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DNA analysis by single molecule stretching in nanofluidic biochips

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ABSTRACT

Stretching single DNA molecules by confinement in nanofluidic channels has attracted a great interest during the last few years as a DNA analysis tool. We have designed and fabricated a sealed micro/nanofluidic device for DNA stretching applications, based on the use of the high throughput NanoImprint Lithography (NIL) technology combined with a conventional anodic bonding of the silicon base and Pyrex cover. Using this chip, we have performed single molecule imaging on a bench-top fluorescent microscope system. Lambda phage DNA was used as a model sample to characterize the chip. Single molecules of λ-DNA stained with the fluorescent dye YOYO-1 were stretched in the nanochannel array and the experimental results were analysed to determine the extension factor of the DNA in the chip and the geometrical average of the nanochannel inner diameter. The determination of the extension ratio of the chip provides a method to determining DNA size. The results of this work prove that the developed fabrication process is a good alternative for the fabrication of single molecule DNA biochips and it allows developing a variety of innovative bio/chemical sensors based on single-molecule DNA sequencing devices.

1. Introduction

Current methods for DNA analysis require chopping each molecule in millions of fragments, replicating each segment, sorting them by size and reconstructing the original DNA strand in time consuming and costly processes. In contrast, DNA stretching methods, which consist in linearising single molecules by confinement in nanofluidic channels, open new possibilities for DNA analysis [1] and bio/chemical sensing, which speeds the process and lowers the cost [2].

Stretching single DNA molecules in nanofluidic channels can be used for the study of physical and biological properties of these molecules [3]. These nanofluidic devices have been used for real-time contour length measurements of the stretched DNA molecules [4–6], single molecule studies of protein–DNA interactions [7], on chip ordered restriction mapping using endonucleases [8], DNA single-molecule barcoding using nanoslits [9], biological studies of conformation, entropic motion and speed of stretched DNA in nanochannels [10,11], and confinement spectroscopy of single DNA molecules in tapered nanochannels [12].

Several innovative processes have been proposed for the fabrication of nanochannels by means of NanoImprint Lithography (NIL) technology and sealing techniques to enclose those channels [13]. NIL is a parallel technology to create nanostructures over a large substrate surface area with both high resolution and throughput. On the other hand, sealing of etched structures to create enclosed channels is a major challenge; previously accomplished by using non-conventional methods such as shadow sputtering deposition [14], sacrificial polymers [15], modified NIL processes [16] or direct bonding [17,18]. Thermal imprinting and polymer bonding technologies have also been recently used to fabricate polymeric nanochannels for DNA stretching in disposable biochips [19]. Furthermore, the fabrication of extremely narrow (sub-10 nm) and long enclosed channels [20,21], together with the implementation of nanogap electrical detectors inside these nanochannels [22,23] opens up new possibilities for the real-time label-free DNA analysis, including low or medium-quality sequencing that could be used as a screening tool for medical diagnosis and monitoring of therapies.

Therefore, the integration of these nanofluidic devices in lab on a chip systems offers the capability for sensitive and rapid analysis of the complex chemical contents of the body fluids, which could enable new medical diagnostic approaches, identifying the markers for disease at a stage at which treatment can be most effective.

In this work, we present the fabrication of a sealed micro/nanofluidic chip for DNA stretching applications, based on the use of the high throughput NIL technology combined with a conventional anodic bonding of the silicon base and Pyrex cover. Using this chip, we have performed single molecule imaging on a bench-top fluorescent microscope system.

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2. Theory and calculations

DNA is a long flexible self-avoiding polymer. In bulk solution, genomic DNA assumes a random coiled conformation with a characteristic radius of gyration $R_g = (PwL^3)^{1/5}$.

P is the persistence length, a basic mechanical property quantifying the stiffness of a macromolecule. It is about 50 nm for a double stranded DNA (dsDNA) molecule under physiological conditions but varies inversely with salt concentration. Staining with intercalation dye increases persistence length (P_{dye}).

w is the DNA width. The effective width (w_{eff}) varies with the ionic environment. It is approximately 2 nm for dsDNA under physiological conditions.

L is the contour length, which is the length of the totally stretched molecule. The value depends on the number of base pairs in a specific DNA strand, usually calculated assuming 0.34 nm per base pair. Staining with intercalating dye increases contour length (L_{dye}).

The R_g for λ-DNA, the model molecules selected for this study, is about 2 μm in the buffer conditions used for our experiments. When DNA molecules are forced to enter in a confining channel with inner diameter D much smaller the R_g it is more favorable energetically to stretch in a series of blobs along the channel. In the de Gennes regime, $R_g \gg D \gg P$, the extension length of the DNA in the nanochannel L_{ext} scales linearly with the contour length L_{dye} according to [24].

$$L_{ext} \cong L_{dye}\left(\frac{w_{eff}P_{dye}}{D^2}\right)^{1/3} \quad (1)$$

Thus, DNA extension ratio L_{ext}/L_{dye} depends on the inner diameter of the channels D that is characteristic of the chip. Therefore, the determination of the extension ratio of a chip provides a method to determine DNA size [8,19].

