The study of adhesive forces between the type 3 fimbriae of *Klebsiella pneumoniae* and collagen-coated surfaces by using laser tweezers

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ABSTRACT

Adherence to host cells by a bacterial pathogen is a critical step for establishment of infection. It will contribute greatly to the understanding of bacterial pathogenesis by studying the biological force between a single pair of pathogen and host cell. In our experiment, we use a calibrated optical tweezers system to detach a single *Klebsiella pneumoniae*, the pathogen, from collagen, the host. By gradually increasing the laser power of the optical tweezers until the *Klebsiella pneumoniae* is detached from the collagen, we obtain the magnitude of the adhesive force between them. This happens when the adhesive force is barely equal to the trapping force provided by the optical tweezers at that specific laser power.

This study is important because *Klebsiella pneumoniae* is an opportunistic pathogen which causes suppurative lesions, urinary and respiratory tract infections. It has been proved that type 3 fimbrial adhesin (*mrkD*) is strongly associated with the adherence of *Klebsiella pneumoniae*. Besides, four polymorphic *mrkD* alleles: namely, *mrkDv1*, *v2*, *v3*, and *v4*, are typed by using RFLP.

In order to investigate the relationship between the structure and the function for each of these variants, DNA fragments encoding the major fimbrial proteins *mrk*A, *mrk*B, *mrk*C are expressed together with any of the four *mrkD* adhesins in *E. coli* JM109. Our study shows that the *E. coli* strain carrying the *mrkDv3* fimbriae has the strongest binding activity. This suggests that *mrkDv3* is a key factor that enhances the adherence of *Klebsiella Pneumoniae* to human body.

Keywords: optical tweezers, adhesive forces, bacterial, trapping force

1. INTRODUCTION

Since the trapping of dielectric particles was demonstrated by Ashkin *et al.* in 1986 through the use of gradient dipole force, this optical-tweezers technique has been developed and is now widely applied in a variety of fields to manipulate micron-size objects. Optical tweezers use focused laser beams to trap and remotely manipulate dielectric particles, including cells and other biological objects.

Adherence to host cells by a bacterial pathogen is a critical step for establishment of infection. In order to study the mechanism of adherence, it is necessary to measure the biological force due to adherence. In the past decade, the gradient force of optical tweezers has been widely applied in the manipulation of a single small bio object. Consequently, optical tweezers become an effective tool for this purpose.

Klebsiella pneumoniae is an important cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics and commonly results in a high fatality rate if untreated. Nonetheless, the vast majority of *K. pneumoniae* infections are associated with hospitalization. It has been estimated that *K. pneumoniae* causes up to 8% of all nosocomial bacterial infections in developed countries, and its colonization in hospitalized patients appears to be associated with the use of antibiotics. Bacterial adherence to host tissues is considered a major factor in determining tissue tropism during infections. The process is generally mediated by a group of bacterial molecules named adhesin. Type 3 fimbriae are produced by uropathogenic or nosocomial isolates of *K. pnenumoniae* and mediate the agglutination of erythrocytes in a mannose-independent way, hence referred to as the Mannose-resistant, *Klebsiella*

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Hemagglutin (Mr/KH). The Mr/K hemagglutination exhibited by type 3 fimbriae is determined by the adhesin protein *mrkD*. It would not be surprising to find that *K. pneumoniae* develops many different forms of *mrkD* to adapt to the ever changing environment. We have identified four novel *mrkD* alleles from the 17 meningitis-associated *K. pneumoniae* isolates collected from the Veteran General Hospital in Taipei. In order to analyze the four *mrkD* variants, surface displaying of the type 3 fimbriae with either of the *mrkD* isoforms on *E. coli* JM109 were constructed. The sequence variation of *mrkD* appeared to affect their capability collagen binding activity.

1.1 Principles

Optical Spring

According to the RO model of Optical Tweezers, we stimulated the relationship between optical trap and micro-particle displacement. We can see from the figure 1 that it displays linear relationship in the red-line area. Simply it is similar the spring model. Therefore the strength of optical trap represents one trap elastic modulus. Mathematically the elastic modulus is the absolute value of trap curve gradient in the figure 1.

