Effect of Medium on Interaction Forces between Atomic Force Microscopy (AFM) Tip and Gold Nanoparticle

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**Abstract.** Atomic Force Microscopy (AFM) has become powerful tool not only to study the surface topography but also the interaction forces between the tip and the sample. In this work, the measurement of the forces was determined by using AFM imaging in air and in liquid environment. Then, the force-distance (FD) curve was analysed to determine the interaction forces between the tip and the samples. The forces was computed from the pull-off forces of FD curve. When the FD curve are required in air, a thin layer of water (liquid contaminant layer) adsorbed on the sample surface exerts a meniscus or capillary force. Instead, when working in a liquid, the capillary force is eliminated and other forces becomes relevant. The force present is the van der Waals force and electric double layer force. The net force between the tip and the samples is the vector sum of several forces. In air, the dominant component of the adhesion force between the tip and the sample is believed to be the capillary force while in liquid, the dominant components present is the van der Waals force. The adhesion force ranging from 10 to 100 nN have been reported for imaging in air and the adhesion force of 5 to 104 nN observed in our studies lies well within this range. Meanwhile, in liquid, the adhesion force obtained from this work is in the range of 0.1- 40 nN and it is lies within the range reported previously which is as low as 100 pN. Detecting forces is an important step toward developing new analytical and biomedical devices such as biosensors.

1. **Introduction**

Atomic force microscopy (AFM) method is part of a scanning probe microscopy (SPM) technique used for surface characterization. It uses a sharp tip for scanning over a sample surface with the motion controlled precisely by a piezoelectric actuator to obtain information about the properties of the sample, including its topography image and surface interaction. AFM has been widely used in studying biomaterial characterization since it can be operated in air, vacuum, various gases and liquid environment. AFM visualises images by “feeling” the structure of a sample surface with a sharp probe rather than “looking” into the surface (Morris \textit{et al.}, 2010).

In recent years, AFM has increasingly been used in nanotechnology especially for the visualization of biomolecules and force measurements. Originally AFM was used to image the topography of surfaces, but by modifying the tip it is possible to measure other quantities and also to perform various types of spectroscopy and analysis. AFM is more than a surface-imaging tool in that force measurements can be used to probe physical properties of molecules, such as molecular interaction. The main advantage of AFM in biology as compared to other methods is that it usually does not require specific
sample preparation and allows measuring in liquid environment. The reason for choosing liquid media instead of air is not only because it is the natural physiological media for biological objects, but also due to the fact that all the interaction forces including unwanted ones are an order of magnitude smaller than in air allowing, for instance, to raise the resolution and to diminish image distortion. Although, it should be noted that measuring in liquids is much more complicated than imaging in air.

In nanobiotechnology, AFM can indicate the interaction of nanoparticles with biomolecules such as protein, DNA and others by measuring the force between the AFM tip and the sample surface via force spectroscopy (FS) measurement. Force spectroscopy or also known as force distance measurement is a method to measure interaction force occurring from the AFM tip to the surface of the samples (Neuman & Nagy, 2008). Force response is detected through beam deflection by a laser beam when a molecule of interest which is anchored at the end of the AFM tip to the surface of the sample is stretched (Ros et al., 2004). Force spectroscopy can be analogized as a fishing rod, to pick up molecules at a sample surface and then to stretch them. It sounds easy but it is not certain whether it is a single molecule or a mass of molecules attached to the tip. Hence, to overcome this limitation, functionalization of the AFM tip to specifically attach to a point of interest in a sample surface must be introduced. The choice of functionalization approach of nanoparticles-biomolecules to the cantilever tip is of importance in order to obtain information on the interaction of nanoparticles with biomolecules.

Gold nanoparticles (AuNP) have been found to be one of the useful tools in biomedical applications because of its unique properties of being inert and less toxic, small size and large surface area to volume ratio, high reactivity to living cells, stability in high temperatures, ease of detection and others (Tiwari et al., 2011). It can be synthesized into different shapes and sizes due to its ability to react and agglomerate with other nanoparticles in the surroundings. Due to its nano-size, it flows easily into various cells in the human body. Conjugation of nanoparticles to biomolecules generates hybrid materials that can be used to let the nanoparticles interact specifically with biological systems. Nanoparticle-biomolecule conjugates bring together the unique properties and functionality of both materials. If the aim is to use AuNP in biomedicine as diagnostic and therapeutic agents in cells or tissues, then, it is necessary to rightly choose the targeting component such as a monoclonal antibody (mAb), and the strategy to attach it on the surface of the particle (Cao-Milán & Liz-Marzán, 2014). Antibody conjugated gold nanoparticle probes allow for the study and visualization of cellular and molecular processes.