3. Materials and methods

3.1. Fabrication process

The conceptual design for this DNA stretching chip is illustrated in Fig. 1. The chip is composed by a silicon base, containing microchannels for fluid transport and nanochannels for DNA stretching, immobilization and detection, and a Pyrex cover that can integrate electrodes for sample movement by electrophoresis and four inlet holes. This design includes two V-type long microchannels, for pressure-driven fluid quick transport, connected by short nanochannels for direct visualization of single DNA molecules.

The nanofluidic network is composed by 100 nanochannels of 100 nm nominal width, 3 μm pitch to ensure optical resolution and 250 μm length. Auxiliary microstructures were also introduced to ensure a right pattern transfer, see Fig. 1.

The schematic cross section of the fabrication process is shown in Fig. 2A. Initially, wafer scale silicon nanochannels are fabricated using NIL technology, including the following steps: spin coating and baking the NIL thermoplastic polymer over the four inches silicon wafer, imprinting process, residual layer etching by an oxygen plasma, pattern transfer to the silicon by anisotropic silicon plasma etching and polymer removal. Then, the microchannels and reservoirs are defined over the silicon wafer using conventional photolithography (including an alignment step) and anisotropic silicon plasma etching. Next, a thin layer of thermal silicon oxide is grown on the silicon base. This oxide layer has a double functionality, on the one side to get electrical isolation and on the other side make the surface hydrophilic. In parallel, a thin layer of platinum is patterned by lift-off over the Pyrex cover in order to define the metallic contact and the inlet holes are created by femtosecond laser ablation. Finally, the silicon base and Pyrex cover are bonded using a conventional anodic bonding process. Details of the fabrication process are given elsewhere [25].

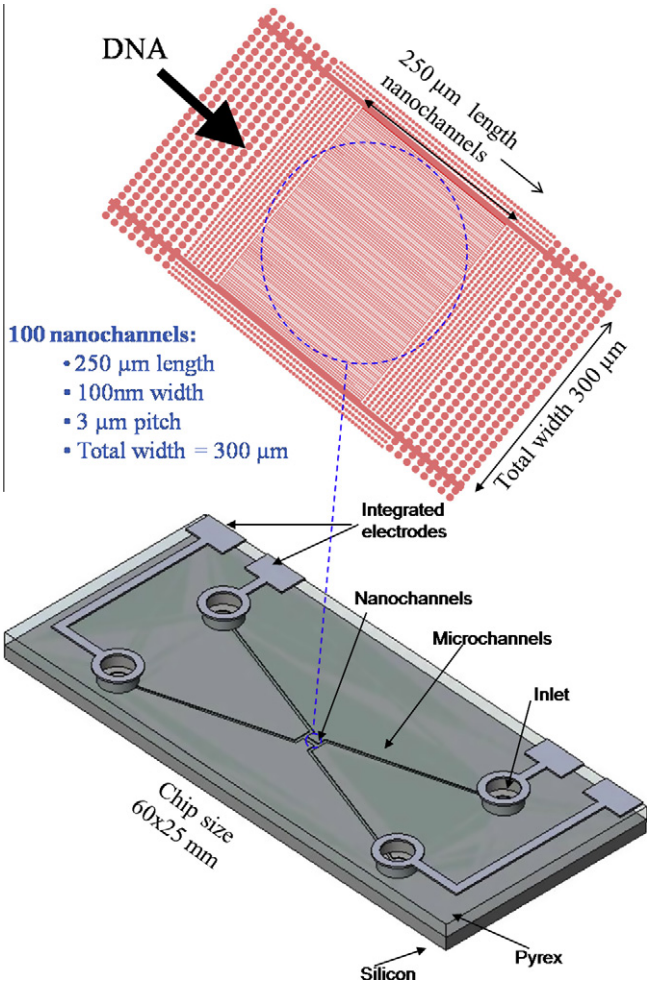

Fig. 1. The conceptual design of the DNA stretching chip showing two V-type microchannels connected through the nanochannel array. The design of the nanochannel array includes micropost arrays to pre-stretch the DNA molecules and facilitate the entry into the nanochannels as suggested by Cao et al. [27].

The fabricated chips were diced and packaged using commercial fluidic Nanoports (Upchurch Scientific, Oak Harbor, WA) to facilitate the contact with the macroscopic world. The Nanoports were sealed to the access holes of the chip to allow pressure driven movement of the sample.

3.2. DNA and buffer preparation

Lambda phage DNA molecules (New England Biolabs, Beverly MA) were stained with the bis-intercalating dimeric cyanide dye YOYO-1 (Molecular Probes) at a staining ratio of 1 dye molecule per 10 base pair and used at a concentration of 10 ng/μl. YOYO-1 dies are among the highest sensitivity fluorescent probes available for nucleic acid staining. The contour length of single λ-DNA individual (48,502 bp) can be calculated from the base pair spacing of 0.34 nm to L = 16.49 μm. The influence of YOYO-1 intercalation of the contour and persistence length has been experimentally investigated [26]. These studies show that L and P increase by 30% at a staining ratio of 1:4. Thus at our staining ratio of 1:10 we will assume a 12% increase in L and P, thus resulting in L_{dye} = 18.3 μm and P_{dye} = 56 nm.