Smooth operators: Teflon microfluidic chips

May 17, 2011 by Stuart Mason Dambrot feature

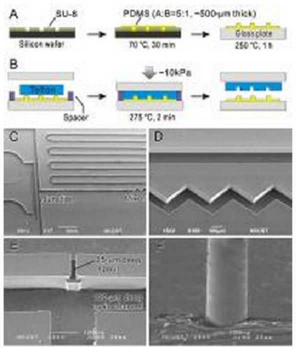

Fig. 1. Molding of Teflon microchannels using PDMS masters. (A, B) Schematics of the preparation of thermally stable PDMS masters and the molding of Teflon channels, respectively. (C-E) SEM images of microfabricated PFA channels (C) for … more

(PhysOrg.com) -- The growing number of research and development efforts focused on microfluidics speaks to the technology's promise of a potentially broad range of applications, largely in highly-integrated single-chip medical devices. However, the materials currently used to fabricate these labs-on-a-chip and other microfluidic devices have significant limitations, including absorption of small nonpolar and weakly polar molecules, adsorption of biomolecules, and the material's molecules leaching into the microfluidic channel. The good news is that researchers have overcome these obstacles using microfluidic channels made entirely of Teflon, which supports cellular activity similar to that found in current materials. Moreover, whole-Teflon microchannels have gas permeability levels that permit cells to be cultured in-channel for extended periods of time.

The researchers, led by Prof. Hongkai Wu at Hong Kong University of Science and Technology's Department of Chemistry, faced a number of obstacles to designing and developing a microfluidic chip that was optimally inert yet suitable to machining. "Currently, there are two major types of materials for microfluidic chips," Wu explains to *PhysOrg.com*. "One is inorganic, such as glass and silicon. Unfortunately, fabrication of micropatterns and bonding chips of these materials are difficult and require sophisticated equipment. The other class of materials is plastics, including polydimethylsiloxane (PDMS) – the most widely-used – poly(methyl methacrylate) (PMMA), and polyurethane. Chips in plastics are easier to fabricate than in glass, but they have their own problems," including the adsorption, absorption and leaching mentioned above, as well as being incompatible with organic solvents, all of which greatly limit their microfluidic chip applications.

"For example," Wu continues, "they will be unsuitable for highly-sensitive analysis because the analyte will be lost by absorption if it's a small, non/weakly polar molecule or by adsorption on channel walls if it's a large molecule. For all of these reasons, we chose Teflon, which is well-known for its high degrees of inertness, non-adhesiveness and resistance to solvents." Moreover, the Teflon compounds Wu used – perfluoroalkoxy (PFA) and fluorinated ethylenepropylene (FEP) – have melting points above 260 °C (one of the highest in thermoplastics) and are optically transparent (although less so than PDMS and glass).

At the same time, Teflon had its own challenges. For example, Wu notes, "Teflon's superior inertness causes two major obstacles: one in micropatterning the material and the other in bonding patterned chips. Prior to our work, there were only several very expensive and complicated lithographic methods using high-energy radiation to effectively micropattern Teflon."

In addition, Wu continues, "tight bonding of Teflon chips is rather difficult. Both bonding temperature and pressure need to be precisely controlled to overcome the problems that come from residue internal stress and plastic flow. "Initially we tried to bond the Teflon channel without pressure (as for bonding glass chips) and with constant pressure (as for bonding PMMA chips)," says Wu, "but neither worked. We needed an effective and convenient method to bond and thereby seal the Teflon channels."

Overcoming these two obstacles led to the team's two key innovations, micropatterning Teflon materials and bonding Teflon chips. "We established a very simple and easy-access method to fabricate three-dimensional Teflon micro- and nanostructures. The Teflon PFA and FEP substrates we used are melt-processable, so they could be hot-embossed using a template – an ideal way is to generate micropatterns in photoresist and then transfer the structure into the mold."

But another problem arose. "Teflon's melting points of substrates are much higher than those of photoresist. We therefore introduced a specially-treated intermediate thermosetting master to overcome the gap between low-melting-point photoresist master and a high-melting-point replica, allowing us to cast the master at milder temperatures into the replica and then use it at elevated temperatures to mold the patterns into Teflon."

Their method was adapted from traditional soft-lithography, which previously wasn't applicable to high-melting-point substrates. "Normal PDMS severely leaches gas above 150 °C, creating bubbles that make it impossible to mold micropatterns into Teflon. With our treated PDMS replica, we now can mold any micropatterns that are formed in photoresist by photolithography into thermoplastics (including Teflon) at as high as 350 °C."