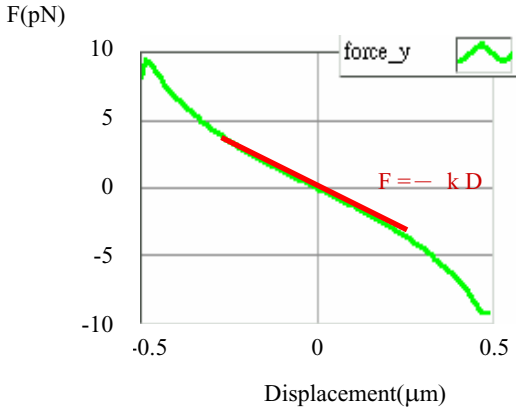


Fig. 1 The relationship between optical trap and micro-particle displacement. The radius of micro-particle is 6.8 m, laser power is 10mW, the wavelength is 514nm, the red line shows the scope of validity of optical trap spring model.

1.2 Drag Force

The trap force, F_{trap}, applied to a particle inside the trap has been shown to be proportional to the displacement, x, of the particle, as long as the particle stays within half it's radius from the focus of the laser:

$$F_{trap} = kx \tag{1}$$

In which, k is the elastic constant of optical tweezers; x is the displacement between the center of particle and laser focus. Our laboratory has successfully implemented the drag force for adjusting the optical trap. In pico-Newton quantity our experimental data of trap exactly like the date in theory. The principle of using drag force to adjust optical trap, however, is to utilize a known drag force for balancing and adjusting unknown the trap force of optical tweezers. We can take an example from one d movement of a micro-particle under the water. We suppose it is under force balance the micro-particle is forced by drag force and optical trap:

$$F_{trap} = F_{drag} = 6\pi r \eta v \tag{2}$$

While the maximum trapping force of the laser used in the project is in the order of hundreds pico-Newton, it is excellent to use this type of tweezers for force measurements in the lower pico-Newton range. To measure the maximum trapping force applied to a trapped particle you have to know the maximum dragging force. In this experiment, we made the current speed to control dragging force, until the laser tweezers cannot trap the micro-particle. From the equation(2), we can get the result of the maximum trapping force.

2. MATERIAL

2.1 Bio sample

Table 1. Bacteria used in this study

Bacteria strain	Relevant genotype
E. coli	
JM109	*RecA1 supE44 endA1 hsdR17 gyrA96 RelA1 thi* Δ (*Lac-proAB*)
JM109pGEM-T Easy	JM109 carrying TA cloning vector, pGEM-T Easy
JM109mrkABC	JM109 carrying plasmid with *mrkA*, *mrkB*, *mrkC*
JM109mrkABCD$_{V1}$	JM109 carrying plasmid with *mrkA*, *mrkB*, *mrkC*, *mrkD*$_{V1}$
JM109mrkABCD$_{V2}$	JM109 carrying plasmid with *mrkA*, *mrkB*, *mrkC*, *mrkD*$_{V2}$
JM109mrkABCD$_{V3}$	JM109 carrying plasmid with *mrkA*, *mrkB*, *mrkC*, *mrkD*$_{V3}$
JM109mrkABCD$_{V4}$	JM109 carrying plasmid with *mrkA*, *mrkB*, *mrkC*, *mrkD*$_{V4}$

2.2 Bead

The 6μm polystyrene bead was added into PBS (Phosphate buffer saline: 137 mM NaCl, 2.7 mM KCl, 10mM Na_2HPO_4, 2mM KH_2PO_4) containing 0.75 mg collagen and incubated at 4 ℃ for 16 h. The bead was then blocked with blocking reagent (2% BSA in PBS) at room temperature for 1 h. After blocking, the bead was washed and resuspended in 100 100 μl PBS. Bacteria were cultured at 37 ℃ in LB broth for 16 h. A dense bacterial suspension (10^6 to 10^7 per ml) in sterile PBS was prepared. 10μl collagen (15 mg/ml) was dropped on the slide. The slide were then stored in PBS until usage.

