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# Probing Temperature on a Microfluidic Chip with Thermosensitive Conjugated Polymer Supramolecules

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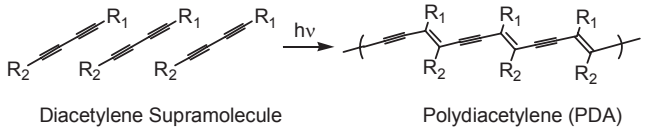
Since the pioneering discovery by Wegner,[1] polydiacetylene (PDA), a family of conjugated polymer, has been extensively investigated in the field of sensors.[2-10] Owing to the unique self-assembled state and the poly(ene-yne) backbone structure, the conjugated polymer displays a phase transition upon environmental perturbations, and this results in a brilliant blue-to-red color change in most cases. Unlike other conjugated polymers which require tedious multistep modification processes, PDA can be readily prepared from diacetylene supramolecules in an aqueous-friendly environment by UV irradiation (Scheme 1). In addition, amphiphilic nature of the diacetylene monomer allows fabrication of various nanostructures including nanotubes, nanowires, nanovesicles, nanohelices, and nanoribbons.

The apparent blue-to-red color transition of PDA has served as a foundation for the construction of many colorimetric chemosensors for the detection of target molecules of interest such as DNAs, proteins, ions, and volatile organic compounds (VOCs).[2-10] PDA has been also used as a probe molecule for the monitoring of temperature.[11]

Another attractive feature of PDA that has been neglected for a long time is its nonfluorescence-to-fluorescence transition upon stimulation.[12-13] In fact, PDA emits red fluorescence when it undergoes a blue-to-red phase transition. Recently, the stimulus-induced fluorogenic change of PDAs has begun to receive substantial attention as an alternative sensing signal.[6,14,15] In addition, the fluorogenic property of PDA allows fabrication of microarrayed and microfluidic PDA sensor systems, which are almost impossible to obtain by using the conventional colorimetric method.[9]

We reported that temperature in a microfluidic channel could be monitored using the thermofluorescent PDA supramolecules.[11] Microfluidic systems have been emerged as powerful tools due to its several advantages such as relatively short diffusion distance, nascent consumption of samples and reagents, large interfacial areas and the capability of continuous analysis. These salient properties enable the microfluidic systems as good alternatives to the conventional batch-type systems.[16-18] However, temperature control or monitoring is an important issue in many applications of microfluidic systems. For example, active temperature control or monitoring is often necessary when performing enzyme-activated reactions, chemical reactions or PCR for DNA amplification on a microfluidic chip.[19-22] Thus, a couple of methods using Rhodamine B[22] or thin film thermocouples[23-25] have been developed to measure temperature on a microfluidic chip. We also reported that PDA can be used as a temperature sensor in the range of 40 ~ 60 °C using its linear relationship between temperature and its fluorescence intensity.[11] It would be more desirable, however, if PDA-based temperature sensor could be utilized in a wider temperature range. As part of our continuing efforts to the development of PDA-based sensor materials, we now report PDA-based temperature sensors derived from different diacetylene monomers for the temperature measurements of a microfluidic channel in different temperature ranges.

Temperature sensor chips and the experimental setup are similar to that used in our previous work.[11] In short, a chip consists of a polydimethylsiloxane (PDMS) substrate bonded to a glass slide and has three inlets and one outlet as illustrated in Figure 1. A blue phase, nonfluorescent PDA solution is injected to the center inlet and corn-oil to two sheath inlets with syringe pumps. PDA droplets are generated by hydrodynamic instability at the inlet junction.[26] While the droplets flow in the main channel, they are subject to a constant heat flux from a microheater integrated on the glass slide. The microheater made of ITO thin film generates Joule heating, resulting in linear temperature




