J. Sci. Math. Lett. UPSI Volume 1 (2013) 28 - 36
ISSN 2462-2052

Resolvability Between Bare and DNA-Grafted Microsphere by Flow Resistance Measurement using Optical Tweezers

Kebolehbezaan antara Mikrosfera Tanpa Salut dan Mikrosfera Bersalut DNA dengan Kaedah Pengukuran Rintangan Alir Menggunakan Penyepit Optik

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Abstract

Optical tweezers have an ability to hold a single optically transparent particle in a suitable medium without mechanical contact. One can measure and exert photonics forces of focused laser power on a particle in confined test cell. They offer a possibility to hold a particle in a flow of surrounding medium. For a bare micro sphere, Stoke's law easily holds. But for 4000-bp DNA grafted micro sphere, the flow resistance is unknown and theoretically difficult to describe. Experimentally, the optical tweezers resolve between bare and the grafted microsphere in phosphate buffer solution (PBS) pH 7.4 by 2.00 ± 0.05 pN at the position of 35 μm from the test cell wall regardless on employed laser power.

Keywords optical tweezers, flow-resistance, DNA

Abstrak

Penyepit optik mempunyai kebolehan untuk memegang zarah tunggal lutsinar dalam medium bersesuaian tanpa sentuhan mekanikal. Daya fotonik oleh kuasa laser terfokus pada suatu zarah dalam sel uji boleh diukur dan dimanipulasikan. Penyepit optik juga berupaya memegang suatu zarah dalam aliran medium persekitaran. Untuk mikrosfera tanpa salut, hukum Stokes adalah terpakai. Tetapi untuk 4000-bp microsfera bersalut DNA, rintangan alirnya tidak diketahui dan amat sukar dijelaskan melalui teori. Melalui uji kaji, penyepit optik dapat membezakan antara mikrosfera tanpa salut dengan mikrosfera bersalut dalam larutan tampan fosfat (PBS) pH 7.4 sebanyak 2.00±0.05 pN pada kedudukan 35 μm daripada dinding sel uji tanpa bergantung kepada kuasa laser yang digunakan.

Kata kunci penyepit optik, rintangan alir, DNA

INTRODUCTION

Light interaction with matter can produce significant mechanical effect especially at micro- and nano-size scale (Kadri, Fujiwara, & Sasaki, 2011). One of such interaction is the use optical tweezers in non-contact micro- and nano-manipulation or rigid and soft matters. The invention of optical tweezers has sparked off a revolutionary in experimental methods

in areas of the physical as well as biological sciences (Ashkin, 1997). The amazing feature of the optical tweezers can be employed to manipulate a micro-sized object as a probe in studying microrheological properties of a material or a physical system of interest (Bishop, Nieminen, Heckenberg, & Rubinsztein-Dunlop, 2004; Valentine, Dewalt, Ou-Yang, 1996).

Meanwhile, the interaction of polymers and a flow field and its physical behavior in a varying solvent has been a research interest since a long time (Sun, 1884). In a flowing medium, polymers deform and perturb the flow pattern. The theoretical treatment of the dynamic behavior of a single polymer tethered on a surface under a flow has been well emerged (Rzehak, Kromen, Kawakatsu, & Zimmermann, 2000). Experimentally, the study becomes possible when the single polymer can be attached to a trapped micro sphere by optical tweezers and the interaction of the polymer with a flow field can be observed by video microscopy (Perkins, Smith, & Chu, 1994). Even though the study of single polymers by using optical tweezers has been extensively studied and kept going on, studies on grafted polymers are still rare. Among the technological implication of the research is the rapid development of microfluidic devices for example in controlling micro valve pump using laser spot.

METHODOLOGY

Optical Tweezers Set-up

The optical set-up is developed by modifying an inversed microscope (Axiovert S 100 TV, Carl Zeiss Jena, Germany) as shown in Figure 1. The solid Nd:Yg continuous infrared wave with wavelength 1064 nm, maximum power 1 W and linearly polarized (LCS-DTL-322, Laser 2000, Russia) passes through bottom TV-base port into microscope. After passing the isolator, a quarter wave plate is employed to produce circularly polarised light to prevent reflection differences between the p- and s- part of the laser beam. The simple beam expander is formed by a combination of a convex lens and microscope in-built tube lens. The expanded beam is fed into the back aperture of the microscope objective (Plan-Neofluor 100x 1.30 Oil immersion, Zeiss, Germany).

