Video Article

# Fabrication of the Thermoplastic Microfluidic Channels

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## Abstract

In our lab, we have successfully isolated nucleic acids directly from microliter and submicroliter volumes of human blood, urine and stool using polymer/nanoparticle composite microscale lysis and solid phase extraction columns. The recovered samples are concentrated, small volume samples that are PCRable, without any additional cleanup. Here, we demonstrate how to fabricate thermoplastic microfluidic chips using hot embossing and heat sealing. Then, we demonstrate how to use in situ light directed surface grafting and polymerization through the sealed chip to form the composite solid phase columns. We demonstrate grafting and polymerization of a carbon nanotube/polymer composite column for bacterial cell lysis. We then show the lysis process followed by solid phase extraction of nucleic acids from the sample on chip using a silica/polymer composite column. The attached protocols contain detailed instructions on how to make both lysis and solid phase extraction columns.

## Video Link

The video component of this article can be found at https://www.jove.com/video/664/

## Protocol

The microfluidic channels are fabricated in a cyclic polyolefin (Zeonex® 690R, Zeon Chemical Inc., Louisville, KY). Zeonex has a glass-transition temperature ($T_g$) of 136°C and is UV transparent, which is essential for the light directed chemistry used in the porous monolith formation. The microchannels are fabricated by micro-hot-embossing with a NiCo electroformed master-mold, which has the negative (inverse) features of the microfluidic platform. The hot-embossing is done as follows:

1. The mold and the polymer substrate are placed between two parallel metal platens.
2. The platens are heated up to the embossing temperature (166°C), which is 30ºC above the $T_g$ of Zeonex.
3. The platens are then pressed together for approximately 5 min and the pressure is maintained at 250 psi.
4. The mold and the substrate are then removed from the heated platens, allowed to cool for 1-2 min, and then manually separated from each other.
5. Wells of 1.5-mm diameter are drilled at the ends of the channels for sample introduction and collection.
6. The embossed plastic substrate is then thermally bonded with another piece of Zeonex to form enclosed microfluidic channels. The embossed substrate and the cover piece can be UV-ozone or plasma treated prior to thermal bonding to enhance the sealing efficiency and ensure the fidelity of the enclosed channel structure (Bhattacharyya and Klapperich, 2007).

**Fabrication of solid-phase extraction (SPE) column within the plastic microfluidic channels**

Fabrication of the solid-phase is a two-step process. At first, the fabricated microchannels are surface modified in order to improve the adhesion of the SPE column to the plastic device. Grafting via surface photopolymerization is used to functionalize the walls of the polymeric microchannels.

The **grafting procedure** is as follows:

1. The microchannels are filled with a 1:1 mixture of ethylene diacrylate (EDA) and methyl methacrylate (MMA) with 3% benzophenone, which is a hydrogen abstracting photoinitiator. The EDA, MMA and benzophenone are purchased from Sigma-Aldrich, St. Louis, MO.
2. The chip is then UV-irradiated for 10 min at 254 nm UV wavelength and 200 mJ/cm$^2$ energy in an ultraviolet exposure instrument (CL-1000 UV Crosslinker, UPV Inc., Upland, CA). The grafting occurs via H-abstraction polymerization and results in surface-tethered polymer chains, which get incorporated within the polymerization mixture used for the preparation of the monolith in the second step.
3. The excess monomer is removed from the channels by rinsing with 15 µL of methanol using vacuum aspiration.

After the photografting process, a microporous polymer monolith is formed within the microchannels that entrap the silica particles for DNA extraction. The **porous monolith** is prepared *in situ* as follows:

1. The surface modified channels are filled with a monolith precursor mixture consisting of BuMA (15% wt), EDMA (10% wt), 1-dodecanol (52.5% wt), cyclohexanol (22.5% wt), DMPAP (1% wt with respect to monomers) and 0.7 µm silica particles (15% with respect to the total volume of the pre-polymer mixture). The silica microspheres were purchased from Polysciences, Inc. (Warrington, PA) and the monomers and porogenic solvents are purchased from Sigma-Aldrich, St. Louis, MO.

 

2. The chip is irradiated with UV in the UV crosslinker at 200 mJ/cm$^2$ for 1.1 min to initiate polymerization, and the excess monomer is removed from the channels by rinsing with 15 µL of methanol using vacuum aspiration.
3. The fluidic connections (NanoPorts™ connections, Upchurch Scientific, Oak Harbor, WA) are then attached on the introduction and collection wells of the microfluidic channels.
4. The porous monolith is then washed again with methanol for at least 30 min using the syringe pump at a flow rate of 100mL/hour.

**Fabrication of CNT lysis monolith within the plastic microfluidic channels**

Fabrication of the CNT (carbon nanotube) lysis monolith is a two-step process. First, the fabricated microchannels are surface modified (grafted) in the same manner as for the SPE columns in the plastic device.

