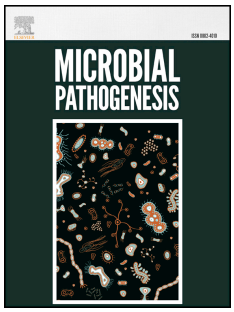
Accepted Manuscript



A nanobiosening method based on force measurement of antibody-antigen interaction for direct detection of enterovirus 71 by the chemically modified atomic force microscopic probe

Chung-Hung Hong, Chung-Fan Hsieh, Chi-Shin Tseng, Wei-Chih Huang, Cheng-Yan Guo, Shiming Lin, Si-Chen Lee

PII: S0882-4010(17)30336-4

DOI: 10.1016/j.micpath.2017.08.051

Reference: YMPAT 2441

To appear in: *Microbial Pathogenesis*

Received Date: 28 March 2017

Revised Date: 18 August 2017

Accepted Date: 30 August 2017

Please cite this article as: Hong C-H, Hsieh C-F, Tseng C-S, Huang W-C, Guo C-Y, Lin S, Lee S-C, A nanobiosening method based on force measurement of antibody-antigen interaction for direct detection of enterovirus 71 by the chemically modified atomic force microscopic probe, *Microbial Pathogenesis* (2017), doi: 10.1016/j.micpath.2017.08.051.

A Nanobiosening Method Based on Force Measurement of Antibody-Antigen Interaction for Direct Detection of Enterovirus 71 by the Chemically Modified Atomic Force Microscopic Probe

Chung-Hung Hong[a,*], Chung-Fan Hsieh[b], Chi-Shin Tseng[c], Wei-Chih Huang[d], Cheng-Yan Guo[d], Shiming Lin[d,], Si-Chen Lee[a,e,***]**

[a]Graduate Institute of Electronics Engineering, National Taiwan University, Taipei, Taiwan
[b]Department of Biochemistry and Molecular Biology, Chang Gung University, Taoyuan, Taiwan
[c]National Taiwan University College of Medicine/Hospital, National Taiwan University, Taipei, Taiwan
[d]Institute of Medical Device and Imaging, National Taiwan University, Taipei, Taiwan
[e]Department of Electrical Engineering, National Taiwan University, Taipei, Taiwan

* Corresponding author. Graduate Institute of Electronics Engineering, National Taiwan University 1 Roosevelt Road, Sec. 4, Taipei, Taiwan

** Corresponding author. Institute of Medical Device and Imaging, National Taiwan University/College of Medicine, No.1, Sec. 1, Jen-Ai Road, Taipei, Taiwan
*** Corresponding author. Graduate Institute of Electronics Engineering/Department of Electrical Engineering, National Taiwan University 1 Roosevelt Road, Sec. 4, Taipei, Taiwan

E-mail address: hongchunghung@gmail.com (C.H. Hong), til@ntu.edu.tw (S. Lin), sclee@ntu.edu.tw (S.C. Lee)

Abstract

Hand, Foot and mouth disease (HFMD) is a common disease with high infectivity for children, and enterovirus 71 (EV71) is one of the main pathogens to cause the type of illness. Therefore, the aim of this study was to propose a rapid and effective technique for detecting EV71 directly based on the mechanism of biological intermolecular force by using atomic force microscopy (AFM). At first, we coated EV71 particles on the mica surface and made the EV71 antibodies (anti-EV71) fixed on the AFM tip by means of several chemical procedures. Then, AFM chemically modified tip was applied to measure the unbinding forces between EV71 and anti-EV71 by contact mode. Finally, by using AFM imaging calculating software, the EV71 particle size (mean±SD) was 31.36±3.87 nm (n=200) and this result was concordance with previous literature. Besides, the force (mean±SD) between EV71 antigen and antibody complex was 336.9±64.7 pN. The force (mean±SD) between anti-EV71 and non-specific specimens was 47.1±15.1 pN and was significantly smaller ($P<0.05$). Apparently, the results show that we can precisely identify EV71 infection among the samples by measuring the force magnitude and observing the occurrence of EV71/anti-EV71 unbinding events. Therefore, the combination of AFM system and the chemically modified tip has the potential to be a rapid and effective method for detecting EV71 directly.

