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Fabrication of a CNT Filter for a Microdialysis Chip

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Abstract

This paper describes the fabrication methods of a carbon nanotube (CNT) filter and a microdialysis chip. A CNT filter can help perform dialysis on a microfluidic chip. In this study, a membrane type of a CNT filter is fabricated and located in a microfluidic chip. The filter plays a role of a dialysis membrane in a microfluidic chip. In the fabrication process of a CNT filter, individual CNTs are entangl-ed each other by amide bonding that is catalyzed by 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS). The chemically treated CNTs are shaped to form a CNT filter using a PDMS film-mold and vacuum filtering. Then, the CNT filter is sandwiched between PDMS substrates, and they are bonded together using a thin layer of PDMS prepolymer as adhesive. The PDMS substrates are fabricated to have a microchannel by standard photo-lithography technique.

Keywords: CNT filter, Microdialysis Chip, Microfluidics

Conventional biochemical analysis for toxicology, toxicogenomics, or toxicoproteomics requires complicated, time-consuming and multi-step processes to prepare and detect target molecules. The recent advent of a microfluidic chip is expected to automate the complicated processes. A microfluidic chip is a miniaturized device where one can conveniently and efficiently perform the analytic processes. The microfluidic chip has miniaturized functional sites for analysis, and the sites are interconnected by micro or nano channels. Thus, the microfluidic chip is often called Lab-on-a-Chip or micro Total Analysis System (μ-TAS)[1-4].

Despite intense efforts to develop a microfluidic chip and to apply it to the analysis of real-world samples, there is a key issue to be solved: on-chip sample pretreatment. For the automation of the complicated processes, the sample pretreatment processes like purification, extraction, concentration, amplification, mixing, reaction, and etc., should be performed on a chip. However, the integration of these functions on to a chip is challenging technically because the specification of a sample pretreatment is too diverse from one sample to another[5]. Thus, the sample pretreatment processes are performed off chips in most cases.

Dialysis is one of frequently used sample pretreatment methods for extracting a desired sample from a complex solution in conventional biochemical analysis. Most previous studies to perform dialysis at chip level utilized microbeads[6] or a nanoporous membrane[7-11] fabricated on a microfluidic chip, and showed a capability of extracting desired samples in a continuous flow system. However, a feasible sample size for dialysis is limited to a micro-scale due to the coarse pore size in the cases of using microbeads, and the pore size of the nanoporous membrane is difficult to control it.

Carbon nanotubes (CNTs) have been widely studied and utilized in science and engineering because of their excellent mechanical, electrical and chemical properties since they were discovered by Ijiima in 1991[12]. Generally, CNTs have a circular cross-section with a diameter of a few nanometers and a length of a few microns. They can be combined readily by chemical treatment[13-19] and be used to make a CNT filter. In addition, the pore size of a CNT filter is expected to be controlled precisely by varying the length and density of individual CNTs.

This paper describes the fabrication method of a CNT filter in detail and presents a microfluidic chip for microdialysis using the CNT filter. Fabrication processes include the chemical treatment of CNTs to interconnect them by covalent bonding and the shaping of a CNT filter using a PDMS film-mold. The microfluidic chip for microdialysis is constructed by bonding two PDMS substrates with embedded microchannels. The CNT filter is sandwiched between the two substrates. A standard soft-lithography technique is used to make the PDMS substrates and the PDMS film-mold.

Fabrication of Microdialysis Chip and CNT filter

Microdialysis chip layout. The microdialysis chip proposed in this study consists of two PDMS sub-

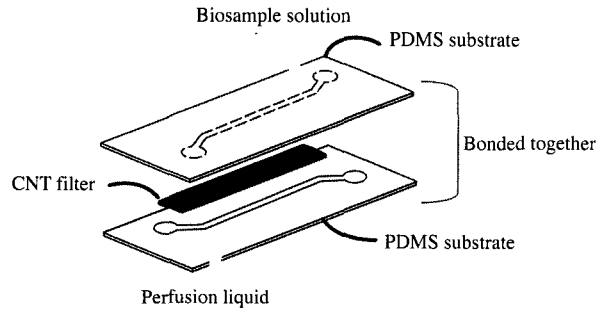

Fig. 1. Microdialysis chip layout.

