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Citation: Applied Physics Letters **92**, 053108 (2008); doi: 10.1063/1.2840175
View online: http://dx.doi.org/10.1063/1.2840175
View Table of Contents: http://scitation.aip.org/content/aip/journal/apl/92/5?ver=pdfcov
Published by the AIP Publishing

Immobilization of layered double hydroxides in the fluidic system for nanoextraction of specific DNA molecules

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(Received 31 August 2007; accepted 15 January 2008; published online 8 February 2008)

The purpose of this study was to immobilize inorganic layered double hydroxides (LDHs) on the poly(methylmethacrylate) substrate as the media to extract the specific DNA molecules through fluidic system to enhance the efficiency of extract specific DNA molecules from extremely low concentration in sample solution. LDH immobilized through solvent swelling and plasma treatment on the polymer surface captured the specific DNA molecules lysed from Escherichia coli (E. coli) cells as the target DNA molecules with 2×10^{-4} g/l of concentration in sample solution mixed biomacromolecules lysed from human blood. The encapsulated DNA molecules released through dissolving of LDHs by slight acid (pH=4–5) solution then amplified by polymerase chain reaction (PCR) process through the primers for E. coli cells. The DNA molecules amplified by PCR process were characterized by gel electrophoresis to recognize the existence of E. coli cells. The results show that immobilized LDHs could be regarded as the specific DNA detector for rapid disease diagnosis through fluidic system. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2840175]

Gene therapy is gaining growing attention for the treatment of genetic deficiencies and life-threatening diseases recently. Originated from microelectronics, it has spread into many other disciplines, such as biology, chemistry, medicine, and therapy for the application of new drugs, clinical disease diagnosis, food security, and the chemical industry.[1–8] The DNA chips contained thousands of microscopic DNA probes have been well investigated to analyze multiple genes simultaneously through biomicroelectronic mechanical system in a lab-on-a-chip (LOC).[9] Recently, it has been demonstrated that layered double hydroxide (LDH) could form a nanohybrid by intercalating with biomolecular anion such as mononucleotides and DNA.[10,11] Layered double hydroxides represented by the general formula $[M^{II}_{1-x}M^{III}_{x}(OH)_2][A^{m-}_{x/m}nH_2O]$ (abbreviated notation $M^{II}_{R}M^{III}/A$ with $R=(1-x)/x$), where M^{II} and M^{III} are di- and trivalent metal cations and A^{m-} are interlayer anions, are entirely degradable at acidic pH.[12]

Such biomolecules can be incorporated between hydroxide layers by a simple ion-exchange reaction to form bio-LDH nanohybrids.[12] The negatively charged biomolecules intercalated in the gallery spaces would gain extra stabilization energy due to the electrostatic interaction between cationic brucite layers and anionic biomolecules. Here, the hydroxide layers can play the role of a reservoir to protect intercalated DNA from DNase degradation. If desired, the hydroxide layers can be intentionally removed by dissolving in an acidic media, which offer a way of recovering the encapsulated biomolecules. This observation prompted us to design and formulate LDHs as DNA extractor with immobilization in the polymeric tunnels able to capture and release DNA molecules more efficiently from diseased blood through fluidic solution. More precisely, our goal was to explore the possibility of formulating the polymeric tunnel with LDHs that were capable of complete extraction from the flowing solution mixed with DNA molecules and macromolecules. Poly(methyl methacrylate) (PMMA) was utilized to be the substrate to fabricate the trenches on the surface by hot embossing process due to manufacturing easily and low price for commercialization.[13,14] In this work, our primary attention was focused on the immobilization of LDH on the surface of trenches fabricated from PMMA to nanoextract the DNA molecule from the mixed solution with human blood and diseased cells through fluidic system.