The team also designed a very simple and highly efficient method that solved the problems that have been encountered for a long time when bonding Teflon chips. This thermobonding process is based on different thermal expansion factors of Teflon materials (slightly higher than that of stainless steel) and the holding scaffold (stainless steel screw clamps) during bonding. "The bonding pressure is automatically controlled," Wu explains. "When temperature is raised and the two Teflon plates are not bonded, Teflon expands more than the clamps and so the pressure is high. Once the two plates are bonded, the pressure is automatically released."

Wu is already looking at future innovations and improvements. "We want to develop a smaller on-chip microvalve. The current nanoliter valve is still relatively large, so we're working on reducing its volume to the picoliter range." The team is also interested in advances in Teflon itself. "The optical transparency of our current whole-Teflon chip is still lower than that of PDMS and glass chips, so optical detection can be performed only when the optical path length of the Teflon chip is less than 2 mm. If the Teflon materials become as transparent as PDMS or glass, we will have more freedom in designing the microchips. But," he acknowledges, "this depends on the design of new Teflon materials."

In terms of the new chip's most promising near-term and future applications, Wu comments that "since the whole-Teflon device is extremely inert and super-clean, it is superior for applications involving corrosive chemicals, strong solvents, high and low temperatures, and pressurized processes. It is antifouling and biocompatible, and therefore well suitable for quantitative and biological analysis. Interestingly, various biological cells can attach and grow well inside the Teflon channels – so it expands the applications of microfluidics to all these areas that were previously difficult. It is particular advantageous when accurate quantitative information is required."

Beyond this Wu sees an even wider range of possibilities. "We also believe that Teflon materials are superior to PDMS for commercial applications of microfluidics due to their outstanding stability and reliability. Teflon could be a next-generation microchip material with very broad use, such as serving as standard equipment for flow reactors, microanalysis and bioassay. Moreover, they can also be used under extreme conditions – for example, on a space shuttle."

Perhaps the ultimate application of Wu's Teflon microfluidic technology will derive from the intersection of further miniaturization and biocompatibility. "We can mold Teflon pattern down to submicron range and fabricate sealed Teflon channels within the scale of 10 um. Since Teflon materials have outstanding biocompatibility and have long been used for implantations in human body, such as catheters, miniaturized Teflon microfluidic devices might be used for in-vivo diagnostics, drug delivery and flow control."

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More information:
* Whole-Teflon microfluidic chips, PNAS Published online before print May 2, 2011, DOI:10.1073/pnas.1100356108
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Hong Kong Univ.: Whole-Teflon microfluidic chips

V. Semetey

The group of **H. Wu** at **Hong Kong University** has developed a convenient strategy for fabricating whole-Teflon microfluidic chips as well as valves that show outstanding inertness to various chemicals and extreme resistance against organic solvents (Figure 8). Compared with poly(dimethylsiloxane) the whole-Teflon chip has a few more advantages, such as little adsorption of biomolecules onto channel walls, and no leaching of residue molecules from the material bulk into the solution in the channel.

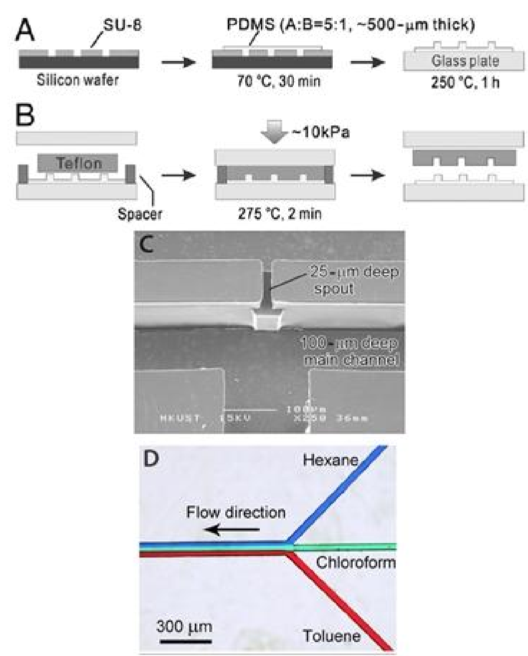

Figure 8: (A/B) Schematics of the preparation of thermally stable PDMS masters and the molding of Teflon channels
(C) SEM images of microfabricated Teflon channels.
(D) Laminar flow of dyed organic solvents in a whole-Teflon chip.