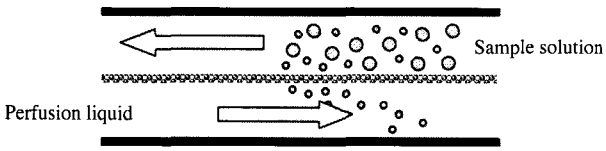

Fig. 2. Microdialysis Concept.

strates with microchannels and a CNT filter sandwiched between the substrates, as shown in Fig. 1. The microdialysis chip has a counter-flow configuration so that a sample solution continuously flows in the upper channel in a direction while a perfusion liquid flows in the bottom channel in the opposite direction. The counter-flow configuration always has a higher efficiency than a co-flow configuration[8]. The CNT filter plays a role of a dialysis membrane as shown in Fig. 2. While a complex biosample solution flows through the microchannel, smaller molecules in the flow than the pores of the CNT filter diffuse into the perfusion channel across the filter. Thus, dialysis continuously occurs as long as the solution flows, and the rate of dialysis depends on the pore size of the CNT filter, flow velocity, channel width, concentration of the sample and etc.

Fabrication of PDMS substrate. The PDMS substrate is fabricated by standard photo-lithography and molding techniques as shown in Fig. 3. To make a mold for shaping a microchannel in a PDMS substrate, a 4-inch Si-wafer is cleaned with acetone and isopropyl alcohol (IPA). SU-8 (Microchem), a photoresist (PR) material, is evenly spin-coated on the wafer at a thickness of 100 microns. The coated wafer is, then, soft-baked at 65°C for 10 min and at 95°C for 40 min in order to evaporate PR solvents and make PR denser since dense PR increases reactivity with UV light. After cooling the soft-baked wafer, the UV exposure of the wafer is carried out using a UV exposer (365 nm, MA6-II, SUSS Microtec) through a

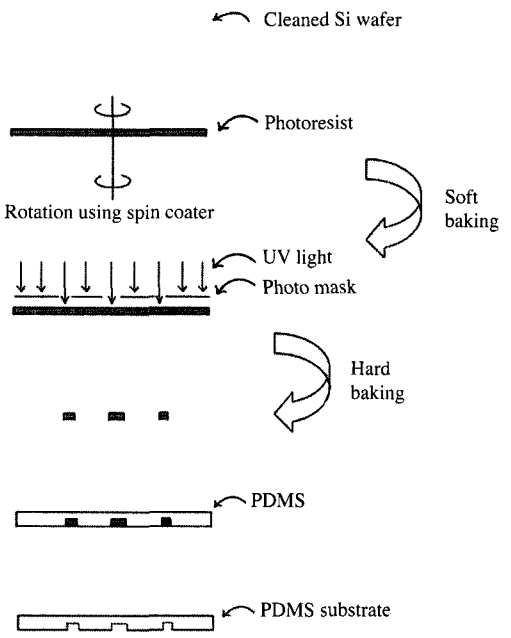

Fig. 3. Photolithography process for fabricating PDMS mold.

film-type photo mask (Han&All Tech) with a patterned microchannel. The photo mask attached on a 4-inch glass plate is laid on the wafer with a soft contact gap of 10 μm. The wafer and the photo mask are inserted into the UV exposer and are exposed to UV with a 675 mJ/cm^2 dosage. Upon the exposure, the prepolymers of PR are crosslinked only in the region exposed through the photo mask. Then, a hard-baking of the wafer is performed at 65°C for 1 min and at 95°C for 20 min to enhance the crosslinking of PR.

The hard-baked wafer is immersion-developed for 10 min in a solution of propylene glycol monomethyl ether acetate (PGMEA). Then, it is washed with IPA and is dried with a nitrogen gun. Unexposed PR is removed from the wafer during the development process, and the mold of a microchannel patterned on the wafer is completed. As the last process for fabricating a PDMS substrate, PDMS (DC-184A, Dow corning) is thoroughly mixed with a curing agent (DC-184B, Dow corning) in a 10 : 1 ratio by volume. This PDMS prepolymer mixture (liquid phase) is degassed in a vacuum chamber for 30 min. After the PDMS prepolymer mixture is poured on to the mold of the wafer, it is cured in an oven at 65°C for 2 hours. Then, the microchannel-patterned PDMS substrate is obtained after it is peeled from the wafer, is washed with IPA and is dried with a nitrogen gun.