1. Introduction

Hand, foot, and mouth disease (HFMD) is commonly seen in countries of the Asia-Pacific region and a number of literatures have indicated that enterovirus is the dominant pathogen [1-3]. Enterovirus was first discovered from neurological disease in the United States in 1969 and caused epidemics in Hong Kong and Malaysia, in 1985 and 1997, respectively [4-6]. Especially in 1998, approximate 120000 people were infected and 78 children even died from enterovirus 71 (EV71) in Taiwan [7]. Even up to now, the complications of HFMD caused by enterovirus infection are still serious threats to children. Because of the rapid progression of HFMD, many clinical conditions usually deteriorate within 24 hours [8]. In some special cases, HFMD even causes some complication such as severe pulmonary edema and respiratory failure within 12 hours [9]. The diagnosis of HFMD that its symptom caused by usually depends on clinical manifestation but such approach to determine the cause of sickness lacks of rapid and accurate specific diagnostic tool [10]. EV71, one kind non-enveloped single-stranded RNA virus, is the most common pathogen that let children suffer from HFMD [11]. To test for disease causative agent so as to assist physicians in diagnosing effectively, it is desired to develop a simple, rapid, and accurate procedure to acquire the information of specimen for early diagnosis.

Traditionally, the diagnosis of enterovirus infection is based on clinical

manifestation and clinician's assessment. However, laboratory methods demonstrated increased sensitivities and specificities to identify the causative agent effectively. The virus culture obtained from the hosts, including throat swabs, anal swabs, sputum and cerebrospinal fluid, is the gold standard for identification the virus and for accurate diagnosis. However, this testing approach mostly takes about 7 days or even more to grow the enterovirus. Nowadays, polymerase chain reaction (PCR) is also a common and rapid method to identify a genetic region in the enterovirus with high sensitivity and specificity. But the accuracy of enterovirus PCR and whether it could replace viral culture are still under debate [12]. The enzyme-linked immunosorbent assay (ELISA), is another method by using qualitative immunoassays for the detection of human antibodies, directed against enteroviruses which were produced by the human immunes system [13]. But it also required at least three days for human antibodies to react after infection. Therefore, we hope to build up a direct method to identify the EV71 with accuracy and timely response. In this study, we use atomic force microscopy (AFM) as a measurement tool to acquire the unbinding force of the EV71/EV71 antibody (anti-EV71) complex based on the antigen-antibody specificity phenomenon during the experimental process. The anti-EV71 was separated from the EV71/anti-EV71 complex so as to obtain the direct evidence that EV71 existed in specimens from the unbinding force of the EV71/anti- EV71 complex.

Atomic force microscopy (AFM) belongs to the family of scanning probe microscopes (SPMs), and it was proposed by Binning, Calvin Quate, and Gerber for the first time in 1986 [14]. AFM possesses some specific capabilities such as high image resolution (nanometer level) and high mechanics sensitivity (pico-Newton (pN) level) so that it has been used widely in the fields of semiconductor physics, molecular biology, etc [15-18]. According to previous studies, the biological intermolecular forces fall in the range between pico-Newton and nano-Newton [19-23]. Therefore, AFM system is a suitable tool to observe the mechanics relationship between virus particles and their antibodies. In this study, we proposed a procedure for detecting EV71 directly based on the mechanism of biological intermolecular force by using the chemically modified AFM tip as a virus mechanics biosensor.

