 1. In this process, a master pattern comprised of lines (50 μm in height by 50 μm in width and 7.5 cm in length) was fabricated through conventional photolithography using SU-8 photoresist. A mixture of poly(dimethylsiloxane), PDMS, and its corresponding curing agent (1:0.07, wt/wt) was poured over this master pattern and allowed to cure at 70°C for 4 hours. The PDMS stamp was then peeled from the master, thereby producing a negative image of the original pattern. The ends of the stamp were then carefully cut with a razor blade to open up the channels and each stamp was cleaned via sonication in ethanol.

When placed on a substrate, the stamp formed small microchannels that were filled with an imprinting solution by capillary action. Prepolymer solutions were generated by mixing monomers (0.375 mmol bisphenol A and 0.455 mmol *p,p'*-diisocyanatodiphenylmethane), crosslinkers (0.250 mmol trihydroxybenzene and 0.195 mmol *p,o,p'*-triisocyanatodiphenylmethane), template molecules (0.0485 mmol anthracene) and porogen (dimethylformamide). Each mixture contained a 1:1 mol ratio of hydroxy to isocyanate functional groups and a 35% mol ratio of cross-linking components to polymer monomers. The templated polymers contained approximately 4 mol % of anthracene, which interact with the aromatic monomers through noncovalent, Π-Π interactions. Control polymers were prepared identically except for the anthracene.

To ensure covalent attachment of the polymer to the substrate, the silicon wafers were previously cleaned in piranha solution (3:1 v/v, conc. $H_2SO_4$ / 30% $H_2O_2$) and were silanized with 3-aminopropyl trimethoxysilane. Subsequent overnight polymerization under ambient conditions and stamp removal left behind imprinted filaments attached to the wafer support. Filaments were visually inspected via an Olympus BHM optical microscope. Filament segments, which appeared to be uniform in composition and geometry, were cleaved for laser coupling experiments using a 543 nm laser.

## Synthesis of freestanding filaments

Freestanding filaments of 20 μm dimension were prepared similarly except that the wafer substrates were spin-coated with PDMS at 1300 rpm for 40 s rather than being silanized. This

thin layer of PDMS (cured at 70°C for 4 h) allows for the easy removal of the 20 μm polymer filaments.  Elastomeric stamps were produced as previously discussed using a master pattern with line dimensions of 20 μm height and 20 μm width.  Stamps were placed on the PDMS coated substrate and filled with either a control or templated prepolymer solution by capillary action.

Following overnight polymerization at room temperature, stamps were peeled from their PDMS support and immersed in a bath of toluene for isolation and extraction.  Fresh volumes of toluene were repeatedly added to the filaments until no further anthracene could be detected.  The filaments were then dried under vacuum for 48 hours and massed.  These filaments were subsequently shaken with a known concentration of anthracene for 24 hours.  The fluorescence of the anthracene solutions was measured using an excitation at 358 nm and an emission at 403 nm, both before and after rebinding.  The decrease in fluorescence intensity was correlated to amount of anthracene bound by the polymer through a linear calibration curve.

## RESULTS AND DISCUSSION

In this study, polyurethane filaments imprinted with anthracene were fabricated for use as optical waveguides.  The imprinting system was adapted from the work of Dickert, where imprinted polyurethane was applied as thin films for the selective detection of polyaromatic hydrocarbons using quartz microbalance (QMB), surface acoustic wave (SAW), and planar waveguide sensors.[10-13]  In these studies, Dickert was able to optimize the polyurethane imprinting conditions such that selective detection of various polyaromatic hydrocarbons in the ppt range was observed.  The fabrication method used in this study offers the possibility of an array of  polyurethane MIPs on a single chip that may lead to simultaneous analysis of multiple analytes.

## **Imprinted waveguides**

Initial attempts at waveguide fabrication proved unsuccessful. The 7.5 cm long capillaries (both 50 μm and 100μm dimension) would not fill by capillary action alone.  They could be forcibly filled, however, with the aid of a small vacuum.  Via water aspiration, the vacuum was able to draw the prepolymer solution through the capillaries.  Unfortunately, the filaments that resulted were of poor quality.  As shown in Figure 2 a-b, the filaments were often cracked, filled with small inclusions, and of varying morphology.  It was determined that surface impurities and defects in the original photolithography master played key roles in the quality of the filaments.  By making sure that the stamps produced were without defects and that all surfaces (stamp and substrate) were free from particulate matter, the homogeneity of the filaments was dramatically improved.