The beam focus is adjusted to be inside a test cell channel to establish the optical trap. After passing through the test cell, the beam is recollimated by a condenser. A beam splitting mirror and a convex lens images the forward scattered light of the micro sphere onto a quadrant photodiode (S5017; Hamamatsu, Japan) for electrical position detection. For video imaging and video position detection, a digital camera (KP-F120, Hitachi, Japan) is used. The set-up utilizes the Fiberoptic-Heim Model LQ1700 Fiber Light Source for illumination. The microscope stage was framed with a nano-positioner (P517.3CD, Physics Instrumente, Germany). A joystick can also manually control this microscope stage. The whole apparatus was mounted on anti-vibration table (Integrated Dynamics Engineering, USA).

Data acquisition and processing

There are two positional detection schemes employed in this project, direct imaging scheme and electrical signal based detection scheme. The direct imaging scheme is achieved

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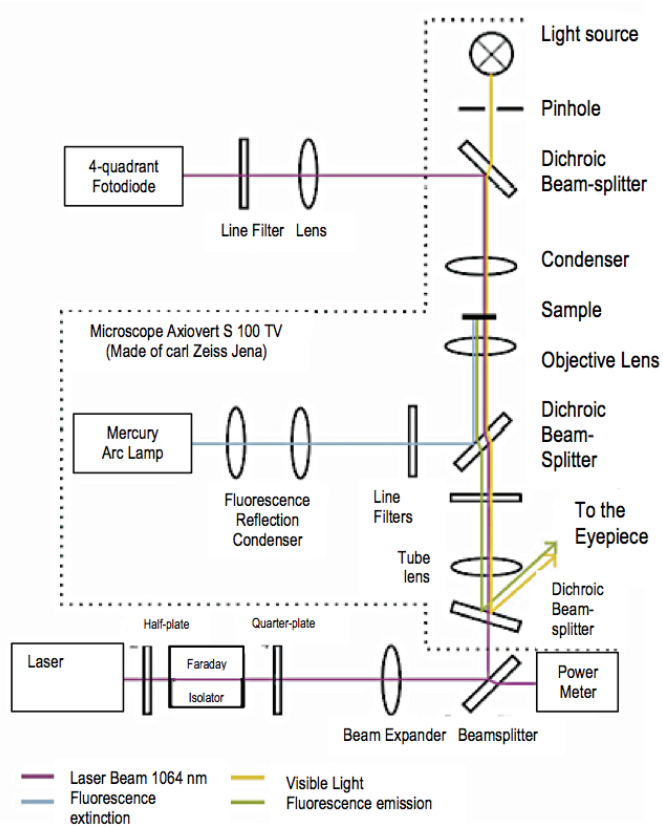

Figure 1 Optical tweezers setup

using video microscopy to record images of the trapped micro sphere. An interactive data language (IDL) environment program was written to calculate the micro sphere position in the recoded image with an accuracy of ±1 nm. Image sequences captured by the CCD camera were sampled and transferred to the computer storage by the controlling program written in LabView 6. Images sequences can be sampled by up to a 30 ms interval. Once the micro sphere displacements were determined, data from image processing were simply processed in OriginLab data processing software.

Preparation

Test cell construction

The test cell (Figure 2) was constructed by sandwiching 2 layers of Parafilms M® (American National Can, USA) with a cover slip No. 1 and a microscope slide. Two small holes were drilled apart on the microscope slide. To avoid dust and dirt that may interfere with later measurements, the sample chamber was prepared in a clean way. Two capillary tubes with 1.05 mm inner diameter were connected to the microscope slide and functioned as inlet and outlet for microsphere injection and solvent flow. Tubes were glued on the microscope slide by using acrylat (2-Hydroxyehtylmethacrylat) treated by ultraviolet ray. All layers

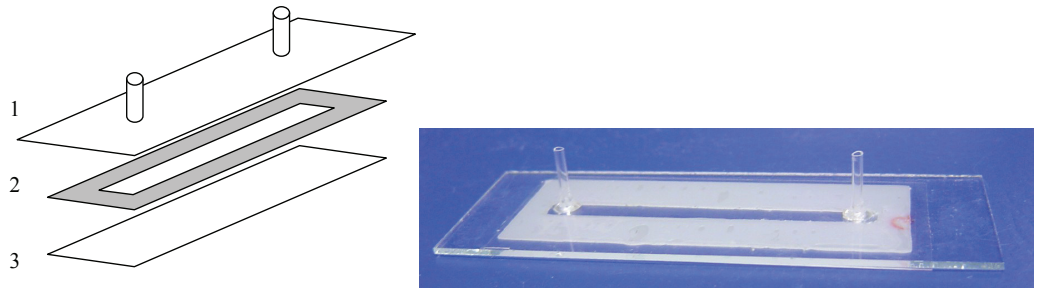

Figure 2 Left: Test cell construction layers; (1) microscope slide with two capillary tubes attached to it, (2) Parafilms M® layers and (3) cover slide. Right: Test cell real look.

were assembled and were thoroughly heated. The Parafilms melted and made those layers stick together. The flow channel formed had a typical thickness of 253.2±17.9 μm.