**Scheme 1.** Formation of PDA from self-assembled diacetylene monomers

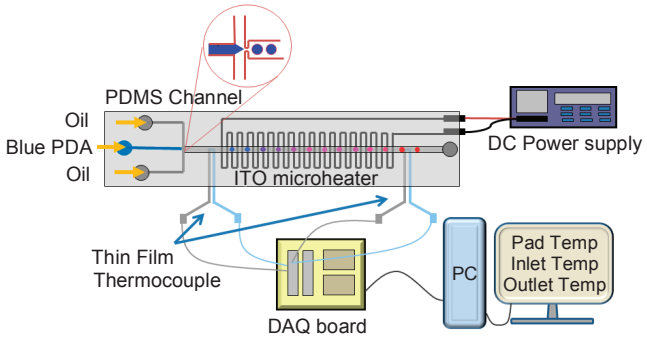



**Figure 1.** Schematic of the PDA temperature sensor microfluidic chip.

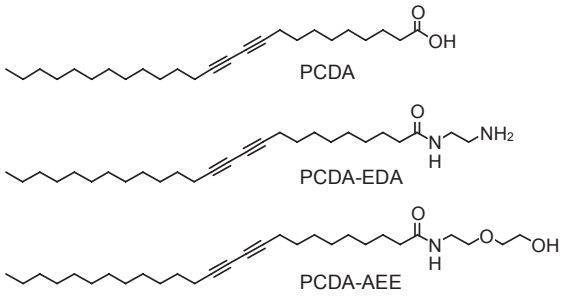



**Figure 2.** Structures of diacetylene monomers: PCDA, PCDA-EDA and PCDA-AEE.

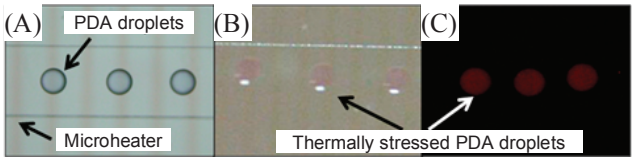



**Figure 3.** Optical (A and B) and fluorescence (C) microscopic images of PDA droplets (PCDA-EDA) in the middle of the main channel before (A) and after (B, C) heat treatment.

distribution along the channel.[27] In addition, we integrated T type thin-film thermocouples at the entrance and exit of the main channel to measure the temperature distribution along the channel so that we can compare the fluorescence intensity of PDA droplets with the temperature. These PDA droplets are monitored by using an inverted fluorescence microscope system (IX71, Olympus).

Among various diacetylene monomers investigated, PDAs derived from three monomers shown in Figure 2 were found to be most efficient in terms of temperature monitoring in the microfluidic device. These three monomers have different head group structures (acid, amine, and alcohol) and should allow different colorimetric sensitivities when they are transformed to polymers owing to the different degree of head group interactions. The ethylenediamine (EDA)-derived diacetylene monomer, PCDA-EDA, was prepared from activated 10,12-pentacosadiynoic acid (PCDA) and EDA. Similarly, the PCDA-AEE was obtained by coupling activated PCDA and aminoethoxyethanol (AEE).

Figure 3A displays an optical image of blue-phase PDA sensor droplets in the middle of the main channel at room temperature (20 °C). Droplets generated at the junction of inlet channels march along the microchannel stably and regularly. The vertical brown lines are the ITO microheater. Thermally stimulated PDA droplets after turning on the heater are shown in Figure 3B and 3C. It is clear that they experienced visible color transition from blue to red, and emitted red fluorescence upon the stimulus.

Images of PDA droplets, derived from PCDA-AEE, are taken 35 mm downstream of the inlet junction, and are displayed in Figure 4A for various channel temperatures. The fluorescence of red-phase PDA droplets becomes stronger with increasing the channel temperature. Figure 4B shows the relations of the temperature and the fluorescence intensities of PDA droplets