Prepared under more rigorous standards, the new capillaries filled freely and immediately without vacuum assistance.  As shown in Figure 2c, the quality of these filaments was also improved.  Filaments appeared to be of uniform geometry and composition.  However, the length of these filaments was much shorter than expected.
During polymerization, the polyurethane would shrink laterally from the open end of the 7.5 cm long capillary.   As solvent evaporated and cross-linking progressed, an approximate 75% reduction in length would occur thereby producing a filament of only 2-3 cm.

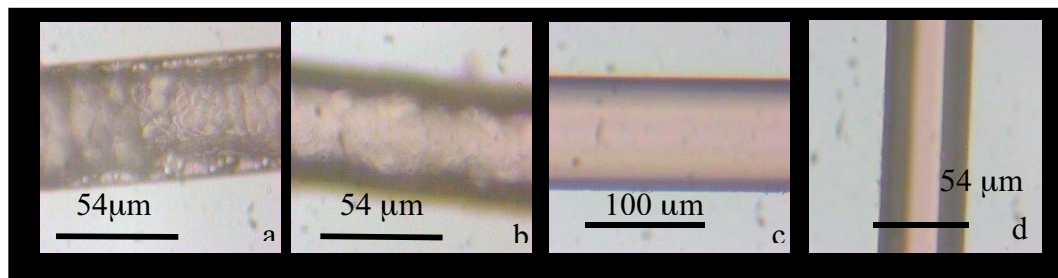To prevent this length reduction, the catalyst TMED, N,N,N',N'-

**Figure 2**. Optical micrographs showing various filament morphologies.

tetramethylethylenediamine, was placed at both ends of a filled capillary. This caused immediate polymerization of the polymer at both capillary ends, thereby making solvent evaporation much more difficult. When the stamp was finally peeled from the substrate, a 7.5 cm length filament was observed along with a significant amount of unevaporated solvent. Although the length of the filament was maintained, it was at the expense of the overall geometry of the filament. As shown in Figure 2d, trapezoidal fibers were often the result of these experiments. For this reason, fiber quality was chosen at the expense of length and the filaments were allowed to shrink. Small segments of imprinted filaments containing no inclusions or defects were cleaved and tested for performance.

In theory, a single wavelength laser can be coupled into such polymer filaments as long as the polymer index of refraction is lower than the index of refraction of the surrounding media and the filament is of high quality. Previous work has already shown successful waveguide performance by nonimprinted polyurethane filaments fabricated through microcontact printing on a silicon substrate.[14] If light can be coupled into the imprinted waveguides, the degree of analyte binding may be measured. As the light is guided through the filament via multiple internal reflections, it can excite and cause an emission from fluorescent molecules, such as anthracene, that bind to the imprinted polymer matrix. The emitted fluorescence from the analytes can be measured and correlated to analyte concentration.

While binding tests have not yet been performed, coupling of a 543 nm and 632 nm He-Ne laser into 0.5 cm waveguide segments has been verified visually. A lens with a focal length of approximately 10 cm was used to focus the laser into the waveguide. Coupling was verified by rotating the waveguide relative to the optical axis such that the light emitted from the fiber was aimed in a direction that was distinct from the illumination light. The distal end of the waveguide lit up as well. Quantitative tests are currently being performed.

**Freestanding Filaments**

Freestanding filaments were prepared in a manner similar to the polymer waveguides. The main fabrication difference was that the substrate surface was coated with a thin layer of PDMS to facilitate removal of the freestanding filaments during isolation. Therefore, the imprinting solution was sandwiched between PDMS on the substrate support and the PDMS stamp. After polymerization, the PDMS stamp was easily peeled from the substrate resulting in well-formed 20 μm filaments inside the PDMS stamp as shown in Figure 3a.

Previous studies involving MIMIC and freestanding MIP filaments employed tetrabutylammonium fluoride, TBAF, in the isolation procedure.[15] TBAF is known to
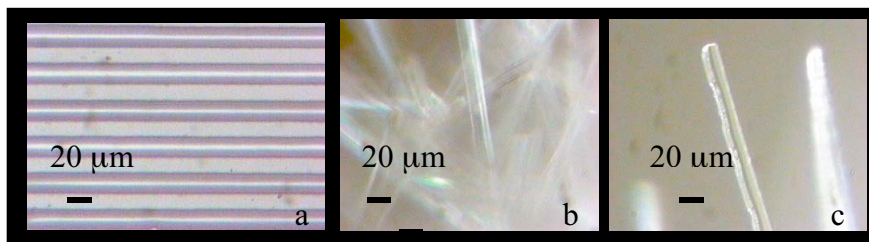
**Figure 3**.    Optical micrographs of  a)  filaments still in the PDMS stamp
b,c)  freestanding MIP  filaments

attack PDMS.[16] Therefore, if a stamp and its polymer-filled channels were placed in a bath of TBAF, the stamp should be dissolved and free-floating MIP filaments should be obtained. Unfortunately, TBAF reacted poorly with the polyurethane filaments.  The TBAF solution caused the filaments to shrink into small, viscous masses.