Naturally, AFM can perform imaging in all environment including in air, liquid and gases. For bioconjugated samples, AFM imaging in air is able to clearly track and illustrate the performance of attaching the biomolecules to the nanoparticle surface for the ratification of the conjugation while in liquid, the AFM provides information with high spatial and temporal resolution related to the real-time changes of shape or structure of bioconjugates (Tessmer et al., 2013). Hence, AFM helps to understand the structural and mechanical properties of biomolecules. In summary, AFM known as a powerful tool for the structural investigation of biomolecular conjugation to AuNP surface and analysis of bio conjugation-based nanostructures. Understanding the bioconjugation and the physical interactions of bioconjugation on the surface of gold nanoparticles opens novel opportunities for their use in biomedicine.

2. Methodology
2.1. Mica surface functionalization
Mica surfaces were freshly cleaved just prior to experiment. The mica substrate should be changed from negative charge to positive charge in order to allow the negatively charged gold nanoparticle particles bound tightly to the surface of the mica. The mica surface was promoted by absorption method using 150-300 kDa of 0.01% Poly-L-Lysine (PLL). All the chemicals were obtained from Sigma Aldrich. The solutions were all prepared shortly before experiments. The PLL solution is prepared by ratio dilution of 1:10 with deionized water (Grobelny et al., 2009). 50µl of dilute PLL solution was dropped on freshly cleaved mica using a micropipette. The solution was allowed to air dry completely under room
temperature. Then, functionalized mica was rinsed with deionized water and dried under room temperature.

2.2. Deposition of AuNP conjugated goat anti-human (AuNP_IgA)
The 40 nm AuNP conjugated goat anti-human IgA of 0.02% sodium azide and 1% BSA was received from NANOBI INFORMM, USM. 50 µl of AuNP_IgA was applied on functionalized mica using micropipette. Then, the substrate was air dried at room temperature. The mica was then rinsed gently with deionized water to remove loosely attached particles. Finally, the mica was air dried under room temperature.

2.3. AFM tip functionalization
The AFM tip was silanized in a 0.1% (v/v) toluene solution of 3-aminopropyl-triethoxysilane (APTES) for 15 minutes to coat it with a self-assembled monolayer of amines. Following the treatment with the amino silane, the tips were directly incubated for half an hour in a solution of 10 mg/ml O,O’-bis[2-(N-succinimidyl-succinylamino)ethyl] polyethylene glycol (bis(NHS)-PEG) in anhydrous chloroform containing 5µl/ml triethylamine as catalyst, and then rinsed using deionized water. Frequent washing step will remove the molecules of PEG that are not tightly bound to the AFM tip. The AFM tip is functionalized to control the place of attachment to the molecule of interest that lie between the tip and the substrate for further compressing and stretching by force spectroscopy measurements. Occasionally, a molecule will be picked up by the tip and stretched, as the sample and the tip are pulled apart. The PEG linker offers better control for the AFM tip to ‘fish’ the complementary molecules on the sample surface and to avoid more than one molecule binding to the tip at the same time, onwards, measuring the adhesion force upon the retraction of the tip.

2.4. AFM imaging
The FD measurement was carried out using triangular shaped OMCL-TR800PSA tip made of silicon nitride. The cantilever was 100 µm long, 13.4 µm wide and 0.8 µm thick with a spring constant of 0.57 N/m. The tip was 15 nm radius with a resonant frequency of 73 kHz. The AFM imaging was carried out in non-contact mode of operation in both ambient air and liquid environment. AFM imaging in liquid environment is the same manner as the AFM imaging in air. Phosphate Buffer Saline (PBS) with pH 7.4 was used in liquid environment. Only the cantilever’s deflection against the Z scanner’s position was measured. Acquisition of the FD curves in some places has been carried out to better repeatability and uniformity in all cases and at all time. In this study, the FD curve was obtained in a series of measurements taken at the same point, as well as at different points on the sample surface. Each time the tip interacts with the sample, a FD curve is captured. After AFM force measurement (obtaining force-vs-distance curves), the pull-off force data were directly extracted by the instrument-equipped software (XEI software).

3. Results and Discussion
Table 1 shows the pull-off force value for FD curve measurement for conjugation of 40 nm AuNP with goat anti-human (AuNP_IgA) on 150-300 molecular weight of 0.01% Poly-L-Lysine (PLL) functionalized mica using nonfunctionalized and functionalized OMCL tip imaging in air and liquid environment. The pull-off force represents the value of retrace data (when the Z scanner retracts) which means the tip is moved away from the sample. The magnitude of the pull-off force was higher when imaging in air environment. This is due to the meniscus force of liquid contaminant layer (water) that formed from the humidity condensation between the tip and the sample surface which acted against the pull-off during imaging in air environment. Basically, at low humidity condition, water on the tip as well as on the surface of the sample will accumulate at the point of contact to generate a meniscus.
These meniscus force is often referring as the capillary force between the tip and the sample surface. By placing the tip into contact with the substrate, capillary forces move the water on the tip and sample surface to the point of contact, and a meniscus forms at the periphery of the tip. In here, the magnitude of the pull-off force is a measure of the attractive attraction between the tip and the sample surface combined with the capillary force. The pull-off force is significantly influenced by the water in order to obtain the accurate values of the tip-sample interaction (Rozhok et al., 2004).