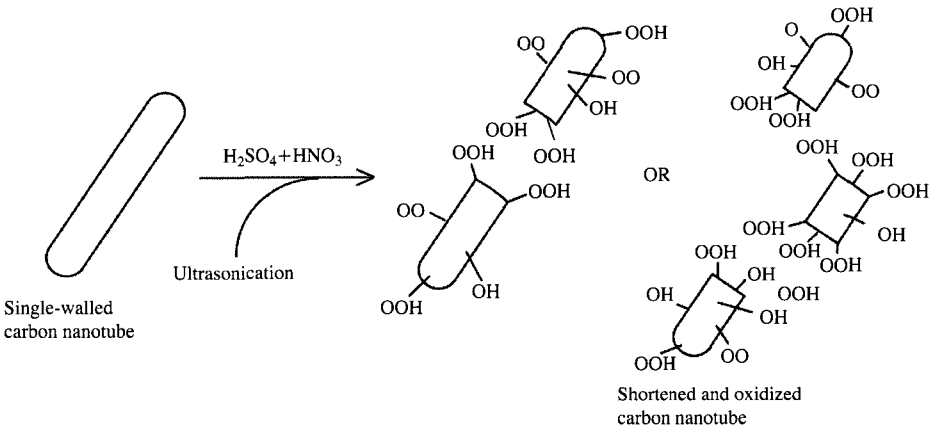

Fig. 4. Carbon nanotubes oxidation.

CNT filter fabrication. A CNT filter is fabricated by CNT oxidation[13-16], a two-step process of diimide-activated amidation[17-19] and a film-molding method. The oxidation cuts CNTs and provides CNTs bonding sites where CNTs are able to be entangled each other as shown Fig. 4. CNTs (ASP-100F, Iljin Nanotech) used for this study are a single-wall type synthesized by arc-discharge process and have a diameter of 1-1.5 nm and a length of 5-20 μm. Purified CNTs[14] of 50 mg are suspended in a mixture of concentrated sulfuric and nitric acids (3 : 1, 98% and 60%, respectively), and are sonicated at 70°C for 6 hours. During the sonication, defect sites are generated on the surfaces of CNTs, and CNTs are cut at the sites by oxidizing acid. In addition, the CNT oxidation produces chemical groups like carboxylic and alcoholic groups on the surfaces of the oxidized CNTs. Among these groups, the carboxylic group is the functional group that enables CNTs to chemically interact each other. After the oxidation, carboxylated CNTs (CNTs-COOH) of about 35 mg are filtered on a hydrophilized polyvinylidene fluoride (PVDF) membrane filter (VVLP04700, Millipore) with the pore size of 0.1 μm, and are washed with deionized (DI) water until the pH becomes neutral. Then, carboxylated CNTs are dried in an oven to obtain a CNTs-COOH powder.

CNTs in the form of a CNTs-COOH powder are entangled through a two-step process. As the first step, CNTs-COOH of 13.5 mg is suspended in 33.75 mL of DI water by sonicating the mixture for 10 min. Then, 6.75 mL of a 500 mM MES buffer solution (M3671-50G, Sigma Aldrich) and 15.53 mL of a 50 mg/mL N-Hydroxy-Succinimide (NHS) (130672-25 G, Sigma Aldrich) solution are added quickly to the CNT solution. While the mixture is stirred at 600 rpm at a room temperature for 30 min, 8.1 mL of a 10 mg/mL N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) (E7750-5G, Sigma

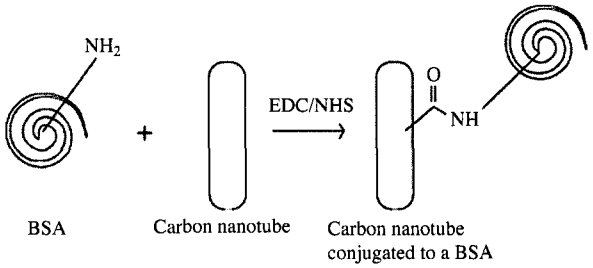

Fig. 5. Intermolecular junction between CNT and BSA.