This problem was avoided by submerging the stamp and its polymer filaments directly into the extraction solvent, toluene.  When introduced to toluene, the PDMS stamp swelled enough that the filaments were removed from the stamp.  Figure 3b-c show freestanding 20 µm filaments, which had been isolated, extracted, and dried.

Dried fibers were vortexed with a known concentration of anthracene for 24 hours.  The fluorescence intensity of the solution was measured before and after rebinding.  The decrease in fluorescence intensity was then correlated to the amount of anthracene absorbed by the polymer filaments.

Initial studies have shown a preference for the templated filaments towards anthracene 1.5 times greater than the control polymers as shown in Figure 4.  While, the amount of analyte absorbed by the templated polymer (2.2 µmol/g) is more than the control (1.5 µmol/g), the difference is much less than that previously reported by Dickert.[17]  Using thin imprinted films and planar waveguide fluorescence measurements, Dickert reported a less than 1% response of the control polymer to the analyte when compared to the templated polymer.  A difference in imprinting conditions as well as the method used to evaluate rebinding may account for this discrepancy. Further optimization of the imprinting parameters are currently being explored.
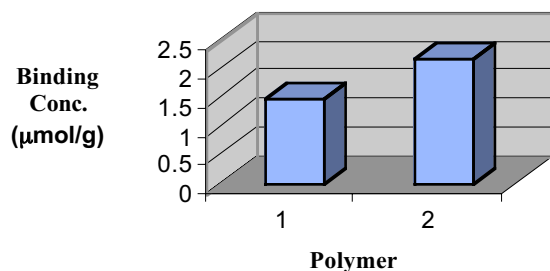


**Figure 4.**  Templated (2) versus control (1) polymers for anthracene binding.

## CONCLUSIONS

While optical waveguides have previously been produced via soft lithography techniques,[18] this study combines MIMIC and molecularly imprinted polymers to fabricate imprinted optical waveguides. The preferential binding of imprinted polymers compared to control polymers for freestanding filaments as well as the fabrication of high-quality filaments capable of coupling light is promising. Studies are underway to test the performance of these waveguides and to further optimize imprinting conditions.

## ACKNOWLEDGMENTS

## REFERENCES

1. G. Vlatakis, L.I. Andersson, R. Muller, and K. Mosbach, Nature **361**, 645 (1993).
2. N. Masque, R. Marce, F. Borrull, P.A.G. Cormack, and D. Sherrington, Anal. Chem. **72**, 4122 (2000).
3. L. Schwartz, M. Petersson, T. Johansson, and S. Nilsson, Journal of Chromatography A **892**, 203 (2000).
4. D. Kriz, C. Berggren, L.I. Andersson, and K. Mosbach, Anal. Chem. **66**, 2636 (1994).
5. F.L. Dickert and O. Hayden, Trends Anal. Chem. **18**, 192 (1999).
6. D. Kriz, O. Ramstrom, and K. Mosbach, Clin. Chem. **69**, 345A (1997).
7. J. Mathew-Krotz and K.J. Shea, J. Am. Chem. Soc. **118**, 8154 (1996).
8. R.J. Ansell and K. Mosbach, J. Chromatogr. A **787**, 55 (1997).
9. G.M. Whitesides and Y. Xia, Angew. Chem., Int. ed. Engl. **37**, 550 (1998).
10. F.L. Dickert, P. Forth, P. Lieberzeitz, and M. Tortschanoff, Fresenius J. Anal. Chem. **360**, 759 (1998).
11. F.L. Dickert and O. Hayden, Adv. Mater. **14**, 311 (2000).
12. F. L. Dickert, M. Tortschanoff, W. Bulst, and G. Fischerauer, Anal. Chem. **71**, 4559 (1999).
13. F.L. Dickert and S. Thierer, Adv. Mater. **8**, 987 (1996).
14. E. Kim, G.M. Whitesides, L. Lee, S.P. Smith, and M. Prentiss, Adv. Mater. **8**, 139 (1996).
15. M. Yan and A. Kapua, Analytica Chimica Acta **435**, 163 (2001).
16. B. Xu, F. Arias, and G.M. Whitesides, Adv. Mater. **11**, 492 (1999).
17. F.L. Dickert, H. Besenbock, and M. Tortschanoff, Adv. Materials **10**, 149 (1998).
18. P. Yang, G. Wirnsberger, H. Huang, S. Cordero, M. McGehee, B. Scott, T. Deng, G.M. Whitesides, B. Chmelka, S. Buratto, and G. Stucky, Science **287**, 465 (2000).