The pull-off force for Table 1 is significantly higher about 3 folds after functionalized the tip. The meniscus force still occurs even though the tip coated with amine monolayer is used during imaging. Regardless, even with a functionalized tip, liquid contaminant layer (water) will move near the point of contact between the tip and sample surface and form a meniscus. During contact, some chemical bonds from the functionalization of the tip or adhesive bonds may result in non-conservative force.

Apparentely, the attractive forces between the functionalized tip and water in the meniscus are weak enough to contribute to the value of the pull-off force (Rozhok et al., 2004). Providing a stable hydrophobic surface via tip functionalization is one example to decrease the formation of liquid contaminant layer (water) on the surface, thereby decreasing the capillary forces (Chen & Soh, 2008). Besides, when obtaining the pull-off force in water, due to the coupling of the adhesion force and the double layer force, the magnitude of pull-off force becomes lower compared in air environment. Hence, the electric double layer on the surface of a samples in water can be influenced by the ionic strength of the liquid.

| AFM tip              | Pull-Off (nN) |
|----------------------|--------------|
|                      | Air  | Liquid |
| Nonfunctionalized tip| 30.47| 0.904  |
| Functionalized tip   | 103.7| 37.10  |

Figure 1 shows force distance (FD) curve for non-functionalized OMCL tip imaging in (A) air and (B) liquid for conjugation of 40nm AuNP with goat anti-human (AuNP-IgA) deposited on glass slide functionalized with 150-300 molecular weight of 0.01% Poly-L-Lysine (PLL) functionalized mica using nonfunctionalized and functionalized OMCL tip imaging in air and liquid environment.

Figure 1 and Fig. 2 (A) explain that the detachment process is composed of single unbinding event between the tip and sample surface. It indicates that only one interaction between AFM tip and the sample is pulled apart as the cantilever retract from the sample surface. Meanwhile, Fig 1. and Fig. 2 (B) explain that the detachment process is composed of several pull-offs. It indicates that more than one or several interactions between AFM tip and the sample is pulled apart as the cantilever retract from the sample surface. The tip and sample seem to have been separated from each other. However, there are a small number of AuNP and antibody molecules remains bound to the bonds that has been pulled tightly until they broke at a certain value. In the consideration that the molecules possibly situated at particular position on the tip as well as the sample, they probably have been separated at distinct distances. Therefore, the FD curve may have presented multiple detachments. Multiple interaction needs
to be minimized when the purpose of the work is to study the individual interaction between the tip and the sample.

Figure 1. Force Distance (FD) curve for non-functionalized OMCL tip imaging in A) air and B) liquid for conjugation of 40nm AuNP with goat anti-human (AuNP_IgA) deposited on glass slide functionalized with 150-300 molecular weight of 0.01 % Poly-L-Lysine (PLL)

Figure 2. Force Distance (FD) curve for functionalized OMCL tip imaging in A) air and B) liquid for conjugation of 40nm AuNP with goat anti-human (AuNP_IgA) deposited on mica functionalized with 150-300 molecular weight of 0.01 % Poly-L-Lysine (PLL)

In this study, the 40 nm spherical shape of AuNP was used since it is a noble metal (exhibits a high chemical stability), easily to functionalize and has extraordinary capability to conjugate with various biomolecules (agglomeration induces a shift of the maximum absorption towards higher wavelength that reflects a color changes when the biomolecules are present in the sample) as well as the spherical AuNP was less toxicity (depends on the type of cells used) compared to the nanorod AuNP (Murphy et al., 2008). Phosphate buffer saline (PBS) is used as a medium in liquid imaging because the pH value of PBS is set to be within the range of 7 to 7.6 as pH of most of biological materials fall between pH 7 to 7.6, but often set to be 7.4 as the pH of the blood nears 7.4. Furthermore, PBS has the osmolarity and ion concentration that matches those of the fluid inside the cells and cause isotonic and non-toxic to most cells.
4. Conclusion
The net force between the tip and the samples is the vector sum of several forces. The pull-off force in air was correlated to both capillary and electrostatic forces. In air, the dominant component of the adhesion force between the tip and the sample is believed to be the capillary force. Meanwhile, the pull-off force in liquid correlated to the Van der Waals force and double layer force. However, in liquid, the dominant component present between the tip and sample is believed to be the van der Waals force. When imaging is operated in ambient air environment, van der Waals forces which consistently appear at interfaces, are usually overshadowed by the emergence of stronger capillary forces resulting from the condensation of water vapour at the point of contact that occurs between the tip and the surface of the sample. Hence, the influence regarding capillary forces can be averted by conducting imaging in liquid environment which removes the capillary force which holds the tip to the sample surface that produces in ambient air environment from contaminant layer on a sample surface, mostly absorbed water, which determines the magnitude of adhesive forces.

Acknowledgements
This study was supported by the Universiti Sains Malaysia through Research University Grant (RU-PRGS) (1001/PFIZIK/845010).

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