LARGE AREA 3D MICROFABRICATION TECHNIQUE BY MULTIDIRECTIONAL PHOTOLITHOGRAPHY FOR A CHROMOSOME EXTENSION CHIP

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ABSTRACT

In this paper, we propose a UV lithography technique for fabricating large-area 3-dimentional structures using multidirectional UV exposure, and report its application to fabrication of a human chromosome extension chip. Human chromosomes are composed of DNA fibers of approximately 2nm in width and 8.3mm in maximum length having complicated three-dimensional conformations in a nucleus. A human chromosome extension chip with simple method proposed previously have a small work area for extension in comparison with the human chromosome size. So, we propose a fabrication technique with large work area and carried out extension tests with human chromosome. We evaluated a fabrication accuracy of the proposed technique and fabricated a large mesh array chip in the diameter of 68 mm, and demonstrated extension of the chromosomes by flow induced by a centrifugal force and compartmentalize them on the microfabricated 3D mesh array.

KEYWORDS: Gene Analysis Chip, DNA fiber, Centrifugal force, Chromosome, Photolithography

INTRODUCTION

Human chromosomes are composed of DNA fibers of approximately 2nm in width and 8.3mm in maximum length having complicated three-dimensional conformations in a nucleus. Extension of chromosomes to a fiber form have attracted attention in the field of DNA analysis, though, conventional techniques have difficulties in positioning extended chromosomes for visualization of them [1]-[4]. Previously, we have proposed a microfabricated 3D mesh array that extends chromosomes by flow induced by a centrifugal force and compartmentalize them at its mesh structures [5]. Although we demonstrated a highly-resolved DNA observation and a short-time gene analysis using the chip, it requires further development for visualization of whole chromosomes in fiber conformations; i.e. fabrication of a large-area chip. To solve this problem, we improved a 3D fabrication technique using multidirectional UV exposure with larger work area and demonstrated chromosome extension in the fabricated large-area chip.

THEORY

Figure 1 shows an extension method of the chromosomes. Firstly, chromosome suspension was dropped to the center of the circular chip. Then, parts of the chromosome fixed to the chip by physical adsorption due to half air-dry of the chromosome. Secondly, the chip are rotated by a spin-coater, and the chromosomes are extended by the shear stress in the flow field induced by the centrifugal force. The extend chromosomes are suspended along the groove at the top of the mesh structures in liquid, and chromosomes fixed by physical adsorption due to half air-dry. Finally, the suspended chromosomes in the liquid are stained by a fluorescent dye or hybridized by multi DNA probes, and observed by a fluorescence microscope. Although we demonstrated a highly-resolved DNA observation and a short-time gene analysis using the chip [5], it requires further development for visualization of whole chromosomes in fiber conformations; i.e. fabrication of a large-area chip. Previous chip has a disk shape of 14 mm diameter in maximum which is insufficient for the extension of long chromosomal fibers. To solve this problem, we improved a 3D fabrication technique using multidirectional photolithography and fabricated a mesh array with a large area of 68 mm in diameter.