3. APPARATUS

3.1 Experiment setting

Our optical tweezers is consisted of laser light source, reflector and lens. The figure 2 displays our experimental setting. Firstly, a beam of laser passes through two 45 degree reflectors, then through beam expander and beam splitter. In the focus of the strong infrared laser small objects can be trapped. The trapping laser was an argon ion laser with a continuous wave. In this project a wavelength of 488nm - 514nm was used. Secondly, we add one slice of dichroic mirror between two 45 degree reflectors in order to separate a laser into blue light 418nm and green light 514 nm. By this way we can simply use a light power for monitoring the laser power. We use CCD camera for monitoring optical tweezers trapping, sending the receiving signal to the computer immediately, taking the pictures and analyzing the datum.

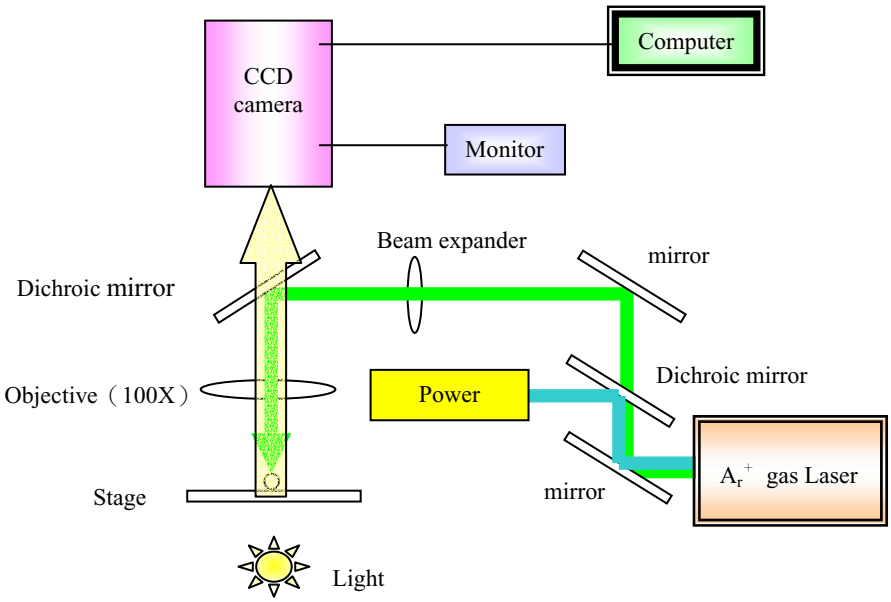


Fig. 2 The setup of our laser tweezers system.

3.2 Image analyzing system

We implemented the Imaq of National Instrument for images collecting and analyzing tool. By Imaq's image software, we can collect thirty pictures per second and calculate the information of the image, for example, coordinates of the micro-particle, moving speed etc.