Aldrich) is added. The suspensions are filtered through a 0.1 μm hydrophilized PVDF membrane filter and are rinsed thoroughly with DI water to remove excessive NHS, EDC and byproduct urea. During this step, the CNTs-COOH chemically treated with NHS/EDC is turned into stable active-estered CNTs by diimide-activated amidation. The stable active-estered CNTs can covalently bind with amine groups as illustrated in Fig. 5. The second step of the two step process starts with resuspending the estered CNTs in 60.75 mL of a 50 mM MES buffer solution, followed by adding 6.75 mL of a 10 mg/mL bovine serum albumin (BSA) (A6003-5G, Sigma Aldrich). After stirring the mixture at 700 rpm for 1 hour, it is kept in the ambient for 12 hours for CNTs and BSAs to interact enough. Because the surface of BSA has amine groups, the estered CNTs are bonded with the BSAs[17-19], resulting in CNT-BSA conjugates. These conjugates are washed thoroughly with DI water to remove unbounded BSA, and are, then, dispersed in DI water.

In order to shape a CNT filter from the dispersed CNT-BSA conjugated solution, a PDMS film mold is prepared using the similar method to that described in the Microdialysis chip layout section. A different part in fabricating a film mold is that to make PDMS as a

thin film the PDMS prepolymer is spin-coated on a wafer with PR (SU-8) patterned to the CNT filter shape. The spin-coating is performed at 800 rpm for 10 seconds. As shown in Fig. 6, a PDMS film-mold is placed on a hydrophobilized PVDF membrane filter (GVHP04700, Millipore) with the pore size of 0.22 μm in the vacuum filtration system. The hydrophobic membrane helps separate a fabricated CNT filter from the membrane. Under a vacuum condition, the suspended CNT-BSA solution is filtered and washed with DI water until no bubbles are observed. Because the PDMS film-mold is hydrophobic, the solution is slipped into the CNT-filter-shape groove of the mold. The fabrication of a CNT filter is completed after it is dried for 12 hours.

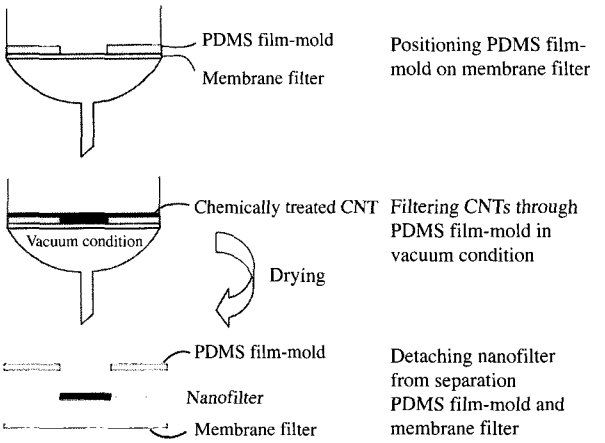

Fig. 6. Shaping a CNT filter.

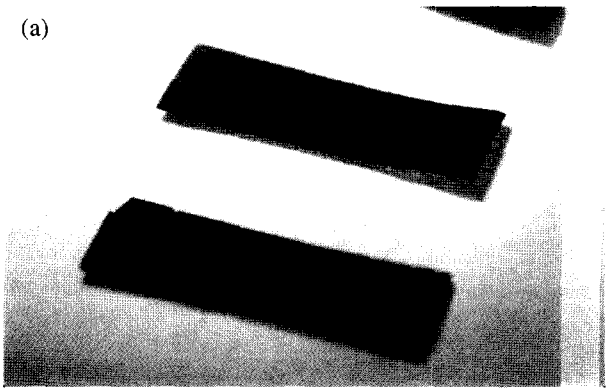

Fig. 7. CNT filter imaged by (a) digital camera and (b) FE-SEM.