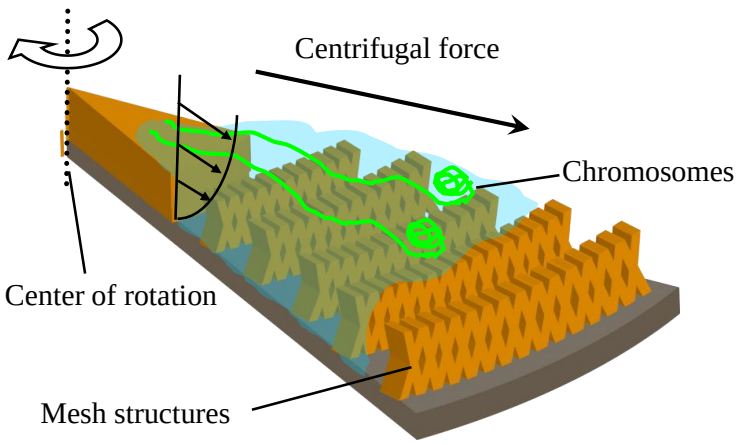

Figure 1. A conceptual diagraph of chromosomes extended by a centrifugal force

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FABRICATION

The proposed mesh array chip is fabricated the multidirectional photolithography. The concentrically micro-mesh structures and four dropping stages on the microchip were simultaneously fabricated by the single process of the rotated/inclined exposure with a fixed mask. The proposed process involves directly spin-coating a thick negative photoresist such as SU-8 on a mask and rotational exposing it from the backside of the substrate with the fixed mask. A large variety of microstructures are fabricated by the combination of the mask patterns of the rotated and fixed masks. We evaluated a precision of dimensions of a chip fabricated with previous exposure condition. The top length of a mesh structure, *a*, was selected as the representative size for the evaluation. The difference from the design value, *r*, was plotted along the distance from the chip center (in Figure 2), showing a large difference of over 40 % in $r < 3$ mm. To reduce it, we optimized a mask pattern and an exposure dose, which brings a high precision less than 30 % in whole area. To demonstrate a large area fabrication, we assembled a setup of rotational inclined exposure as shown in Figure 3 that is available for exposure of up to 4-inch substrate, based on the estimation of the uniformity of UV light field and the precision of the pattern. Using the setup, we fabricated a mesh array chip of 68 mm in diameter with a mesh size of 2μm in width as shown in Figures 4 and 5.

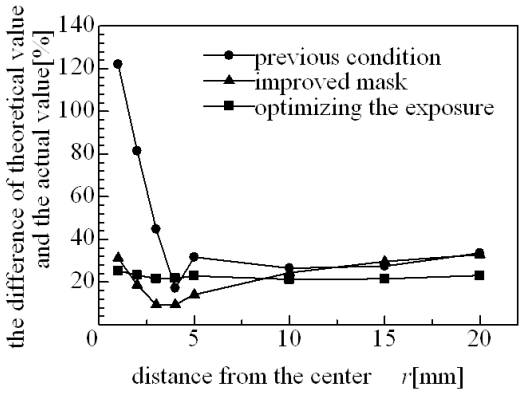


Figure 2. Accuracy of dimension of the fabricated mesh structures on the chip

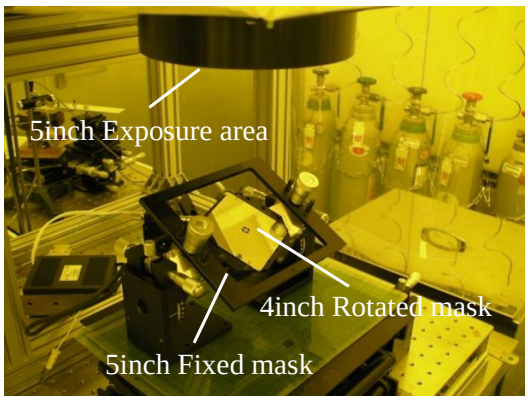
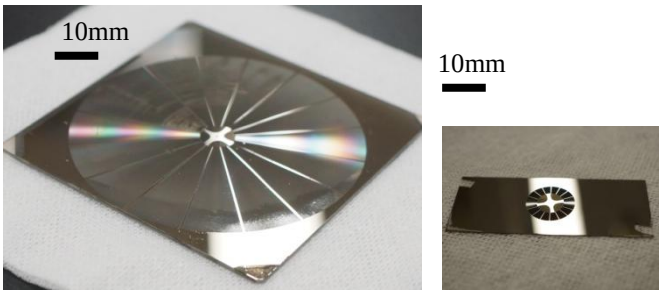
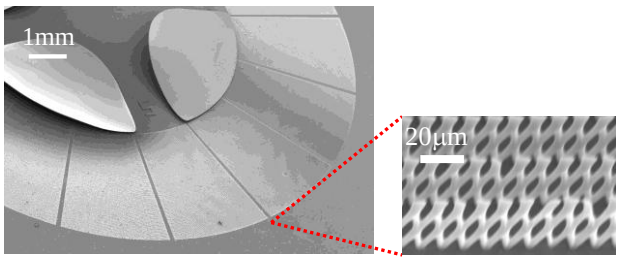


Figure 3. A Photograph of a multidirectional exposure system for 4-inch in work area



(a) A Large mesh array chip *(b)* A previous *chip*

Figure 4. Photographs of the mesh array chips



(a)Whole image *(b)Micro mesh structures*

Figure 5. SEM images of the device fabricated by the multidirectional photolithography

RESULTS AND DISCUSSION

We evaluated the extended length of the chromosomes as the function of the number of rotation for optimizing the extend conditions of the chromosome. Chromosome extension was performed using HeLa cell samples. First, the cells were ruptured by chemical treatment and the solution containing chromosomes were dispensed onto the center of the mesh chip. Then, the chip was rotated with the speed of 4000-6000 rpm to induce a flow on the chip by the centrifugal force, resulting in chromosome extension by its shear force.

