NON-INVASIVE HANDLING OF CHROMATIN FIBERS ISOLATED FROM INDIVIDUAL CELLS IN A MICROCHANNEL UTILIZING AN OPTICALLY DRIVEN MICROTOOL −TOWARD DIRECT EPIGENETIC ANALYSIS BY MICROSCOPY−

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ABSTRACT

We report a new method for investigating individual intact chromatin fibers isolated from individual cells with a custom-designed microfluidic device and a novel technique for non-invasive handling of individual chromatin fibers under a fluorescence microscope.

KEYWORDS: Chromatin fiber, Single cell, Optical tweezers, Microtool

INTRODUCTION

In eukaryotic cells, DNA molecules form chromatin fibers with histone proteins; gene expression is regulated by the degree of chromatin folding and histone tail modifications such as methylation, acetylation, and phosphorylation [1,2]. Epigenetic phenomena are determined by the partial unfolding/tight folding and histone modifications along the chromatin fibers, and by the timing of these events. There are few methods for investigation of individual intact chromatin fibers isolated from single cells, because intact chromatin fibers (typically several hundreds of microns long) break easily due to hydrodynamic shear during isolation and handling. Although a microfluidic device has been used to suppress hydrodynamic fragmentation of genomic DNA [3,4], few studies have applied it to intact chromatin fibers. We aimed to develop a method for non-invasive handling and isolation of chromatin fibers from individual cells with optically driven antibody-conjugated microspheres.

PRINCIPLE

To directly investigate intact chromatin fibers from individual cells, we developed a microfluidic device and microtool, depicted in Figure 1. The device consists of a single main channel bearing side micropockets and micropillar arrays. The micropockets can be used as reaction chambers for gentle isolation of native chromatin fibers and subsequent experiments. Solution conditions in the micropockets can be changed quickly by replacing the solution in the main channel and allowing exchange to occur via diffusion. At this scale, the relaxation time for the change in concentration of a small molecule is <10 s. The micropillars are used to tether the chromatin fibers with stretched form under flow in the main channel. Tethering enables direct and space-resolved observation of the response of individual chromatin fibers to changing conditions. To translocate isolated chromatin fibers from the micropockets to the micropillars for tethering, we developed antibody-conjugated microspheres that bind certain proteins in the chromatin fibers specifically and can be manipulated by optical tweezers. This microtool functions as an optically driven microgripper coated with certain protein-specific glue. Figure 1B shows how isolated chromatin fibers are tethered by one of two methods. The first is to place an antibody-conjugated microsphere bound to chromatin fibers in the gap (smaller than the diameter of the microsphere) between micropillars; the other is to wind chromatin fibers around a micropillar and pinning them at 2 positions along the fiber via microspheres. Thus, micropillars and microspheres are used together to tether and elongate chromatin fibers under flow. The microfluidic device and optically driven microtool

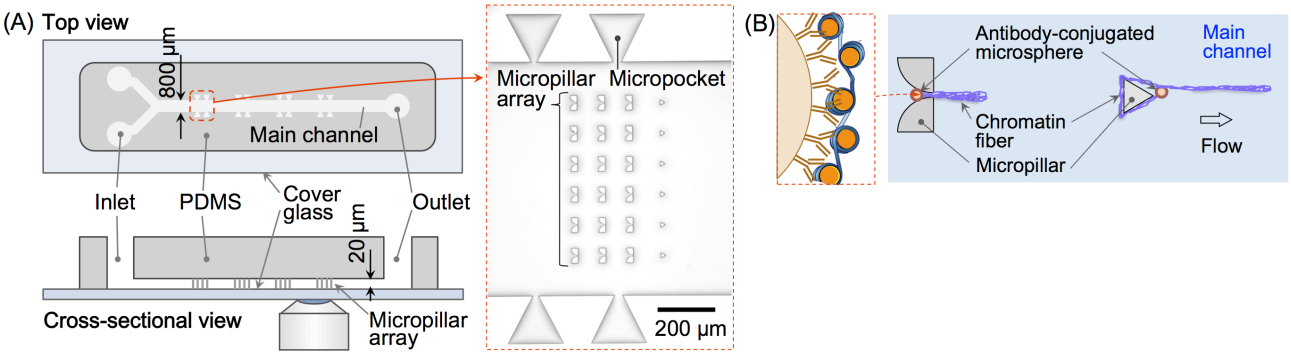

Figure 1: (A) Schematic illustration of the microfluidic device. Inset: Magnified image of the mold. (B) Tethering chromatin fibers. Inset: Magnified illustration of the surface of the antibody-conjugated microsphere, which captures chromatin fibers by specific binding to proteins in the chromatin fiber.