Furthermore, authors have used these Teflon microfluidic chips to culture various biological cells. Adherent cells can attach to the channel bottom, spread, and proliferate well in the channels (with similar proliferation rate to the cells in PDMS channels with the same dimensions). The moderately good gas permeability of the Teflon materials makes it suitable to culture cells inside the microchannels for a long time. **L. Malaquin** and **J.L. Viovy** at **Institut Curie** [2] are currently using a similar strategy to produce microfluidic chips hot embossed from a commercial fluorinated thermoplastic polymer (Dyneon THV).

In terms of the new chip's most promising near-term and future applications, the whole-Teflon devices are extremely inert and super-clean, they are superior for applications involving corrosive chemicals, strong solvents, high and low temperatures, and pressurized processes. These fluorinated polymers are transparent optically. However the optical transparency of the whole-Teflon chip is still lower than that of PDMS and glass chips, so optical detection can be performed only when the optical path length of the Teflon chip is less than 2 mm.

*[1] "Whole-Teflon microfluidic chips" ; K. Ren, W. Dai, J. Zhou, J. Su, H. Wu : PNAS **108**, 8162 (2011).*
*[2] "New family of fluorinated polymer chips for droplet and organic solvent microfluidics" ; S. Begolo, G. Colas, J.L. Viovy, L. Malaquin : Lab on a Chip **11**, 508 (2011).*

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Musings from Physics Today's online editor

Implementing an obvious idea isn't always obvious

By Physics Today on May 9, 2011 2:40 PM | No Comments | No TrackBacks

Some biochemical experiments entail processing small batches of a precious solution under different, controlled conditions. To meet that need and others, scientists and engineers have developed a technology called microfluidics.

The most sophisticated microfluidic devices are known as labs-on-a-chip. They consist of networks of tiny pumps, pipes, and reaction vessels. Like a computer chip, a lab-on-a-chip is fabricated lithographically, usually from a single material. The ideal material is chemically inert, has a low coefficient of friction, and is clear so that you can use an optical microscope to monitor the progress of an experiment.

Polydimethylsiloxane (PDMS) is a popular material for microfluidic applications. It's clear, chemically inert, and soft enough to form into different shapes. For biochemical applications, PDMS has the additional advantage of being nontoxic to cells, but it has serious disadvantages, too. Large biomolecules tend to stick to its surface, small molecules can seep in and out of its bulk, and it can't be used with organic solvents.

Aware of those disadvantages, Hongkai Wu of the Hong Kong University of Science and Technology sought an alternative. His choice, Teflon, might seem obvious. The material—or, more accurately, the family of materials—is famously unsticky. The family members are also chemically inert; two of them, perfluoroalkoxy (PFA) and fluorinated ethylenepropylene (FEP) are clear.

But PFA and FEP aren't easy to stamp or mold into microfluidic devices, in part because of their relatively high melting temperatures (around 260°C). To implement an obvious idea, Wu and his team had to come up with a nonobvious solution, which they described in a recent paper in the *Proceedings of the National Academy of Sciences*.

Nonobvious might even be an understatement. Ideally, you'd heat PFA or FEP above its melting temperature then stamp a pattern into it using a master made from an easily shaped plastic. At first glance, making the master out of PDMS might seem like a nonstarter. The material's highest operating temperature is cited as just 150°C.

That upper limit, Wu realized, is not due to the melting temperature of PDMS, but to the out-gassing of small molecules from the bulk. By adjusting the ratio of the two precursors used to make PDMS, Wu found he could raise its operating temperature to at least 350°C—high enough to use as a master for PFA or FEP.

Wu and his team made and tested PFA microfluidic devices. Like PDMS devices, the PFA devices proved to be nontoxic to cells. Unlike PDMS devices, the PFA devices could be cleaned and reused, thanks to their nonstickiness. Wu concludes his paper with a hopeful note:

> By combining the ease of fabrication and the special properties of Teflon materials, we expect that they can be the materials for the next generation of microfluidic chips and will greatly expand the applications of microfluidics to a wider range of substances.

Charles Day

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Studies from Hong Kong University of Science and Technology Further Understanding of Science.

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Science Letter
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"Although microfluidics has shown exciting potential, its broad applications are significantly limited by drawbacks of the materials used to make them. In this work, we present a convenient strategy for fabricating whole-Teflon microfluidic chips with integrated valves that show outstanding inertness to various chemicals and extreme resistance against all solvents," scientists in Hong Kong, People's Republic of China report (see also Science).

