

Supporting Information

Enzyme entrapped nanoporous scaffolds formed through flow induced gelation in microfluidic filter device for sensitive biosensing of organophosphorus compounds

*Donglai Lu, Guocheng Shao, Dan Du, Jun Wang, Wanjun Wang and Yuehe Lin**

Microfabrication Procedures

Soft lithography was used for the microfluidic device fabrication. SU-8 mold was made by a typical UV-lithography process. A thin layer of SU-8 10 was spin coated on a 4" silicon wafer at the speed of 1800 rpm for 30 second. The SU-8 layer was then baked at 65°C for 3 minutes and at 95°C for 8 minutes. The resulting SU-8 layer was then fixed underneath a high resolution mylar mask (Fineline Imaging) and exposed at the UV dosage of 400 mJ/cm² (365 nm). After post baking at 65°C for 1 minutes and 95°C for 3 minutes, the SU-8 mold was developed in SU-8 developer for 8 minutes. Before the molding process, SU-8 mold was first silanized in Hexamethyldisiloxane vapor for 3 hours. The microstructures of the SU-8 mold were characterized by using a Zygo GPI™ interferometer. The typical results for 10 μm ridges (these ridges will be gaps in the final microfluidic device) were shown in Figure S1. The average height of the microfluidic filter is 35.44 μm. A polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning) prepolymer was prepared by mixing silicon base and its curing agent in a 10:1 w/w ratio, poured over the pattern in a Petri dish, and then heated at 80 °C for 2 hours to harden the PDMS. The cured PDMS was peeled off from the SU-8 mold and cut into slabs. A clean, dry glass slide and the patterned side of the PDMS were placed in a plasma chamber for a minute to increase their surface energy and facilitate covalent bonding, after which they were tightly pressed against each other to form the microfluidic device. The bonded microfluidic device was placed into 80 °C oven for 2 hours.

Medical-grade syringes with a 15 ml volume (BD) and 27 G 1/2 needles (BD) were used to inject the surfactant solution into the microchannel using a KD scientific syringe pump. To eliminate contamination from the lubricant oil used in the syringes, all syringes and needles were sonicated in isopropyl alcohol (Sigma Aldrich), rinsed thoroughly with nanopure water, and dried at 110 °C before being used in the experiments. A Keyence (VH-Z100R) microscope was used to characterize the 2-D structure of the microfluidic filter device and observe the flow induced gelation process. We designed and tested three types of microfluidic filter devices with different gap size, as shown in Figure S2. And we only observed the gelation happened in the device with 3 μm gaps.

Flow Injection Amperometric Detection of Paraoxon.

Flow injection amperometric detection of paraoxon was performed with the flow injection system, as shown in Figure S3. First, 10 μL of a desired concentration of ATCh substrate was injected to obtain the initial response of the biosensor after a steady-state value was obtained. Then, 10 μL of a specific concentration of paraoxon was injected. After the paraoxon reached the cell, the flow was stopped at a desired inhibition time. Following the inhibition step, the response of the nanogel biosensor to the same concentration substrate was recorded again. It was noted during the inhibition that the electrochemical recording of current via time was paused. The regeneration of the nanogel biosensor after a complete measurement was realized by successive injections of 200 μL of 5 mM 2-PAM and ATCh at a flow rate 100 $\mu\text{L}/\text{min}$.