Sample for bare microspheres

Polystyrene latex standards with a diameter of 2.0 μm ±5% were supplied by G. Kisker GbR, Germany as a 2.5% (w/v) suspension. The suspension was diluted in phosphate buffer solution (PBS) pH 7.4 to decrease micro spheres concentration to about 1:10000 and mixed well before injected into the test cell.

Sample preparation for a grafted microspheres

DNA is used as it is semiflexible polymer which can help us to understand the microrheology of polymer at microscopic level (Rajkumar & Ali, 2008). 4.5 μl of solution containing 4000 base pairs of double stranded DNA end labeled with digoxygenin and biotin at their 5' end (solution of 1.1 μl DNA in 3.4 μl PBS prepared using Polymerase chain reaction (PCR)) and 5.5 μl streptavidin coated polystyrene micro spheres (1.27% w/v) of 2.1 μm diameter (Polysciences Europe, Eppelheim) resuspended in PBS were mixed together in 0.5 ml PCR tube. Then the sample was incubated for 15 minutes at room temperature for efficient grafting of the micro spheres. Approximately, each micro sphere was grafted with 1000 molecules of 4000 bp of double stranded DNA with biotin/digoxygenin handles. Later the mixture was resuspended again in PBS to reduce particle concentration.

RESULT AND DISCUSSION

The first step in measurements with optical tweezers is to calibrate the system. Several methods have been suggested to accomplish this task for example using Boltzmann statistics, corner frequency, mean square displacement and Stokes friction (Ashkin, 1997). In this project, Stokes friction method was employed.

A triangle modulation of varying velocity was applied to the microscope stage in x-direction (see Figure 3). The test cell fixed on the stage will also move together. While the optical trap stayed in the same position, the test cell was moved relative to the trap. We can infer that the solvent inside the test cell and the stage were moved together with

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the same velocity relative to the optical trap. This temporary fluid flow caused the trapped micro sphere to move due to Stokes friction.

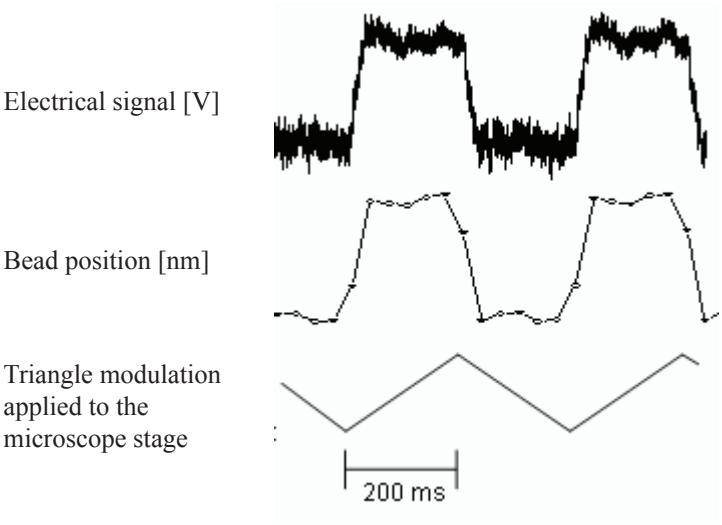

Figure 3 Responses of the trapped microsphere to a triangular modulation input at the microscope stage position in x-direction.

For a microsphere that is far away from influences of another microsphere and wall, the micro sphere velocity is equal to the solvent velocity.

$$v_{bead} = v_{solvent} = v_{stage} \tag{1}$$

Using Stoke's law of friction, force acting on a spherical micro sphere is given by a simple equation:

$$F_s = 6\pi\eta r v \tag{2}$$

where η is solvent viscosity (PBS at room temperature, $\eta = 1.005 \times 10^{-3}$ Pa s, r is micro sphere radius and v is micro sphere velocity relative to the surrounding.