2. Material and methods

2.1. A brief introduction of the experiment purpose and procedure

To acquire the unbinding forces between EV71 and anti-EV71 so as to obtain the direct evidence that EV71 particles exist in samples, we establish an experimental environment which makes anti-EV71 interact with EV71 directly by AFM system. Besides, this setup needs two preparations for measuring the unbinding forces between EV71 and anti-EV71; one is to make EV71 particles coated on the mica substrate (as described in 2.2.), and the other is to make AFM tips modified (as described in 2.3.). After finishing above-mentioned works, we can measure the unbinding forces by AFM system (as described in 2.4.).

2.2. An approach to make EV71 coated on mica substrate surface

In this study, mica was used as the substrate for making EV71 particles coated on cleaved mica surface because the surface possesses flatness level that is usually below 1 nm. Negative electrical property appears on cleaved mica surface, but such property couldn't make EV71 particles fixed on the substrate surface firmly because virus particle surface shows negative electrical property similarly [24]. To overcome this problem, we use 0.02% poly-L-lysine solution to change mica surface electrical property from negative electricity to positive electricity so that EV71 particles could

be coated on substrate surface stably, and the detail procedure is shown in Fig. 1A.

2.3. Functionalization of AFM tips coated with anti-EV71

First, AFM tips were immersed in 65% nitric acid solution for 20 minutes and then all were taken out from the solution. The second step is to make AFM tips immersed in 5% ethanol solution for 20 minutes at room temperature, and rinsing the AFM tips simultaneously. The third step is to make AFM tips immersed in 5% 3-Aminopropyltriethoxysilane (APTES) solution for 50 minutes, and then were taken out. Next, we make AFM tips immersed in 5% ethanol solution and rinse AFM tips thoroughly for 10 minutes. The fourth step is to make AFM tips immersed in 2.5% glutaraldehyde solution and then were taken out. Finally, AFM tips were immersed in anti-EV71 solution (Anti-Enterovirus 71 antibody [10F0] ab36367, Abcam), and then the tips were put in $4^{o}C$ refrigerator for overnight. The above-mentioned procedure is shown in Fig. 1(B). In addition, the AFM tip is composed of silicon, and such material is very hard. After a series of procedures as described in above content, the antibodies coated on tip were fixed firmly. On the whole, the chemical property change only occurred on the tip surface, and silicon is very hard so that the tip structure will not change after antibody fixation process. The only change about tip surface morphology was that tip surface was covered with antibodies (anti-EV71).

2.4. AFM force measurement procedure

After finishing the preparation works (as shown in 2.2. and 2.3.), we could proceed with AFM force measurement, and all AFM force measurements were performed with a SPA-300HV scanning probe microscope controlled by an SPI 3700 probe station unit (Seiko Instruments Inc., Chiba, Japan). During the force measurement procedure (Fig. 2), the piezo-scanner is moved toward the AFM tip by piezo-expansion, and the moving velocity keeps constant until the scanner is brought into contact with the AFM tip (point 2 in Fig. 2). As the forward motion continues, the cantilever is pressed into sample surface until a maximum load point is reached (point 3 in Fig. 2). Finally, the direction of motion is reversed, and then the piezo-scanner withdraws from the AFM tip.

3. Results and Discussion

3.1. Images of EV71 acquired by AFM

AFM has two main modes to observe the sample surface; one is contact mode, and the other is tapping mode evolved from non-contact mode [25-28]. Fig. 3A and 3B show the operation mechanism of contact mode and tapping mode, respectively. Compare to contact mode, tapping mode is more suitable to be used to acquire the images of soft matters such as cell, protein, and virus, because contact mode makes the AFM tip and the sample too close to each other continuously. Therefore, contact mode is easy to destroy the surface structures of samples, soft matters especially [29]. In order to prevent surface structures from being destroyed, we acquire images by using tapping mode in this study.

Fig. 4A and 4B show freshly cleaved mica surface and mica surface coated with poly-L-lysine, respectively. Fig. 4C shows the AFM image of EV71 particles, and we can find that the virus particles are uniformly distributed on the mica surface. By using AFM imaging calculating software, the size of EV71 particles was 31.36$\pm$3.87 nm (mean$\pm$SD, n=200), and such result is in agreement with previous literatures [30-34].