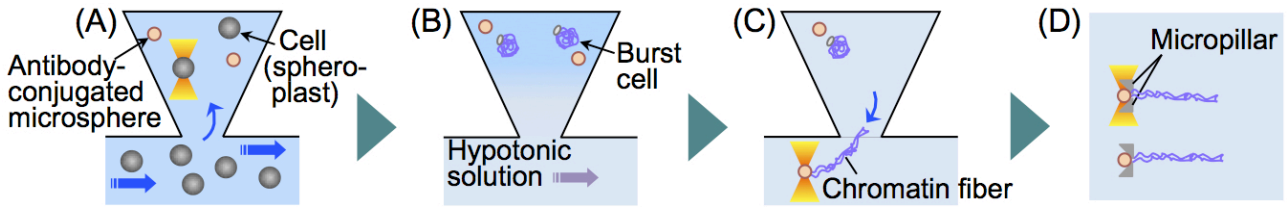

Figure 2: Experimental procedure of isolation and handling of individual chromatin fibers from single cells.

are used as follows: 1) Cells and antibody-conjugated microspheres are moved with optical tweezers into the micropockets from the main channel (Figure 2A). 2) Chromatin fibers are isolated from cells in micropockets by introducing a hypotonic solution containing detergent into the main channel (Figure 2B). 3) Isolated chromatin fibers are transported to the micropillar array region with optically driven antibody-conjugated microspheres (Figure 2C). 4) Chromatin fibers are immobilized on micropillars in the main channel; solution exchange and investigations can then be performed (Figure 2D).

EXPERIMENTAL

We used spheroplasts of fission yeast *Schizosaccharomyces pombe* (*S. pombe*), which has chromosomes of 3.5, 4.6, and 5.7 Mb (1.2, 1.6, 1.9 mm in the length of B-form DNA respectively). The *S. pombe* cells were engineered to express red fluorescent protein (RFP)-tagged histone H3 and green fluorescent protein (GFP)-tagged Cnp1 (a CENP-A homolog in fission yeast) [5]. Histone H3 is distributed uniformly throughout chromatin fibers, and Cnp1 is a histone H3 variant and exists in the centromere region. The optically driven microtool was comprised of anti-RFP antibody-conjugated microspheres prepared with biotinylated anti-RFP antibody (from Abcam) and streptavidin-coated microspheres (6-µm diameter; Polyscience, Inc.). Thus, isolated chromatin fibers were captured by the microspheres via RFP tagged to histone H3. To isolate chromatin fibers from the spheroplasts, we prepared a hypotonic solution containing Triton X-100 (Sigma-Aldrich) for lysis and dithiothreitol (DTT) as a reducing agent to prevent protein cross-linking, and a protease inhibitor (complete, Mini, EDTA-free; Roche Applied Science) to protect against GFP/RFP degradation. The minor-grove binding fluorescent dye 4',6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) was used to visualize DNA. The lysis and isolation buffer contained 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 10 mM DTT, 1 µM DAPI, 0.1% TritonX-100, and 1× protease inhibitor. For the demonstration experiment, immunostaining to enhance the fluorescence of GFP-tagged Cnp1 was conducted using anti-GFP, IgG, Alexa Fluor® 488 (here after, Alexa488) conjugate (Life Technologies). The microfluidic device was fabricated by standard soft-lithography with Polydimethylsiloxane (PDMS; Dow Corning) [6] and flow in the main channel was controlled manually by connecting a variable volume micropipette to the outlet [7].

RESULTS AND DISCUSSION

When the spheroplasts in the micropockets were exposed to the hypotonic solution, they burst and each spheroplast yielded a random coil of chromatin fibers. Maintenance of nucleosome structure in the isolated chromatin fibers was indicated by fluorescence of RFP tagged to histone H3, which showed random coils and no diffusion of fluorescence into the micropocket. Then, the microtool was touched to the random coil of chromatin fibers for 10–15 s using optical tweezers and stable binding was established; the fibers were then non-invasively translocated to the micropillar array (Figures 3A–C). After the microsphere immobilization, the chromatin fibers were elongated by flow and tether stability was confirmed (Figure 3D).