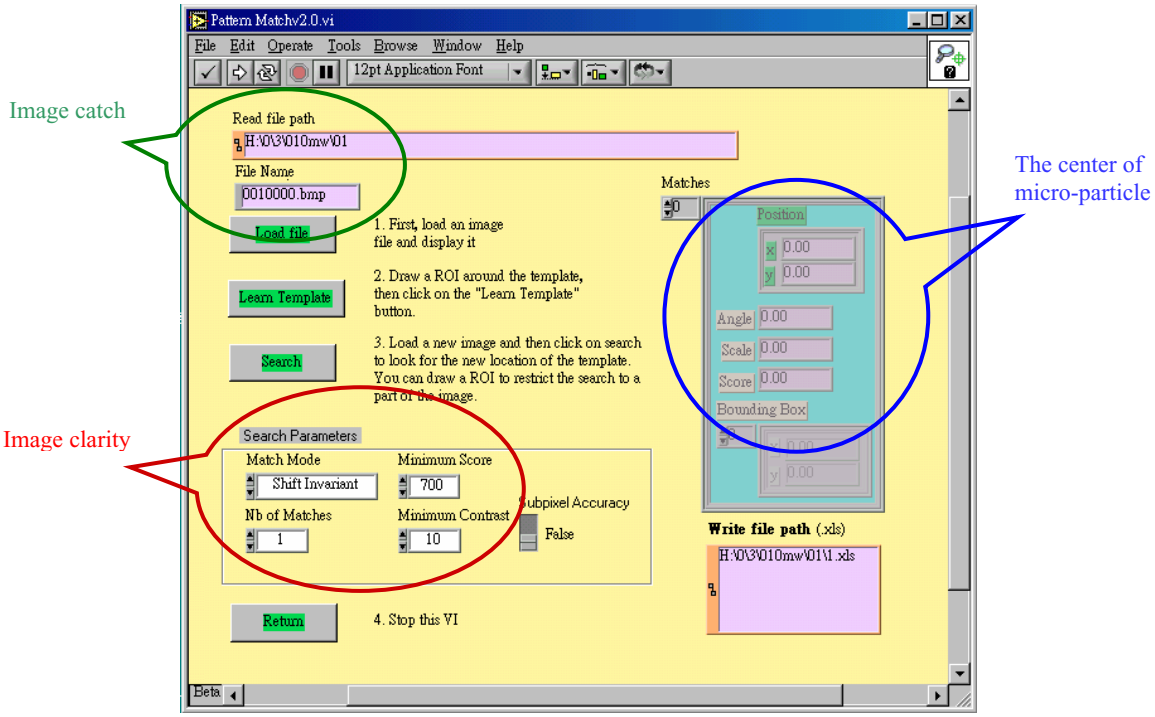
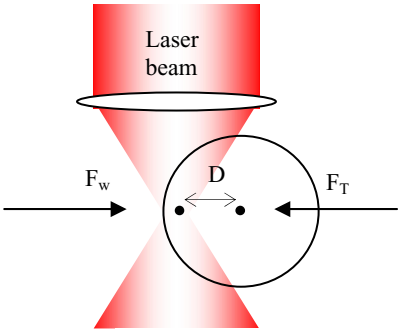


Fig. 3 The control panel for the position of micro-particle.

3.3 Calibration in the relationship between trap force of optical tweezers and micro-particle displacement

We put sample under a controllable stage in order to produce drag force for calibrating trap force, and then move the stage in the same velocity. When optical tweezers traps the micro-particle it is not able to move with the stage. Therefore the drag force which direction is the same as flow influences the micro-particle. The strength of drag force and the move velocity of the stage show direct radio and the direction is reverse. So in the velocity of the stage is 0 the micro-particle is not affected by the drag force, and the trap force is 0. Then we change the velocity of the stage movement; the micro-particle is affected by drag force and trap force of optical tweezers. The drag force is increased by velocity of the stage. The trap force of optical tweezers has the characteristic similar to the spring, therefore the micro-particle, which is trapped by optical tweezers, has different displacement as it is influenced by different drag force. We can see from the figure 4 and figure 5 that when we alter the drag force step by step, we are able to calibrate the relationship between the displacement of micro-particle and trap force.



v : Velocity of the micro-particle

F_w : Drag force of water current

F_T : Trap force of optical tweezers

D : Displacement of the micro-particle

Fig. 4 Calibration in the relationship between trap force of optical tweezers and micro-particle displacement

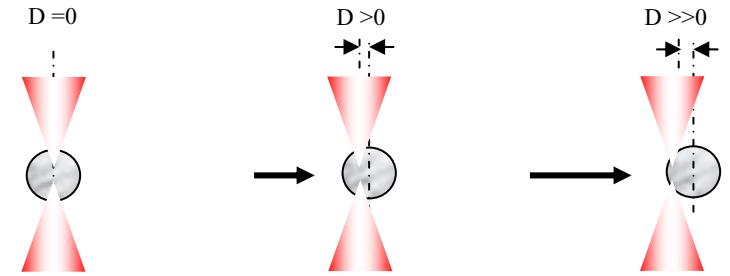


Fig.5 The relationship between trap force of optical tweezers

3.4 Calibration in the maximum trap force of optical tweezers and laser power

Theoretically the relationship between trap force of optical tweezers and laser power shows direct ratio. The stronger laser power is, the bigger capability the micro-particle is. It means that the micro-particle is able to take more flow speed and drag force. In this experiment we implement the optical tweezers to trap the micro-particle in the water, then by different flow speed to wash away the micro-particle which we have trapped. We calculate the biggest flow force is the maximum trap force of optical tweezers. On the other hand, we can get the data of the relationship between the maximum trap force of optical tweezers and laser power by altering the strength of laser power.

