IN-SITU SYNTHESIZED AND PATTERNED NANOWIRE ARRAYS IN MICROFLUIDIC CHANNEL FOR PARTICLE TRAPPING AND CELL LYSIS APPLICATIONS

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ABSTRACT

Cell trapping and preparation (ex. lysis, separation, filtering etc.) becomes important tasks for bioassays as the needs of single cell study are increased. We introduce a novel and simple nanowire-integrated microfluidic device that can be applied to the biological cell assay. Nanowires were grown in the microchannel by in-situ nanowire synthesis method or conventional hydrothermal method on microscale post arrays. We demonstrated preliminary results of particle trapping and cell lysis with nanowire-integrated microfluidic devices. Nanowire bundle-based particle trapping shows good trapping performance. Also, cell lysis by using nanowire-integrated microfluidics shows good mechanical lysis performance.

KEYWORDS: Nanowire, microfluidics, cell trapping, cell lysis

INTRODUCTION

Cell trapping and lysis are essential steps for intracellular analysis and single cell study. Therefore, various BioMEMS devices with integrated micro/nanostructures for cell trapping [1, 2] and lysis [3, 4] have been developed. However, the integration of nanostructures in microfluidic devices involves complicated synthesis and assembly processes. Also, it is hard to integrate nanostructures on microstructures with ex-situ methods. In this paper, we introduce a novel platform for particle trapping and cell lysis that consists of arrays of ZnO nanowires within a microfluidic channel fabricated by either in-situ synthesized [5] or conventional hydrothermal synthesis on PDMS microchannel.

EXPERIMENTAL

Cell lysis and particle trapping microfluidic device was fabricated by in-situ nanowire synthesis method [5] or conventional hydrothermal synthesis on PDMS microchannel. Firstly, the device fabrication by the in-situ synthesis is as follows: (a) The ZnO seed pattern was made by e-beam evaporation of Cr (50 nm, adhesion layer) and ZnO (10 nm, seed layer) thin films on photoresist patterns on glass substrate. (b) After lift-off process, seed pattern (ZnO/ Cr dual layer) was annealed at 150 °C. (c) PDMS channel (height = 20 μm and width = 150 μm) was fabricated by soft lithography process. (d) PDMS channel block was aligned and bonded to the glass substrate with patterned seed layer. After device fabrication, the patterned array of ZnO nanowires was synthesized by in-situ hydrothermal synthesis process [5] (figure 1(a)).

Secondly, hydrothermal synthesis of nanowires on PDMS channel with microscale posts was conducted by the following steps: (a) PDMS microchannel with microscale post array was fabricated by soft lithography process. (b) ZnO nanowire seed

Figure 1. Schematics of nanowire fabrication within microchannel by (a) in-situ ZnO nanowire synthesis on seed-patterned substrate and (b) conventional hydrothermal synthesis along microscale post arrays.
solution was dropped on the PDMS block and gently rinsed by ethanol for uniform coating of seed layer (repeated 3 times).
(c) The surface of PDMS to be bonded onto the glass slide was covered with a thin PDMS layer by stamp-and-stick method to prevent growth of ZnO nanowires [6]. (d) ZnO nanowires were synthesized on the microchannel and microscale posts by hydrothermal method. (e) Final device was prepared by assembling ZnO nanowire-grown PDMS microchannel on the glass slide by stamp-and-stick method (figure 1 (b)).

We demonstrated the applicability of the patterned ZnO nanowire arrays to particle trapping and cell lysis in microfluidic channel. For particle trapping, ‘U’ shaped porous micro-cages based on ZnO nanowires were fabricated in the microchannel by in-situ synthesis method. Silver-coated hollow microspheres were suspended in ethanol with 0.001 g/mL concentration. Ethanol-based particle solution was flown through the nanowire-integrated microchannel at a flow rate of 1μL/min.