In the same time, the optical trap tends to confine the micro sphere into its centre. While the flow brings the microsphere away from the optical trap centre, the optical potential opposes the movement. This results in equilibrium between Stokes frictional and optical forces. The net force from this balance condition locates the micro sphere somewhere out of the optical trap centre as illustrated in Figure 4.

$$F_t = F_s \tag{3}$$

$$\kappa \Delta x = 6\pi\eta r v \tag{4}$$

From above equations, the trap stiffness κ, in a particular direction can be determined.

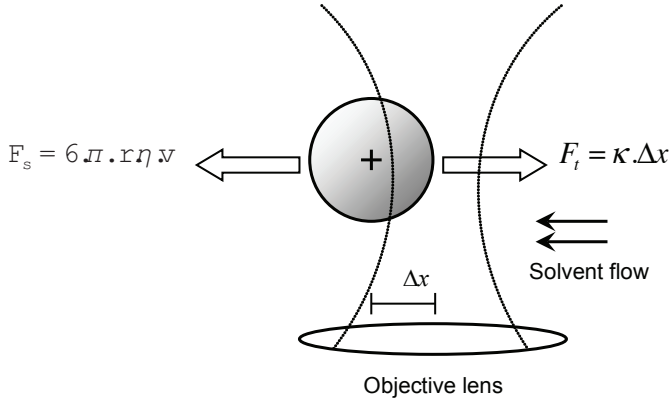

Figure 4 Illustration on how the trapped bead displaces out of the optical trap centre.

Figure 4 shows an apparent deviation between the displacement profile of the blank micro sphere and its grafted form with 4000 bp of DNAs. While Figure 5 shows graph of displacement of blank and DNA-grafted (4000 bp) polystyrene beads (2.1 µm) in PBS versus flow velocity and Figure 6 is representation of Figure 5 in term of the net force acting on the trapped bead. These deviations become more significant at higher flow velocity. The trap stiffness for the trap distance of 35 µm at laser powers of 200, 300 and 400 mW are 7.006×10^{-2}, 10.135×10^{-2} and 14.074×10^{-2} pN/nm respectively according to the

$$\frac{F_{grafted} / v}{F_{blank} / v} = \frac{6.\pi.r^{*}.\eta}{6.\pi.r.\eta} = \frac{r^{*}}{r} \tag{5}$$

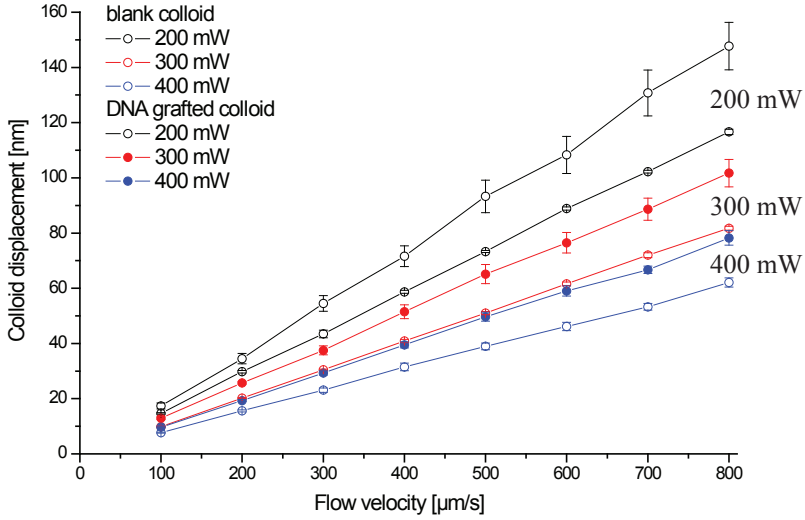

Figure 5 Displacement of blank and DNA-grafted (4000 bp) polystyrene beads (2.1 µm) in PBS versus flow velocity. The colloid is hold in a distance of 35 µm from the cell ground.

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calibration graph (Figure 7). Two distinct slopes for the DNA-grafted micro sphere and the blank micro sphere can be deduced. The net force acting on the trapped micro sphere is independent of laser power but linearly proportional to the flow velocity. The ratio between the two slopes in can be calculated to estimate an effective radius of the grafted micro sphere (Ven, 1989).