4. EXPERIMENTAL METHODS

4.1 Bio method

Collagen-binding assay. This assay was detected as previous study. The wells of flatbottom microtiter plate were coated following incubation overnight at 4°C with collagen (5 μg/ml). Prior to incubation with bacteria, non-specific binding sites were blocked by incubation for 2 h at 22°C with a 1% (wt/vol) solution of bovine serum albumin. Subsequently, 100 μl bacterial suspension (10^{10} cfu/ml) was added to each of the wells. Following incubation for 2 hr at 22°C with gentle shaking, unattached bacteria were removed by washing three times in 0.05% Tween-20 in of PBSs. The attached bacteria were washed off by 0.1% Triton-100 X and adhesion of the bacteria to collagen was measured by recovery of colony-forming units.

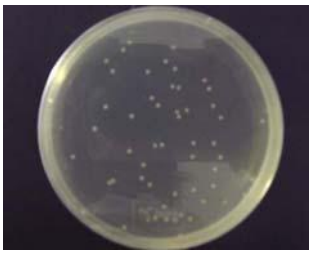


Fig. 6 The planting of *Klebsiella pneumoniae* adhesive to Collagen V

4.2 Optical tweezers trapping

Producing sample: following these steps we can calibrate optical tweezers trapping. We choose any micro-particle diluting 1000 times and drop it between the cover glass and carry glass. The size of the micro-particle and bacteria is near to submicro, so we have to use 100X objective. Turn on the laser and adjust stage to focus one single micro-particle. Then we use computer to catch continuous images and use image analyzing system for analyzing the displacement of the micro-particle. Increase the speed of sample stage stably until the optical tweezers is unable to trap the micro-particle. The finally, we alter different laser power and measure the relationship between optical trap and laser power.

We put the bacterial and micro-particle applying college into the sample, and trap the bacterial by different laser power in order to adhesive the micro-particle in five minutes and then we separate it. The last, we decrease the laser power until we cannot separate it, then recording the data of laser power. According to the data, we acquire the adhesive force between the bacterial and college.

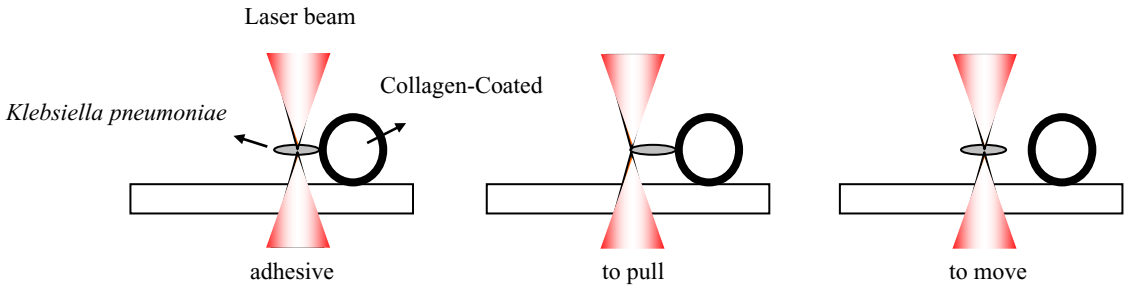


Fig. 7 Move *Klebsiella pneumoniae* by Use of our laser tweezers system.

5. RESULTS AND DISCUSSIONS

The main purpose of this paper is trying to find more efficient ways of bio force measurement. We compared the method of optical tweezers with traditional method. Figure 8 displays traditional bio method. The transverse presents different category bacteria. The vertical coordinates presents collagen binding activity. Figure 9 illustrates optical tweezers method. The transverse presents different category bacteria. The vertical coordinates presents the binding force between the bacteria and collagen, each point is one bacterium. Comparing to those two methods, we can find the type 3 pili which tends to be the maximum adhesive force. Our experiment proved that the type 3 pili is the main factor of bacteria adhesive to collagen.