3.2.AFM force measurements

AFM force measurements were conducted to check whether the unbinding force between EV71 and anti-EV71 exists; and further, to measure the unbinding force magnitude. Besides, the detailed procedure for measuring the unbinding force has been described in 2.4.

The results are divided into two groups; one is control group, and the other is experimental group. In order to check the phenomenon of unbinding event occurs only in the interaction between EV71 and anti-EV71, we design two kind control groups to evidence that the specificity only occurs in the intermolecular force between EV71 and anti-EV71. One is the evidence of the interaction between a clean AFM tip and a neat cleaved mica surface (as shown in Fig. 5A), and the other is the evidence of the interaction between a chemical modified AFM tip coated with anti-EV71 and the mica substrate surface coated with the normal samples acquired from healthy subjects (as shown in Fig. 5B). In addition, Fig. 5C shows the specific event which occurred in the interaction between EV71 and anti-EV71. However, compare to the result as shown in Fig. 5C, the results as shown in Fig. 5A and 5B show that no specific unbinding forces occurred in these control experiments. Therefore, a series experimental and control results (as shown in Fig. 5A-C) evidence that the specific unbinding force between EV71 and anti-EV71 exist. Besides, the result of Fig. 5C

shows the specificity relationship from the existence of the unbinding event between EV71 and anti-EV71. Therefore, we can use the chemical modified AFM tip coated with anti-EV71 to detect whether EV71 particles exist. In addition, the number of antibodies coated on the AFM tip end (as shown in Fig. 6A) is not usually only one, because the number mainly depends on the surface area of the tip end and the antibody size. In this study, the surface area of AFM tip end is approximately 25 nm^2, and the contact area between a single antibody and tip end surface is usually less than or approximately equal to 10 nm^2. So the number of antibodies fixed on the tip end by estimated is about one or two under the normal condition. As long as one of the EV71 antibodies coated on AFM tip end touched EV71 particle, we could detect the phenomenon of unbinding force event as shown in Fig. 2 and Fig. 5(C). In addition, sometimes we could observe two unbinding force events as shown in Fig. 6B, this phenomenon showed that two antibody molecules coated on tip end surface touched the EV71 particle coated on mica substrate during the AFM force measurement process.

3.3. Statistical analysis

In this study, an important finding of the obviously statistical difference between the samples include EV71 and the samples from the normal subjects is worthy of attention, and the finding is the force magnitude between chemical modified AFM tips and the mica substrate surface coated with specimens (as shown in Fig. 7). Fig. 7A shows the force distribution between EV71 and anti-EV71 and the force distribution between specimens from normal subjects and anti-EV71. All forces were calculated from 400 force-distance curves; the amount of EV71 specimens is 200, and the amount of normal specimens is also 200. Fig. 7B shows the force (mean±SD) of the EV71/anti-EV71 is 336.9±64.7 pN, and the force (mean±SD) of anti-EV71/samples from the normal is 47.1±15.1 pN. We can find that the force distribution of EV71 and anti-EV71 is much large than the force distribution of the normal specimen and anti-EV71 from the results. Apparently, the above results are reasonable because specificity forces are much larger than non-specificity forces. Besides, the statistical data show the significant difference between the two groups ($P<0.05$).

3.4. Comparisons of different approaches for detection of EV71

Rapid identification of the EV71 and early management would be helpful in the pediatric population. The viral culture and reverse transcription-polymerase chain reaction (RT-PCR) with EV71 type specific primers are nowadays the direct detection tools for identification of EV71. The virus culture usually takes more than 7 days to grow the enterovirus. Although polymerase chain reaction (PCR) is a rapid method to identify the enterovirus, more evidences were needed to prove the superiority of PCR than viral culture [12]. The diagnosis of EV71 infection could be made by Enzyme-linked immunosorbent assay (ELISA) with indirect manners. The EV71 antibodies, like IgM and IgG, were generated by human immune system and became detectable by ELISA after EV71 infection for several days. In this study, we utilized AFM system and developed the chemically modified probe technique to detect the existence of EV71 timely by measuring the unbinding forces between EV71 antigen and antibody. The experimental results showed that we can precisely and rapidly identify EV71 by using AFM. In the future, we can apply this detection technique to analyze different kinds of specimens, including throat swabs, rectum swabs, blood and cerebrospinal fluid (CSF). More clinical data will be collected to standardize the use of AFM system and the application of the chemically modified tip.