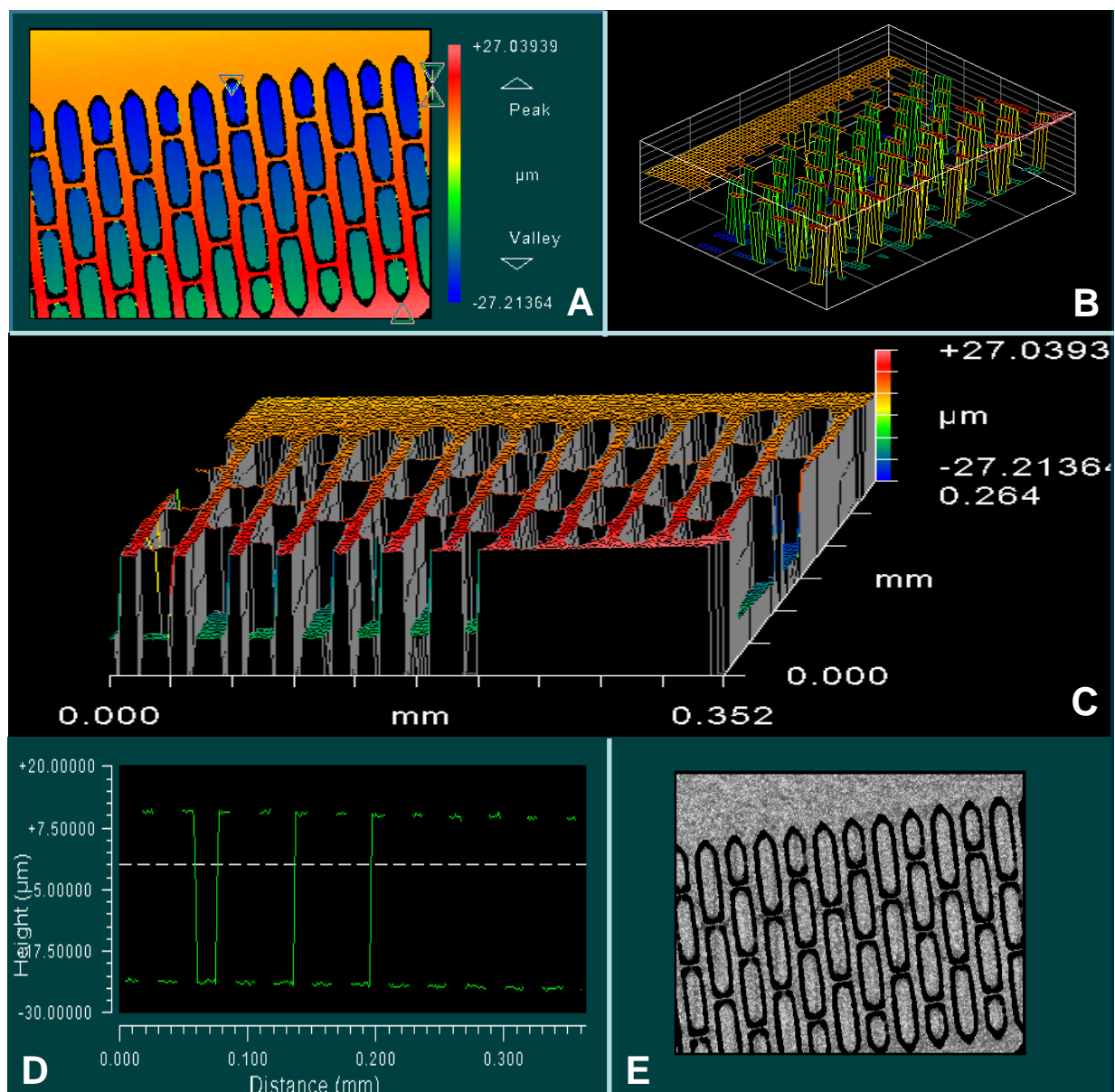


Figure S1: Typical characterization results by using Zygo interferometers for microfluidic filter microstructure using SU-8 mold. (A). Surface map (B). 3-D Plot (C). Oblique plot (D). Surface profile (E). Solid plot.