"Compared with other microfluidic materials [e. g., poly(dimethylsiloxane) (PDMS)] the whole-Teflon chip has a few more advantages, such as no absorption of small molecules, little adsorption of biomolecules onto channel walls, and no leaching of residue molecules from the material bulk into the solution in the channel. Various biological cells have been cultured in the whole-Teflon channel. Adherent cells can attach to the channel bottom, spread, and proliferate well in the channels (with similar proliferation rate to the cells in PDMS channels with the same dimensions)," wrote K.N. Ren and colleagues, Hong Kong University of Science and Technology.

The researchers concluded: "The moderately good gas permeability of the Teflon materials makes it suitable to culture cells inside the microchannels for a long time."

Ren and colleagues published their study in Proceedings of the National Academy of Sciences of the United States of America (Whole-Teflon microfluidic chips. Proceedings of the National Academy of Sciences of the United States of America, 2011;108(20):8162-8166).

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HIGHLIGHT

Research highlights

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Teflon lab-on-a-chip

Microscale technologies have explored many materials for biological applications, from rigid glass and PMMA platforms to the widely used elastic PDMS devices.[1] Most of these materials are useful for some applications, yet they all have certain drawbacks. Rigid materials preclude the use of on-chip valves and flexible membranes, while biological samples such as cells and proteins tend to adsorb onto certain polymer surfaces. Furthermore, many polymers are sensitive to organic solvents.

In an effort to develop a microfluidic platform that can incorporate flexible elements, but is robust to solvents and cell adhesion, Hongkai Wu and colleagues[2] selected Teflon as their material of choice. The authors first created a special PDMS mold at a base : curing agent ratio of 5 : 1 and cured it at 250 °C. The Teflon was subsequently thermomolded using the PDMS master at 350 °C. The molds exhibited high replication fidelity down to 100 nm for both rounded and rectangular channel cross-sections. Finally, two Teflon layers were bonded at a high temperature. In this step, the layers were sandwiched between PDMS-coated rigid substrates and pressed together with stainless steel screw clamps and then heated in an oven. The thermal bonding process required precise control over both pressure and temperature (260 °C at 100 kPa). Inadequate parameters were shown to yield misshaped or collapsed channels, or weak bonding of the layers. This fabrication method was highly successful for channel aspect ratios of up to 1 : 5.

The paper examines several experimental applications of the Teflon device; among others, the efficiency of on-chip micro-valves and -pumps. The authors fabricated soft, 15 μm thick valve membranes and operated on-chip pumps continuously for 7 days at 1 kHz. The valves closed off moderate flow at 0.1 MPa, which is similar to PDMS-based valves, and the pumps regularly propelled 30 nl volumes over the course of the experiment. In contrast to PDMS, resistance of the Teflon chip to hexane, chloroform, toluene, and acetone was documented, as no swelling of the materials was observed after a 24 h exposure. The device was also shown to be reusable. When filled with green fluorescent protein and subsequently rinsed, no protein residue was observed, contrary to PDMS and polystyrene chips. Similarly, it was shown that cells (HepG2) seeded inside Teflon channels spread and proliferated, but did not leave any residue after lysis. Finally, a 5-day cell culture inside closed Teflon channels indicated good oxygen and CO_2 permeation, as a large fraction of the cells was viable at the end of the study.

These observations indicate that Teflon is easy to process, highly biocompatible and in addition reusable and resistant to organic solvents, making it superior to a variety of materials commonly used in microfluidic device fabrication.

Label-free observation of ligand-membrane binding

Membrane proteins are involved in signaling events between cells, but they also help regulate inner cell processes by aiding the transduction of ligand molecules across the membrane. Thus, they are the focus of many drug screening studies.[3] Most studies, however, require the isolation of membrane proteins or modification of the membrane by attaching labeling molecules to it. Fluorescing labels, for example, allow for the use of observation methods like fluorescence microscopy or TIRF. The resulting observations are therefore not fully representative of the native membrane proteins and the membrane behavior. Bornhop, Finn and colleagues have recently offered a solution to this problem by developing a procedure for label-free observation and quantification of interactions between membranes and ligands such as drugs.[4] This procedure utilizes backscattering interferometry (BSI) inside a microfluidic channel and takes advantage of the difference in refractive index between solutions containing bound and unbound ligands (Fig. 1).

The authors prepared small unilamellar vesicles from lipid components or cellular membranes incorporating functional membrane proteins. These vesicles were suspended in solution together with ligand molecules and introduced into a microfluidic channel, which functioned as a resonance cavity. A HeNe laser illuminated the filled channel and the incident coherent light was collected with a CCD camera. The recorded interference pattern was then Fourier-transformed into a functional pattern of the refractive index of the solution. Changes in the index of refraction for samples and

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