4. Conclusion

In this study, we used AFM chemically modified tip as a label-free biosensor to measure the unbinding force between the EV71 antibodies coupled to AFM tips and EV71 particles coated on the mica substrate surface, and the experimental results show that AFM is a high sensitive force (pico-Newton level) instrument. Therefore, AFM is a very suitable tool for measuring intermolecular force between EV71 and anti-EV71. Besides, this study shows high resolution images of EV71 coated on the mica substrate surface by using tapping mode to prevent EV71 particles from being destroyed.

In summary, we proposed two procedures to detect directly whether EV71 particles exist in specimens under test in this study; one is to use AFM tapping mode to check the pathogen size (as shown in Fig. 4C), and the other is to observe the specificity relationship between EV71 and anti-EV71 by the chemically modified tip technique. In this study, we used AFM system and the chemically modified tip as a dynamic and label-free biosensor to observe specificity phenomenon between EV71 and anti-EV71. The experimental results revealed that we could directly detect the existence of EV71 in specimens by this proposed technique of chemical modified tips coated with anti-EV71 (as shown in Fig. 1B). Finally, we hope that the study results will pave the way to new research of EV71 detection technique which will help improvement in the

approach of therapy for HFMD.

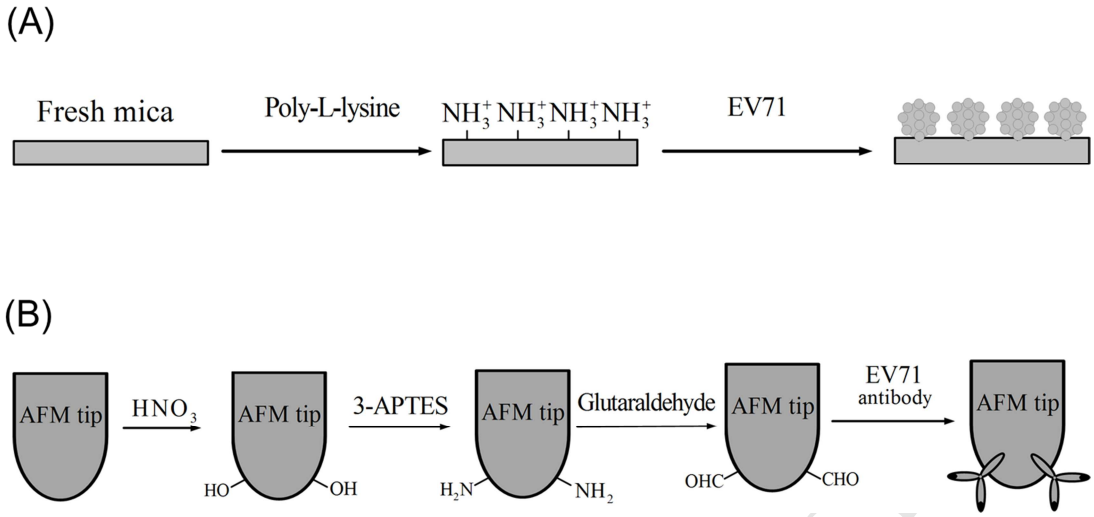


Fig. 1. Schematic illustrations of the chemical modified procedures about (A) adsorption of EV71 particles (with negative charges) on the positively charged poly-L-lysine coated mica surface and (B) AFM tip coated with EV71 antibodies.