This optical tweezers measurement is a way to observe the adhesion of single bacillus. After confirming adhesion, we measured the force. From here we can see it is an objective measurement. According to observation, we found that *Klebsiella pneumoniae* can float on the water. The plating method, however, is to daub collagen on the bottom of the culture. The probability of contact of bacteria and collagen and the force of adhesion, therefore, could affect the result.

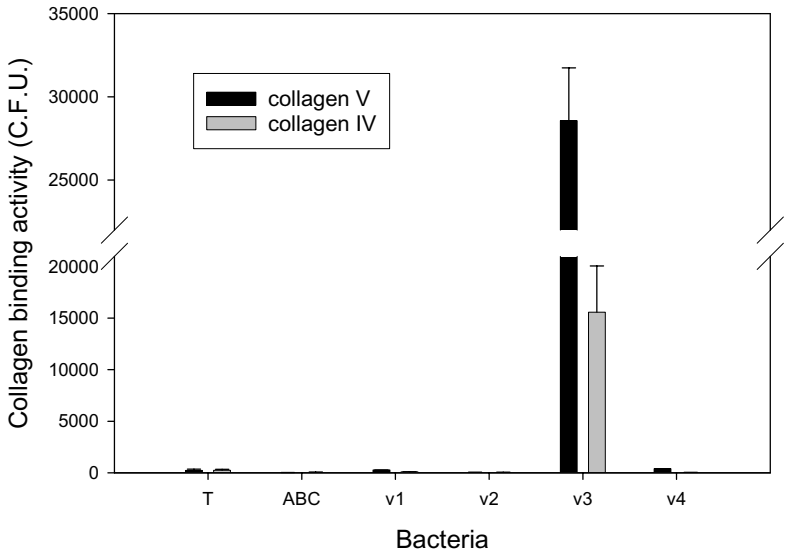


Fig. 8 Bio method：The planting of *Klebsiella pneumoniae* adhesive to Collagen

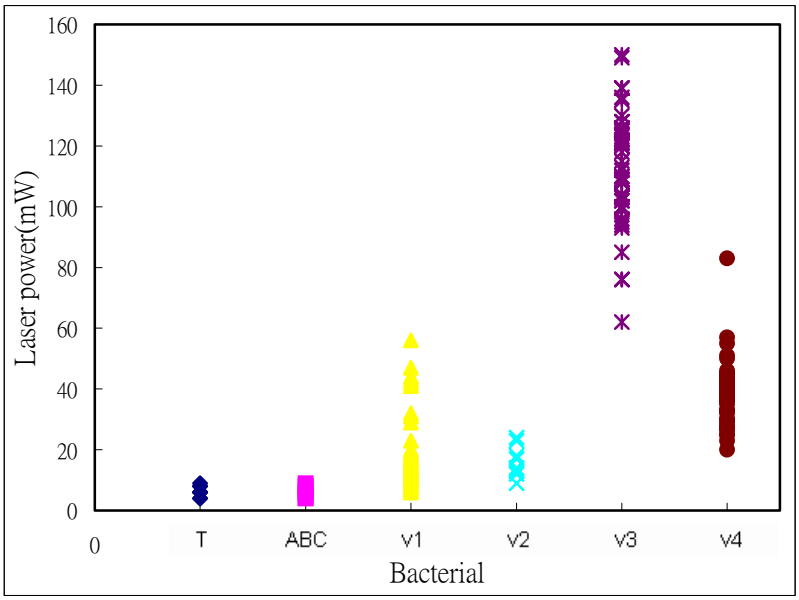


Fig. 9 Laser tweezers method：adhesive forces
Between *Klebsiella pneumoniae* and collagen-coated surfaces.

By the experiment we found that we cannot adhesive it too long, because over two minutes we cannot separate it anymore.

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