The schematic in Fig. 1 illustrates the process immobilized the LDHs on the PMMA substrate to capture and release the DNA molecule through surface treatment. The amorphous PMMA substrate was immersed in the tetrahydrofuran (THF), the good solvent to PMMA, to swell PMMA chains on the surface.[15] The end of PMMA chains extended into the swell layer caused from the permeation of THF into the PMMA chains on the surface. After removing the THF from PMMA substrate, the sample was then subjected to oxygen plasma treatment. The remaining end of PMMA chains was chemically modified and rendered strongly hydrophilic. The sample was then coated with the solution mixed LDHs and surfactant on the surface. Due to the hydrogen bonding and polymer entanglement, the LDHs were immobilized rapidly and rigidly by the end chains of PMMA with strong hydrophilic property after dehydration of heating process. The surface immobilized with LDHs was then cleaned by surfactant solution for LDHs to remove the LDHs without immobilization on the PMMA surface. The 2×10^{-4} g/l of concentration for DNA molecules of Escherichia coli (E. coli) cells were utilized as the target of DNA molecules for nanoextraction from the fluidic solution mixed with human blood and buffer solution utilized to lyse the cells in the solution in channel with immobilized LDHs.[16] The solution with biomacromolecules and DNA molecules from lysed cells was injected into the channel on PMMA

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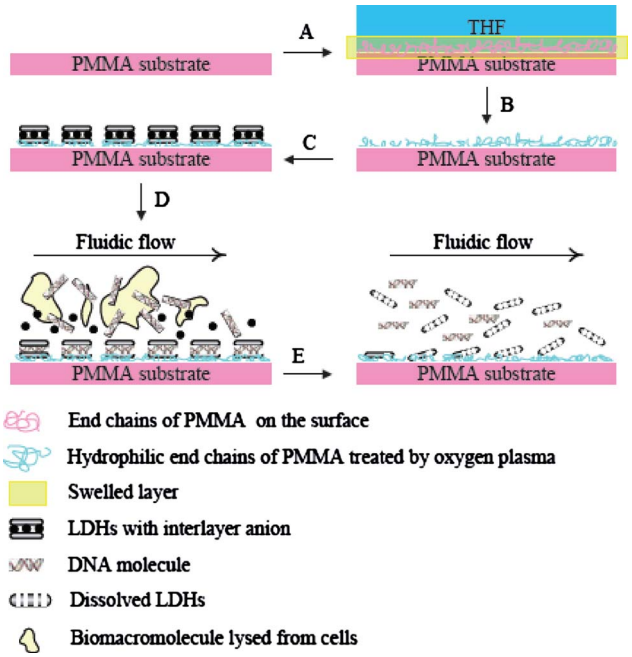

FIG. 1. (Color online) Schematic of the process used to immobilize LDHs on the PMMA surface for extract the specific DNA molecules from the sample solution mixed biomolecules from lysing cells. (A) The amorphous PMMA substrate was immersed in the THF to arise the end of PMMA chains from the surface as the pillarlike structure. (B) Oxygen plasma treatment was used to chemically modify the surface with arisen end of polymer chains to enhance the adhesion between PMMA surface and LDHs as the THF was removed from the surface. (C) LDHs dispersed in the surfactant solution were spun on the surface treated by oxygen plasma for immobilization by entanglement from the ends of polymer chains. (D) The sample solution mixed target DNA molecules and biomacromolecules lysed from the human blood were injected into the channels for extraction through a simple ion-exchange reaction to form bio-LDH nanohybrides on the PMMA surface in fluidic system. (E) The slight acid solution (pH=4–5) was injected into the channels to dissolve LDHs for releasing the encapsulated DNA molecules after the biomacromoles lysed from human blood were removed from the channels by fluidic system.