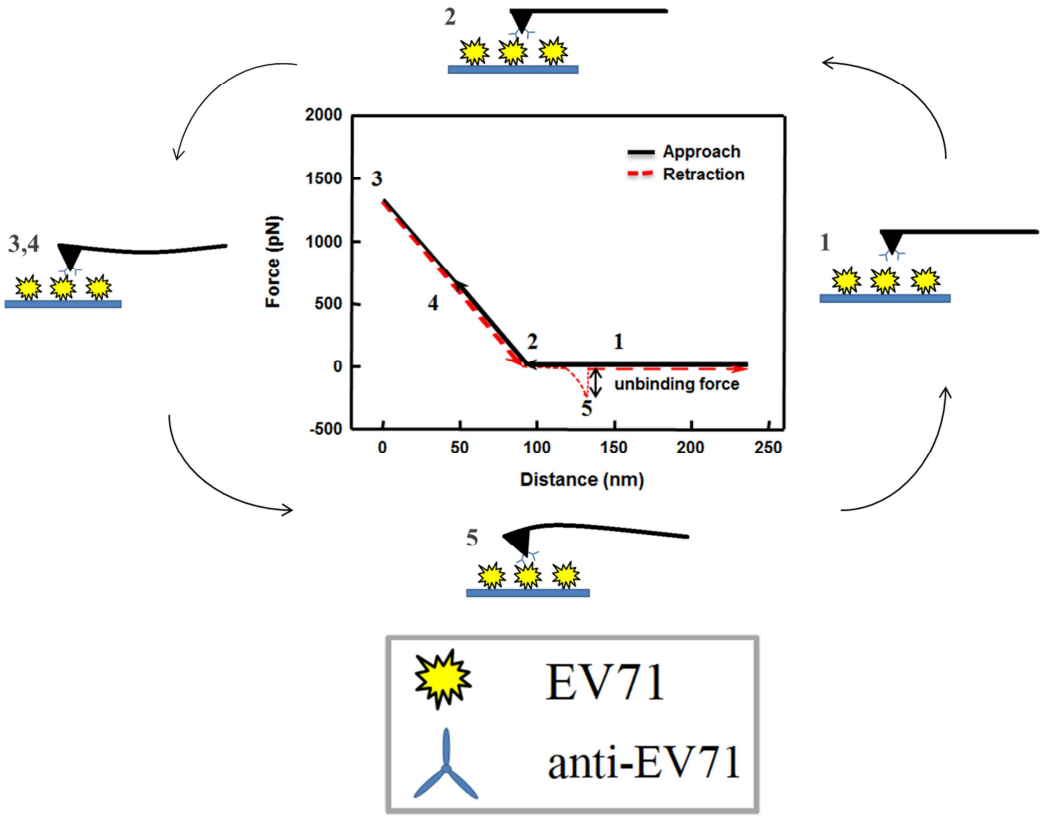


Fig. 2. A schematic diagram of a force measurement cycle by using AFM system as an intermolecular force-based biosensor to measure the unbinding force to break the EV71/anti-EV71 bond formed on contact. On the status of 1, the cantilever is without bend, and the cantilever was influence by the attractive force continually until the AFM tip jumped at contact with the sample surface at the status of 2. Then, the cantilever started to bend upward until the AFM tip reached point 3 (the state of maximum bent extent). As the tip reached 3, the cantilever began to retract (status of 4). Finally, the EV71/anti-EV71 complex started to break from the position of 5.