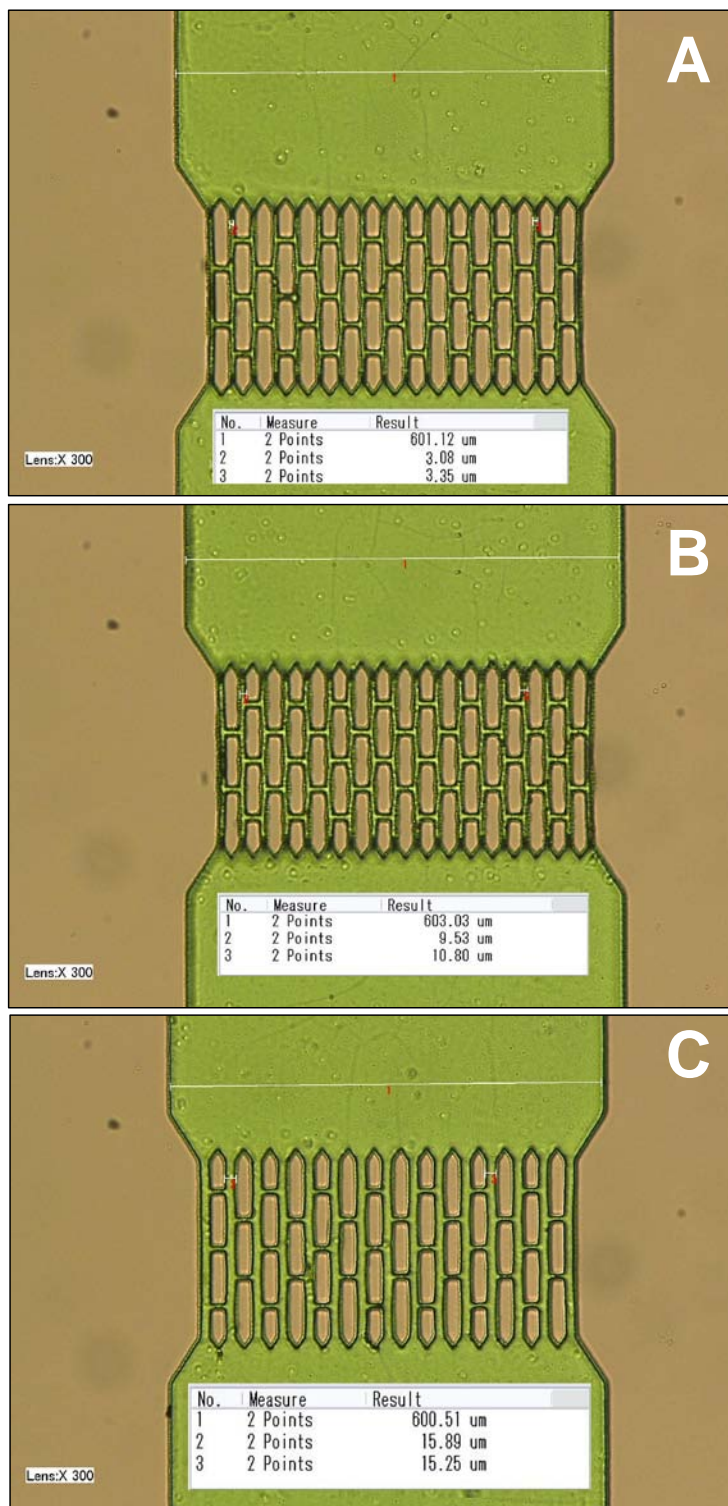


Figure S2. Microscope images of microfluidic filter devices with different gap sizes, 3 μm (A), 10 μm (B) and 15 μm (C).

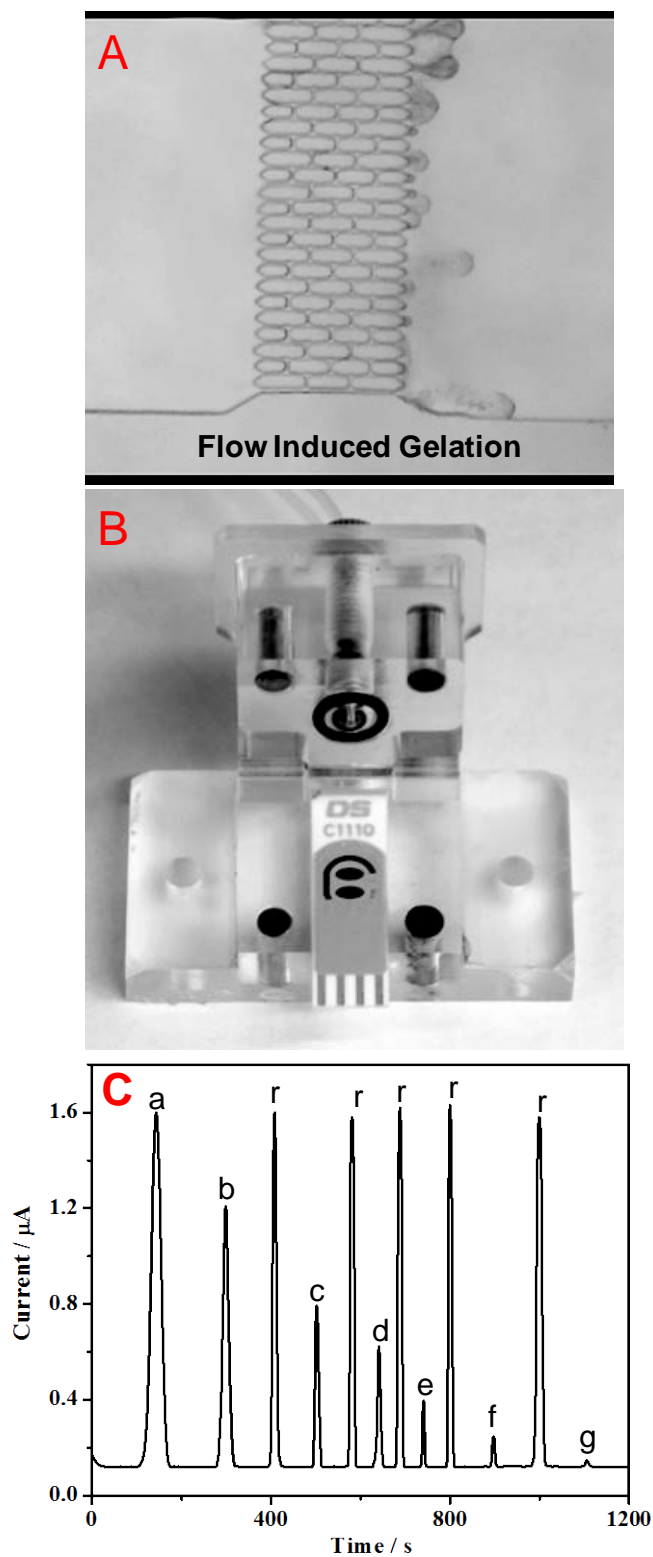


Figure S3. Microscope images of flow induced gelation (A) and flow injection analysis system (B) and typical amperometric responses (C).