To evaluate the potential capability of this device to cell-related applications, we conducted cell lysis experiment. For the confirmation of cell lysis, HaCaT cell (diameter ~ 20 μm after being trypsinized) was flown with rhodamin-phalloidin, which can label intracellular actin filaments when cells are ruptured. The cell solution (800 cells/μL) was flown with a flow rate of 1-5 μL/min for 30-60 min for lysis.

After passing through the microchannel, the concentrations of protein and nucleic acid in the cell solution was measured with a spectrophotometer (NanoDrop, ND-1000).

RESULT AND DISCUSSION

Fabricated nanowire arrays are shown in figure 2. The shapes of ZnO nanowire patterns follow designed seed patterns. Various patterns of ZnO nanowire bundles ranging from simple line or dot arrays (figure 2 (a), (b)) to arbitrary letter patterns (not shown) could be conveniently fabricated within the microchannel [5]. The heights and widths of the nanowires were controlled by the synthesis time (h = 1.5 μm after 30min, h = 6.2 μm after 200 min). Compared to the conventional bulk synthesis, continuous supply of fresh precursors allowed much faster growth of ZnO nanowires in microfluidic channel. Nanowires could also be grown on the PDMS micropost array within the PDMS microchannel (figure 2 (c)) by conventional hydrothermal reaction in the precursor solution bath.

![Figure 2](image1.png)

Figure 2. SEM images of (a) ZnO nanowire arrays that were in-situ grown in microchannel and (b) hydrothermally grown nanowires in PDMS microchannel with microscale posts.

For particle trapping, U-shaped nanowire bundle-like cages were fabricated by in-situ synthesis method. As shown in figure 3, they allow efficient trapping of microparticles (silver-coated hollow microspheres in ethanol, diameter ~ 10 μm). Here, smaller fluidic resistance, although not measured, is expected than solid walls due to the porous network structure of ZnO nanowire bundles that imposes less obstruction to the fluid. Microparticles were entrapped in the nanowire cages because of the geometric confinement by the morphology of cages when the they encountered nanowire-based micro-cages while flowing in streamline. This experiment shows the applicability of these structures to cell trapping and separation tasks.

![Figure 3](image2.png)

Figure 3. (a) SEM image of U-shaped nanowire-based micro-cages and (b) microscope image of trapped particles.

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Cell lysis was performed with two kinds of nanowire array platforms. Cells passing through the microchannel with ZnO nanowire arrays were ruptured and labeled with red fluorescence dye while those passing through empty microchannel did not express any fluorescence (figure 4 (a)). It is believed that sharp tips and high-aspect ratio of ZnO nanowires enable easy breakage of cell membrane, leading to mechanical cell lysis as a consequence. The fluorescence images show that nanowires grown on the microscale posts are more effective in the cell lysis than those grown on the channel substrate by in-situ synthesis method. In order to quantify the cell lysis efficiency, the concentrations of protein and nucleic acid of cell solutions collected from the outlet of the microchannel were measured. Empty microchannel and microchannel with microscale post only were used as control samples. Protein concentration of cell solution that was flown through microchannel with nanowires grown along micropost arrays was higher than those from control samples by 76%. Furthermore, higher concentration of nucleic acid from nanowire-integrated microchannel shows that not only cell membranes but also cell nuclei were mechanically ruptured by of the nanowires without any help of chemical reagents (figure 4 (b)).

Figure 4. Cell lysis result by (a) fluorescence labeling and (b) concentration measurement of protein and nucleic acid of cell solution.

CONCLUSION

In this article, we introduced a novel nanowire-integrated microfluidic device for particle trapping and mechanical cell lysis. It is expected that the nanowire bundle structure may induce smaller fluidic resistance than solid walls due to the porous nature of the structure, while maintaining efficient trapping performance. Also, mechanical cell lysing by using nanowires within microchannel allows much simpler and economical lysis, potentially with less contamination of intracellular molecules than chemical cell lysis method. Although we demonstrated only these two applications here, we believe that nanowire integrated microfluidics can be widely utilized for a variety of lab-on-a-chip and BioMEMS applications.

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