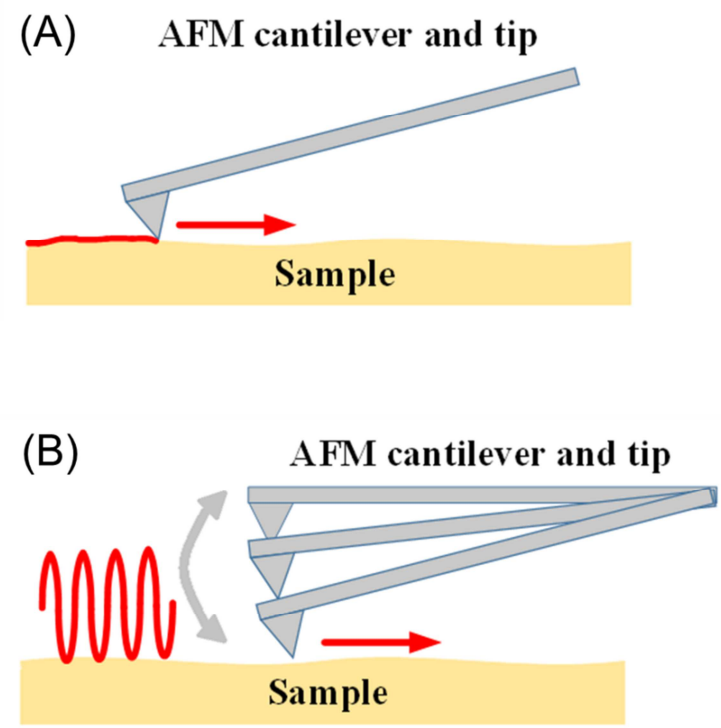


Fig. 3. Schematic diagrams of (A) and (B) are contact mode and tapping mode, respectively. The mechanism of contact mode is to make a tip and the sample surface keep repulsive force continuously because of too close distance between a tip and the sample surface while scanning; tapping mode differs from contact mode by smaller surface damage because the AFM feedback system makes the cantilever keep vibrations during the scanning process (red arrow represents scanning direction).