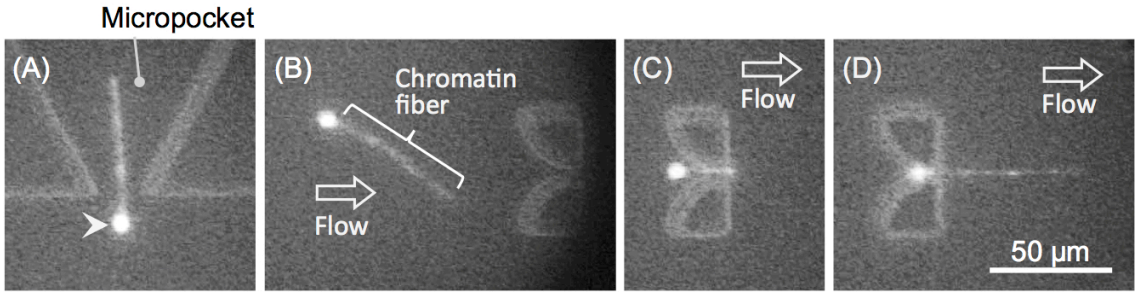

Figure 3. Immobilization of isolated chromatin fibers from a single cell. A: Translocation from a micropocket to the main channel. White arrowhead indicates the microsphere being manipulated by optical tweezers. B: Approach of the free end of the tethered chromatin fibers to a gap between micropillars. C: Release of the microsphere by stopping of the optical trapping. D: Immobilization of the microsphere between the gap by flow. Chromatin fibers were visualized by RFP-tagged histone H3 throughout the process.

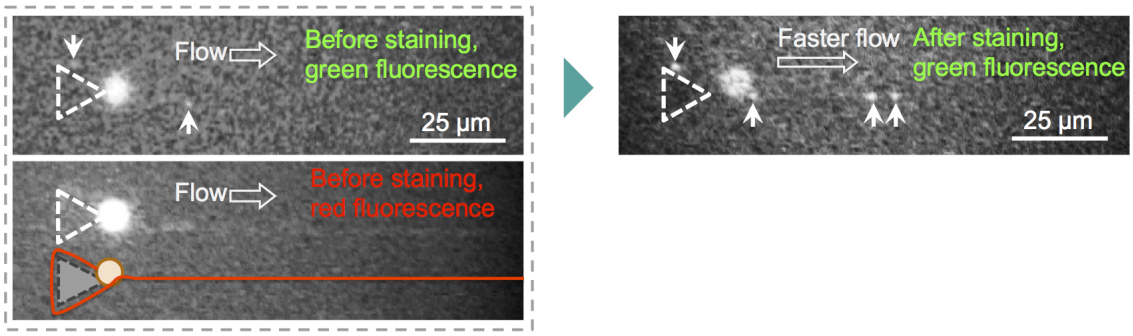

Figure 4: Immunostaining of the tethered chromatin fiber. Left top: Green fluorescence image before immunostaining. Left bottom: Red fluorescence image of the chromatin fiber before immunostaining. Right: Green fluorescence image after immunostaining. White arrows indicate 2 faint fluorescent spots of GFP-tagged Cnp1 (Left top) and 4 brighter spots of Alexa488-labeled anti-GFP antibody (Right).

Figure 4 shows a demonstration experiment in which tethered chromatin fibers in the microfluidic device were immunostained. Before immunostaining, the fluorescence of GFP-tagged Cnp1 on the tethered chromatin fibers was quite faint and difficult to observe (Figure 4, left). For immunostaining, Alexa488-labeled anti-GFP antibody was introduced into the main channel; 15 min later, the solution was replaced with wash buffer. After immunostaining, the fluorescence of GFP-tagged Cnp1 was clearly observed with the aide of Alexa488-labeled anti-GFP antibody (Figure 4, right). Typically, 3−6 green fluorescent spots were observed after immunostaining. In *S. pombe*, over 75% of the cells were in G2 phase, which is just prior to mitotic phase; at this point, there should be 6 chromosomes and, thus, 6 centromeres. The irregularity in the number of fluorescent spots may be due to overlapping centromeres caused by entanglement of the chromatin fibers. This is because the tethering point was non-specific and un-controllable when chromatin fibers were captured by antibody-conjugated microspheres.

CONCLUSION

We demonstrated a method for isolation of intact chromatin fibers from individual cells and non-invasive handling of the isolated chromatin fibers under the microscope utilizing optically driven antibody-conjugated microspheres in a microfluidic device. The method has potential utility for direct investigations of the epigenetic profile of intact chromatin fibers at the level of the individual cell.

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