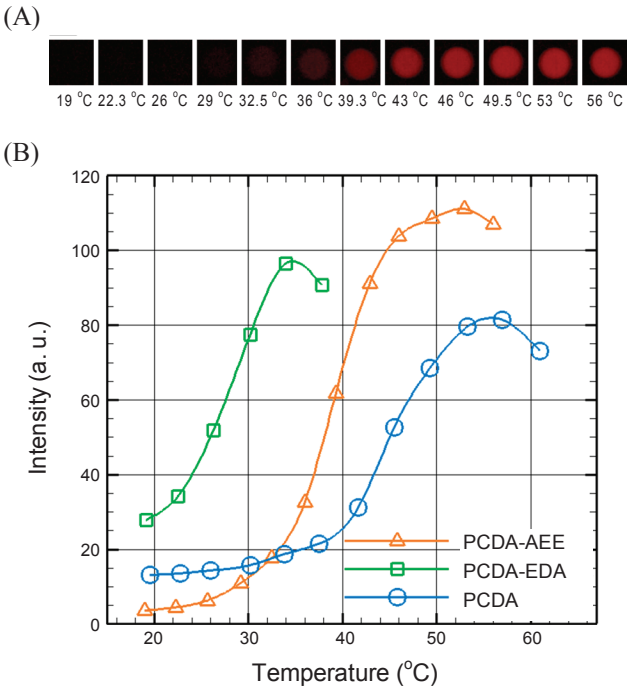



**Figure 4.** Fluorescence microscopic images of PDA droplets derived from PCDA-AEE (A) and fluorescence intensity variations of PDA droplets with respect to channel temperature (B). Images of PDA derived from PCDA-EDA or PCDA are omitted for simplicity.

derived from PCDA, PCDA-EDA and PCDA-AEE. It is evident that each PDA has a different temperature range where the relation is linear. PCDA-EDA resulted in the lowest range from 23 to 34 °C, and PCDA-AEE has a linear range of 33 to 46 °C. Finally, PCDA has the highest temperature range from 41 to 53 °C. The highest temperature range obtained with PCDA-derived PDA is presumably due to the strong headgroup interaction among carboxylic groups in the polymerized supramolecules.

In conclusion, the results described above demonstrate that temperature monitoring in a microfluidic channel is possible with PDA supramolecules. In addition, current study addresses that precise temperature monitoring is possible by choosing proper diacetylene monomers. Thus, PDAs derived from three different diacetylene monomers, PCDA, PCDA-EDA, and PCDA-AEE displayed different temperature ranges where the temperature is linearly proportional to the fluorescence intensity of PDA droplets.

## Experimental Section

**Materials.** 10,12-Pentacosadiynoic acid (PCDA) was purchased from GFS Chemicals. *N*-Hydroxysuccinimide (NHS) and 2-(2-aminoethoxy)ethanol (AEE) were purchased from Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) was purchased from Tokyo Chemical Industry. PCDA-EDA was prepared according to the known procedures.[28] PCDA-AEE was synthesized following modified procedure described in the literature.[29] 1 g (2.67 mmol) of 10,12-pentacosadiynoic acid (PCDA), 0.46 g (4 mmol) of *N*-hydroxy-

succinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were dissolved in dichloromethane and stirred for 2 h at room temperature. After concentration under vacuum, the residue was dissolved in ethyl acetate and washed with water. The organic layer was dried over $MgSO_4$ and concentrated under vacuum to obtain *N*-hydroxysuccinic ester of PCDA (PCDA-NHS), yielding 1.21g (96%). 1.21 g (2.57 mmol) of PCDA-NHS, and 2.56 mL (25.70 mmol) of 2-(2-aminoethoxy)ethanol (AEE) were dissolved in dichloromethane and stirred for 3 h at room temperature. After concentration under vacuum, the residue was dissolved in ethyl acetate and washed with water. The organic layer was dried over $MgSO_4$ and concentrated under vacuum to give a residue which was subjected to silica gel column chromatography using ethyl acetate/methanol as eluent, yielding 0.98 g (83%) of the desired product.

**PCDA-NHS:** m.p. 67 - 68.5 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ = 0.88 (t, 3H), 1.25-1.57 (32H), 2.24 (t, 4H), 2.60 (t, 2H), 2.85 (t, 4H).

**PCDA-AEE:** m.p. 72 - 74 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ = 0.88 (t, 3H), 1.25-1.57 (32H), 2.06 (t, 1H), 2.18 (t, 2H), 2.24 (t, 4H), 3.48 (q, 2H), 3.56-3.60 (m, 4H), 3.76 (q, 2H), 5.87 (s, 1H).