After the photografting process, a microporous polymer monolith is formed within the microchannel that is impregnated with multi-walled carbon nanotubes. The **porous monolith** is prepared *in situ* as follows:

1. Multi-walled nanotubes are resuspended in cyclohexanol at a concentration of 2.27M. This resuspension is ultrasonicated for 30 minutes at 50% amplitude and 50% duty cycle with a sonifier cell disruptor (Sonifier S-250D, manufactured by Branson Ultrasonic, Danbury, CT). The sonication provided an effective and stable 0.5M suspension of CNTs. The CNTs were purchased from Nanolabs (Brighton, MA).
2. The surface modified channels are filled with a similar monolith precursor mixture except that now the solution of cyclohexanol + CNTs is used. The monolith mixture consists of BuMA (15% wt), EDMA (10% wt), 1-dodecanol (52.5% wt), and cyclohexanol+CNTs (22.5% wt). To this solution the photoinitiator DMPAP is added (1.13% wt with respect to monomers). The monomers, porogenic solvents and DMPAPA are purchased from Sigma-Aldrich, St. Louis, MO.
3. The chip is irradiated with UV in the UV crosslinker at 120 mJ/cm$^2$ for 0.9 min to initiate polymerization. The device is flipped 180 degrees in the crossliker and irradiated for another 0.9 min at 120 mJ/cm$^2$. The excess monomer is removed from the channels by rinsing with 50 µL of methanol using vacuum aspiration.
4. The fluidic connections (NanoPorts™ connections, Upchurch Scientific, Oak Harbor, WA) are then attached on the introduction and collection wells of the microfluidic channels.

**Bacterial Sample Preparation for use with CNT lysis monolith**

The sample preparation for a bacterial sample involves taking an optical density measurement at 600nm to ensure that the sample is not extremely concentrated so as to avoid clogging the channel.

1. Take an OD reading at 600nm with the bacteria in media, this is done with a biophotometer (Eppendorf BioPhotometer, Eppendorf Scientific, Inc., Westbury, NY). Dilute the sample until the OD readings fall within the range 0.18-0.25 A.
2. Centrifuge the sample at 6,500 rpm for 5 minutes and decant the supernatant.

Lysis

1. Resuspend bacteria in GuSCN* + .01%SDS + 4% Proteinase K (0.8mg/ml). Vortex briefly (1ml of bacteria in media should be resuspended in 1ml GuSCN). Proteinase K was added to the solution to aid with denaturing proteins that are attached to the bacterial DNA and to inhibit DNAases and RNAases. An additional benefit of the proteinase K is that it also aids with breaking down the cellular wall proteins which is desirable since both gram-positive bacteria and gram-negative bacteria cell walls contain large amounts of protein (although gram-negative bacteria typically have more protein). The chaotropic buffer additionally aids the proteinase K with denaturing proteins while the detergent disrupts the cell wall. Proteinase K and guanidinium thiocyanate (GuSCN) were purchased from Qiagen Inc. (Valencia, CA). SDS (Sodium Dodecyl Sulfate) was purchased from Pierce (Rockford, IL).
2. Immediately load the sample in a syringe connected to PEEK tubing. Aspirate the syringe so that no air is in the system. Connect the tubing to the nanoport and channel.

A KDS100 syringe pump (manufactured by KD Scientific, Holliston, MA) was used for the experiments and the flow rate used is 450 µL/hour for all the steps. Flow the sample lysis channel at 450ul/hr.

1. Collect the sample and proceed to the DNA extraction via SPE (see DNA extraction protocol). NOTE: This bacterial sample will not need to follow Load Step 2, since the suspension is GuSCN*.

**DNA Extraction Protocol**

The extraction procedure consists of 3 steps:

1. Load.
2. Wash.
3. Elute.

KDS100 syringe pump (manufactured by KD Scientific, Holliston, MA) was used for the experiments and the flow rate used was 300 µL/hour for all the steps.

**Load**

1. Condition the channel with chaotropic buffer (GuSCN*, guanidinium thiocyanate) for 1-2 min before starting the experiment.
2. Mix the sample with chaotropic buffer in 1:1 ratio.
3. Flow sample mixture through the channel for 3 min.

**Wash**

1. Wash the channel with 70% ethanol for 2 min.
2. Wash the channel with 100% ethanol for 2 min.

**Elute**

1. Elute the extracted DNA in millipore water for 3 min. Collect 15 µL of purified, PCR-ready DNA.

*The GuSCN from the Qiagen kit (Buffer RLT) was used.

## Disclosures

The authors have nothing to disclose.

## References

1. Bhattacharyya, A. and Klapperich, C.M., 2007. Mechanical and chemical analysis of plasma and ultraviolet-ozone surface treatments for thermal bonding of polymeric microfluidic devices. Lab Chip 7, 876-882.

 