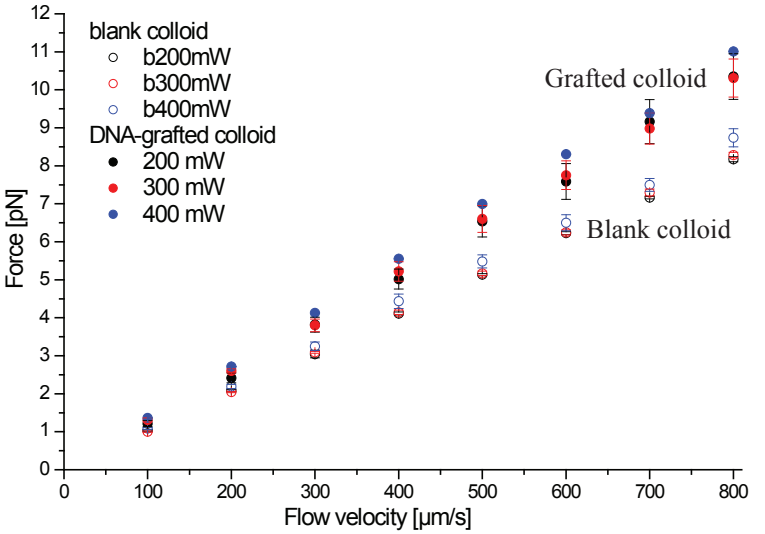

Figure 6 Representation of **Figure 5** in term of the net force acting on the trapped bead.

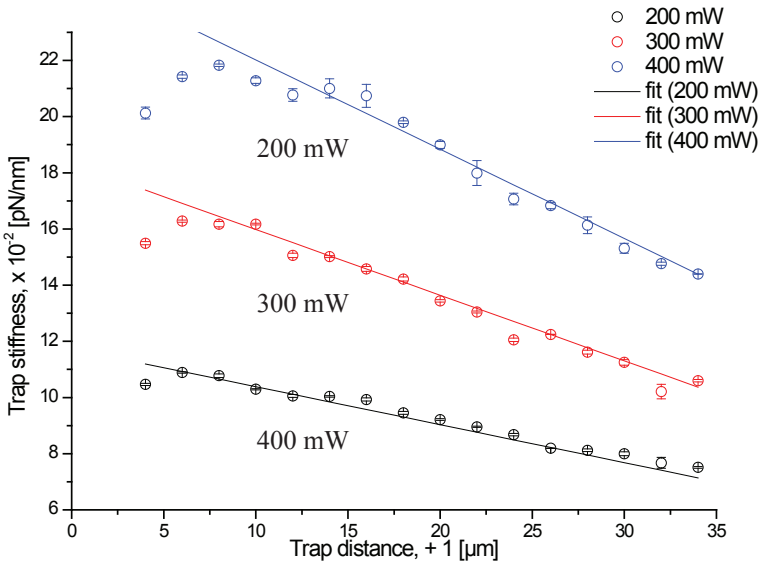

Figure 7 The trap stiffness independent of the trap distance from the glass surface of the test cell for employed laser power of 200, 300 and 400 mW in PBS at room temperature. The bead diameter is 2.0±5% µm.

The ratio of effective radius of grafted micro sphere to that of a blank micro sphere were found to be 1.228, 1.259 and 1.260 at laser powers of 200, 300 and 400 mW respectively according to equation (5). These values suggest an average ratio of 1.249±0.018 for all the three cases. The blank micro sphere has a diameter of 2.1±0.01 μm. If DNA molecules were assumed to form porous shell layer around the blank micro sphere, then the effective thickness of the layer is about 260±16 nm. Whereas the stretch length of the single double stranded 400 bp DNA chain is 1.36 μm. It is known that the DNA chains have a natural tendency to collapse into a condensed form (Perkins, Smith, & Chu, 1997). Therefore, instead of having multiple stretched DNA chains around the micro sphere, the experimental data showed the possibility of the DNA layer to condense up to 80% from its stretch length on the surface.

CONCLUSION

The flow-resistance of *single* DNA-grafted beads (grafting density of approximately 1000 DNA-chains per bead) is performed using the described experimental-setup. It turns out that in the broad flow velocity range between 100 μm/s to 800 μm/s, the grafted bead shows a flow resistance obeying Stokes law with an increased effective hydrodynamic radius. The increase in the flow resistance for tethered DNA-chains of 400 bp is in the factor of 1.26±0.6 with respect to blank beads which shows resolvability between bare and grafted-DNA microsphere. From the general knowledge of the flow resistance, one can have a better understanding of polymers deformation and interaction with the flow field.

ACKNOWLEDGEMENT

This work was partly done under the grant of RAGS UPSI code 2013-00-102-72 and Special Training Scheme for Academic Staff Administration (SLKKAP-UPSI).

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