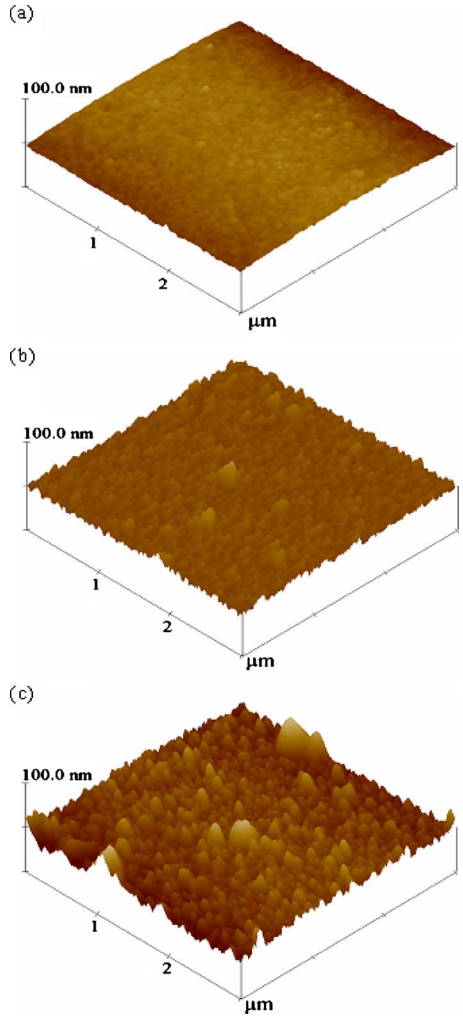

FIG. 2. (Color online) Atomic force microscopy images for the amorphous PMMA surface. (a) Original surface of PMMA substrate. (b) Pillarlike structure arose by good solvent (THF). (c) Pillarlike structure surface treated by oxygen plasma.

substrate by air micropump for controlling the fluidic velocity and direction. The DNA molecules suspended in the solution mixed biomacromolecules were captured rapidly by LDHs from the fluidic system through anion exchange with interlayer anion for 5 min. Then, the unexpected biomacromolecules were removed from the channels of PMMA to approach the nanoextraction by fluidic system. The LDHs would then be removed slowly in the solution with slight acidic (pH=4–5), then, the encapsulated DNA molecules were released in the fluidic solution. The system was preceded with the fluidic flow in the channel inside the PMMA substrate.

The morphology of PMMA the surface treatment by THF swelling was investigated by atomic force microscope, as shown in Fig. 2. The amorphous surface of PMMA substrate without surface treatment showed the smooth structure with 1.686 nm of the roughness, as shown in Fig. 2(a). THF permeated into the amorphous surface to arise the ends of PMMA chains as the PMMA substrate immersed in the THF, which increased the roughness from 1.686 to 4.645 nm of the PMMA surface, as shown in Fig. 2(b). The PMMA surface swelled by THF was chemically modified and rendered strongly hydrophilic or polar by oxygen plasma treatment, causing the increase of roughness with 8.566 nm, as shown in Fig. 2(c). The values of contact angle for the PMMA surface increased from $54° \pm 5°$ to $82° \pm 5°$ after the amorphous surface immersed in THF due to the formation of pillarlike structure.[17,18] The PMMA surface with pillarlike structure rendered strongly hydrophilic property, causing the value of the contact angle for water was closed to 0°.

Figure 3 showed the microfluidic chip system with the channels of 1 mm feature size on PMMA substrate with LDHs. The sample for specific DNA extraction was injected into the helixlike channel located near the left side of the edge. The LHDs were immoblized around the straight channels on the middle of the microfluidic chip system. The sample solution mixed specific DNA molecules and biomolecules was flowed into the channels with the immobilized LDHs for specific DNA molecule extraction. The solution sample was driven forward and backward for 4 cycles in the

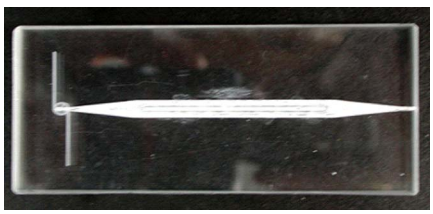

FIG. 3. (Color online) Optical microscope image of the fluidic system with the straight channels immobilized LDHs for extraction of DNA molecules. The helixlike structure at left side was the injected inlet for sample solution.

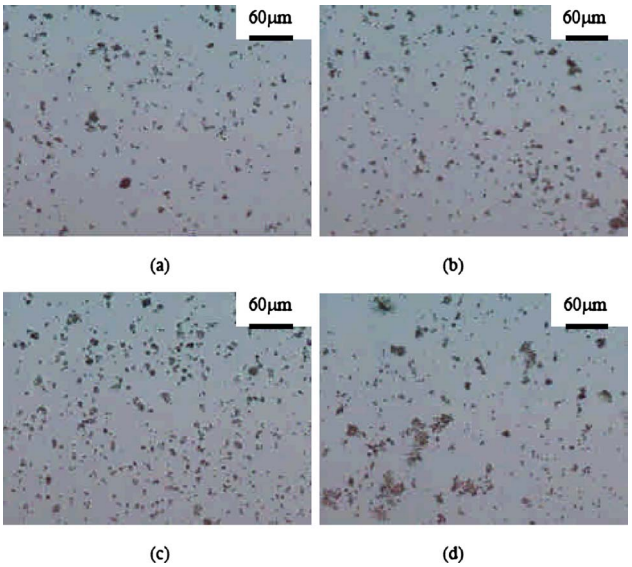

FIG. 4. (Color online) Optical microscope images of the channels immobilized LDHs with (a) 5.0, (b) 10.0, (c) 15.0, and (d) 20.0 wt % of concentration in the surfactant solution on PMMA surface.