The CNT filter has enough rigidity so that it can be handled with tweezers when it is removed from the PDMS film mold. Also, the distribution of CNTs becomes homogeneous due to the vacuum filtering. This is evident from images taken with a digital camera and a field-emission scanning electron microscope (FE-SEM), as shown in Fig. 7. The CNT filter is rectangular ($17 \times 5\ mm^2$) and about 100 μm thick.

Assembly of Microdialysis Chip

The two PDMS substrates and a CNT filter are bonded together using a thin layer of PDMS prepolymer as glue[20]. Fig. 8 schematically illustrates the bonding procedure of a microdialysis chip. PDMS prepolymer is dropped onto the surface of a slide glass. Swabbing the PDMS prepolymer with a sharp

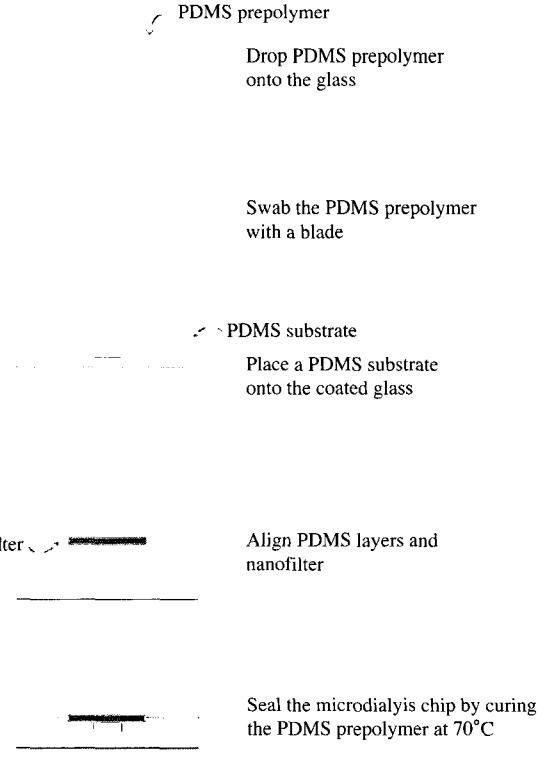

Fig. 8. Assembly of microdialysis chip.

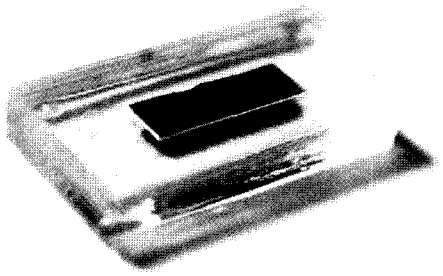

Fig. 9. Microdialysis chip.

blade results in a thin layer of glue. Then, a PDMS substrate with a microchannel is gently placed onto the coated slide glass. The area of the embossed PDMS substrate is in contact with the thin layer. The thin glue is transferred to the PDMS substrate when it is removed from the slide glass. Then, a CNT filter is aligned between two PDMS substrates coated with the glue, and is sandwiched between them. After the three pieces are brought into good contact, they are placed into an oven at 70°C for 2 hours to cure the PDMS prepolymer. The resulting microdialysis chip is shown in Fig. 9.

Summary

This paper described the fabrication processes of a CNT filter and a microdialysis chip in detail. A PDMS substrate consisting of the chip was fabricated by standard photo-lithography technique, and the filter was made of CNTs by chemical treatments: CNT oxidation and the two step processes. The CNT oxidation help the raw CNTs are turned into functionalized CNTs, and the two-step process enables the functionalized CNTs to covalently bond each other. A CNT filter was shaped using a PDMS film-mold technique and a vacuum filtering. Finally, the microdialysis chip was assembled by bonding the two PDMS substrates and a CNT filter using a thin layer of PDMS prepolymer as glue.

For future work, we will study characteristics of the CNT filter like the pore size distribution and surface hydrophilicity as well as will control the pore size of the filter by varying CNT density or length. Experiments of microdialysis of a real-world bio-solution are also scheduled.

Acknowledgements

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