**PDA preparation.** PDA solutions used in this study were prepared as follows. A diacetylene monomer was dissolved in 2 mL of chloroform. The organic solvent was removed by purging with $N_2$ gas to make a thin film inside the vial, and deionized water (30 mL) was added to the vial to yield a total monomer concentration of 1 mM. The sample was then heated at 80 °C for 15 min and probe-sonicated for 15 min. The resulting solution was filtered through a 0.8 μm filter, and the filtrate was allowed to stand at 4 °C for 12 h before being mixed with ethylene glycol (EG) (PCDA : EG = 98 : 2, v/v) to increase the viscosity. Polymerization was carried out at room temperature by exposing the solution to 254 nm UV light (1 mW/cm$^2$) until the absorption at 640 nm reaches a maximum (*ca.* 5 min).

**Fabrication of microfluidic chip.** A microfluidic chip is composed of a PDMS substrate bonded to a glass slide. The PDMS substrate contains a microchannel network whereas the glass slide has an ITO film microheater and thin film thermocouples. The PDMS substrate is fabricated by using standard soft lithography and molding techniques described in our previous report.[30]

Fabrication processes for the microheater and thermocouples are as follows. First, a photoresist (PR) (AZ 4620) is spin-coated for 10 s at 500 rpm and 40 s at 2500 rpm to a uniform thickness of 3 μm on the glass wafer that is intensively cleaned with acetone, isopropyl alcohol (IPA), and deionized (DI) water. It is soft-baked at 100 °C for 4.5 min. Next, the wafer is exposed to UV light (Jaesung Corp.) for 8.5 min through a film mask with a microheater pattern, is developed for 2.5 min, and is thoroughly cleaned with DI water. Then, the ITO is sputtered (Sputter System, Sorona) on to the wafer to a thickness of 200 nm. Finally, an ITO film microheater with a width of 50 μm is completed by the lift-off process that strips off PR with acetone.

Thin-film thermocouples are integrated on the glass wafer by sequentially depositing copper and constantan. Copper lines of a 200 nm thickness were first patterned with E-beam evaporator (EBX1000, Ulvac) after spin-coating and developing PR on the wafer by the same method as described beforehand and depositing a 20 nm Ti film by E-beam evaporating (EBX 1000, Ulvac) for a better adhesion of copper to the glass. Using the lift-off process, PR was stripped off to complete the copper patterning. Next, constantan lines were patterned with the same procedure as the copper lines with careful alignment (MA6-II, Suss MicroTec). Only exception is that it was deposited by thermal evaporating (Daeki Hi-Tech) to a height of 400 nm. As the final process, the microheater and thermocouples were electrically insulated by sputtering ONO ($SiO_2$ 250 nm, $Si_3N_4$ 300 nm, and $SiO_2$ 250 nm) of 800 nm in thickness on the wafer (Daeki Hi-Tech).

**Sensor test and temperature measurements.** Fluorescence emitted from thermally stimulated PDA droplets were imaged using an inverted fluorescence microscope (IX71W, Olympus) with a 4x objective and a color CCD camera (DP70, Olympus). PDA solutions and corn oil are inserted to a microfluidic chip by using two syringe pumps (KDS120, KD Scientific): one for the PDA flow and the other for the oil flows. The flow rates of a PDA solution and an oil flow are maintained at 0.02 mL/h and 0.12 mL/h, respectively. Syringes were connected to the inlets of the microchannels through capillaries and microfluidic fittings (LabSmith). Finally, a thermocouple amplifier (SCXI-1102, National Instruments) and LabView software (National Instruments) were used to acquire voltages out of thin film thermocouples.

**Temperature calibration.** Thin-film thermocouples were calibrated to determine their Seeback coefficient which is known to be different from those of the bulk thermocouple.[31] The coefficient correlates the voltage of a thermocouple with the temperature difference between the sensing position and the other end of the thermocouple. We found that the Seeback coefficient is 16.8 μV/K. By using this coefficient, we were able to measure temperatures at the entrance and exit of the main channel. Then, we determined a temperature at an arbitrary position in the channel by interpolating the two temperatures.[27]

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