Chromosome fibers were suspended between the micromesh structures, where they were spontaneously aligned with constant spacing between each fiber due to the mesh as shown in Figure 5. The fiber lengths were evaluated by staining them with fluorescent dye as shown in Figure 6, showing 200-300 μm extension, which is over 20-times extension compared to a chromosome in a cell. The results also show the higher rotational speed allows the longer extension. These results show the successful extension of human chromosomes with a large area 3D microstructure chip.

Then, chromosome extension on large mesh array chip was rotated with the speed of 5000 rpm in 30 seconds. The extended chromosome was 1.4mm in length on the large mesh array chip as shown in Figure 7. Since the maximum of the extended chromosome length is 0.68mm on the previous chip, the chromosomes on the proposed large mesh array chip were extended longer than one on the previous chip.

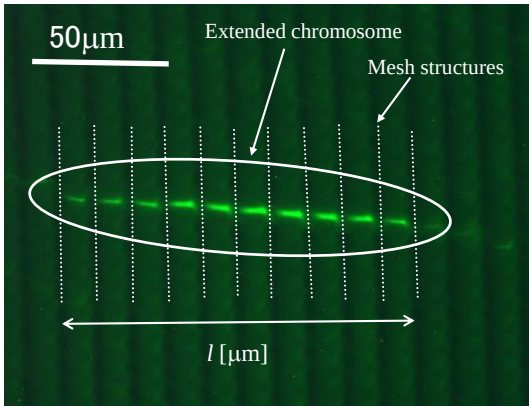


Figure 5. A fluorescence image of extended chromosomes

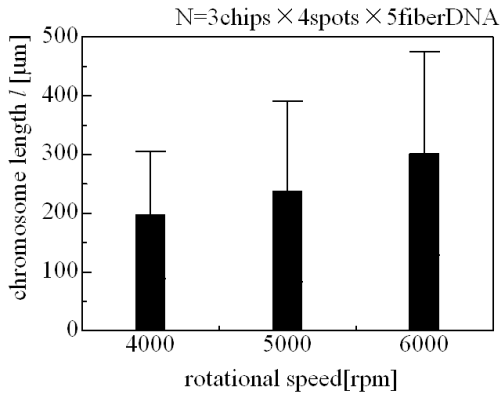


Figure 6. Relationship between rotational speed and extended chromosome length

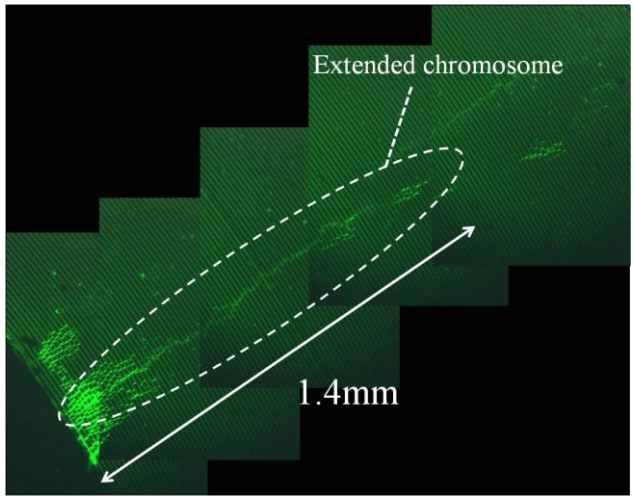


Figure 7. A fluorescence image of the extended chromosomes on the large mesh array chip

CONCLUSION

We evaluated a precision of dimensions of a mesh array chip fabricated by the multidirectional photolithography with the large work-area, and fabricated a large mesh array chip of 68 mm in diameter. The chromosome extended by the large mesh array chip was twice longer than one extended by the previous chip.

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