straight channels to enhance the extracted efficiency due to the extreme low concentration. The morphology of the immobilized LDHs on the channels was shown in Fig. 4 with various concentrations of the LDHs in the mixed surfactant solution, which the LDHs were dispersed on the straight channel surface. The 5, 10, 15, and 20 wt % of concentration for LDHs coated on the PMMA substrate aggregated in the range of 1–50 μm feature size.

The encapsulated DNA molecules lysed from human blood cells and E. coli cells as the target were obtained from the channel in the solution for amplification by polymerase chain reaction (PCR) process to enhance the quality of target DNA molecule for identification. The forward and reverse primer sequences to the target DNA molecule of E. coli cells for amplification through PCR process were 5A-CAGGATTAGATACCCTGGTAG-3A and 5A-TTCCCCTACGGTTACCTTGTT-3A from E. coli cells, respectively. The length of target DNA molecule after amplification by PCR process of 1 cycle of 5 min at 95 °C, 40 cycles of 30 s at 95 °C, 40 s at 58 °C, and 40 s at 72 °C, and one cycle of 10 min at 72 °C through specific primer. The DNA molecules amplified through the primer by PCR process corresponded to the amplified 16S RNA in the DNA fragments of E. coli genomic DNA. The DNA ladders were then utilized as the marker in the gel electrophoresis for quantitative experiment by a bioanalyzer. The results of specific DNA molecule extraction from the sample were shown in Fig. 5. The marker demonstrated the all well-known DNA molecule database to compare with the results from other specimens through the agarose gel electrophoresis.[19] Lanes (1) and (2) represented positive control to confirm the gel electrophoresis process. Lanes (3), (4), (5), and (6) represented 5.0, 10.0, 15.0, and 20.0 wt % of LDHs concentration in surfactant solution to immobilize on the PMMA surface, respectively. The specimens released from LDHs with various concentrations in coating solution demonstrated the same result of 620 base pair amplified by PCR but different brightness. The brightness demonstrated in agarose gel electrophoresis diagram increased with the concentration of LDHs at the range from 5 to 20 wt % due to the quantity of the

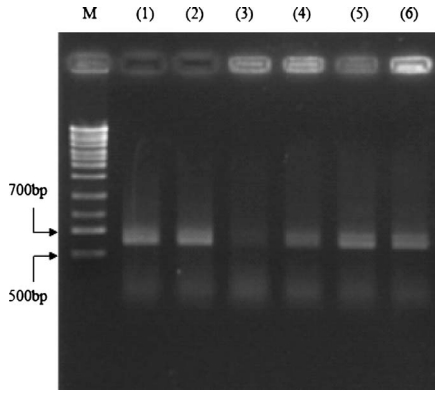

FIG. 5. Agarose gel electrophoresis diagram of the specific DNA molecules released from LDHs, then amplified by PCR process. Lanes (1) and (2) were positive control. Lanes (3), (4), (5), and (6) were 5.0, 10.0, 15.0, and 20.0 wt % LDHs in surfactant solution for spin coating, respectively.

specific DNA molecules captured from LDHs. Enlarged aggregation of the LDHs (Fig. 4) impeded the exchange of interlayer anions with DNA molecules for 20 wt % of LDHs, caused the similar brightness between lanes (5) and (6). The DNA molecules shorter than 620 base pair obtained from PCR process were the primer or incomplete reacted DNA molecules for PCR process, as shown in the end of the lanes in Fig. 5.

In conclusion, the key feature of this approach is the use of polymer entanglement enhanced by the solvent swelling and plasma treatment to immobilize LDHs on the PMMA surface but buried LDHs in the PMMA. The efficiency of encapsulation of DNA molecules is reduced as the LDHs buried in PMMA substrate due to polymer entanglement surrounding. The results obtained from agarose gel electrophoresis suggest that the immobilized LDHs on the surface could regard as the rapid detector for specific DNA molecule in the human blood by one droplet human blood to approach the LOC system.

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