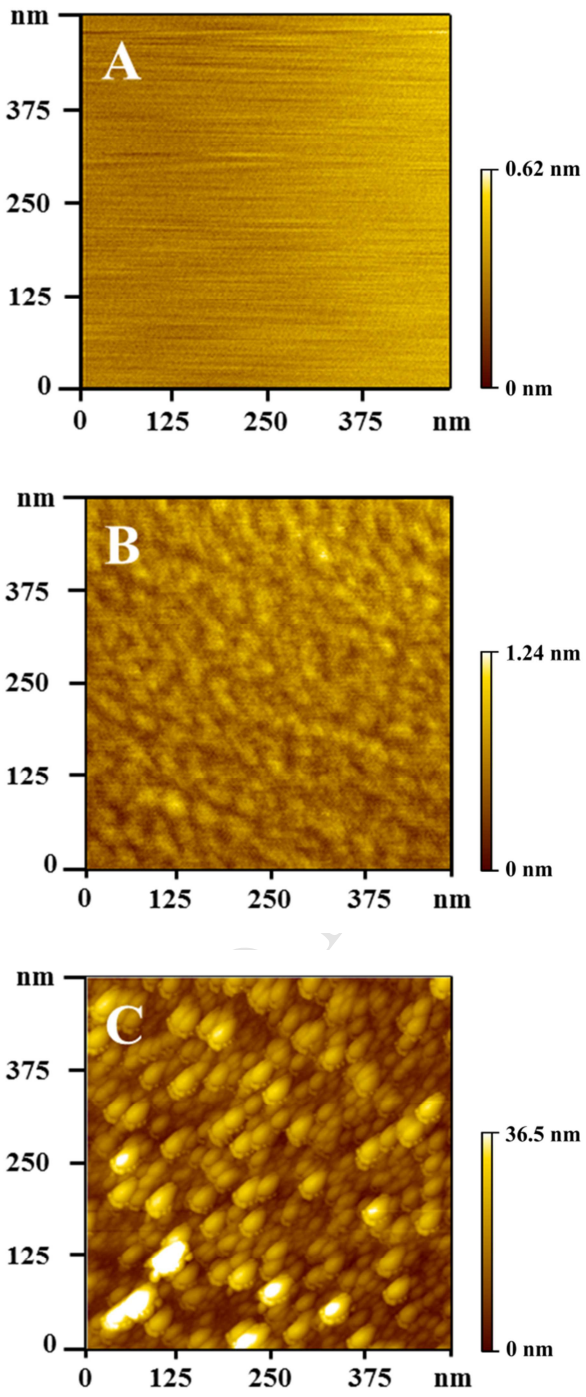


Fig. 4. AFM topographic images acquired by tapping mode. (A) A two-dimensional topographic image of a freshly cleaved mica surface (scanning area: 500 nm x 500 nm); (B) A two-dimensional topographic image of a freshly cleaved mica surface coated with 0.02% poly-L-lysine solution (scanning area: 500 nm x 500 nm); (C) A two-dimensional topographic image of EV71 particles which are distributed over the mica surface (scanning area: 500 nm x 500 nm).

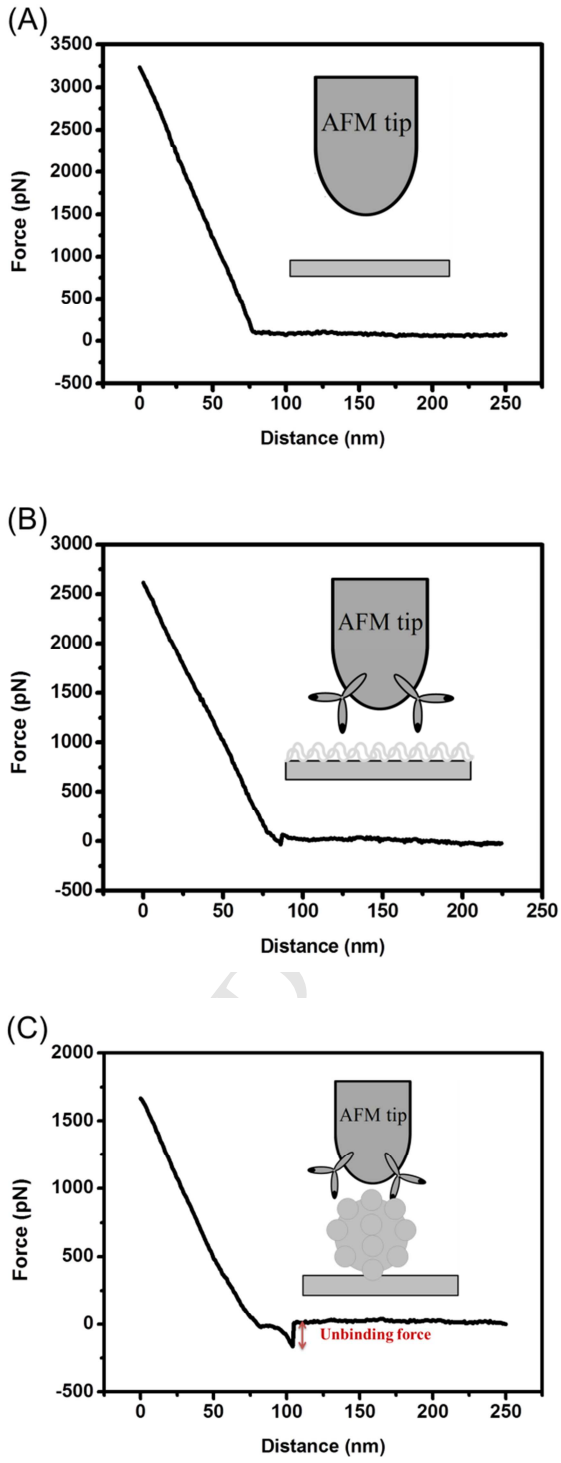


Fig. 5. Typical intermolecular force measurements acquired from AFM contact mode. Force-distance curves of no unbinding events appeared during the cantilever retraction process, which were obtained by use of (A) AFM tip/mica and (B) EV71 antibody/healthy specimen without EV71 particles as two control experiments. (C) The figure shows a force-distance curve of EV71/anti-EV71 complex system with an unbinding event existing during the cantilever retracting process.

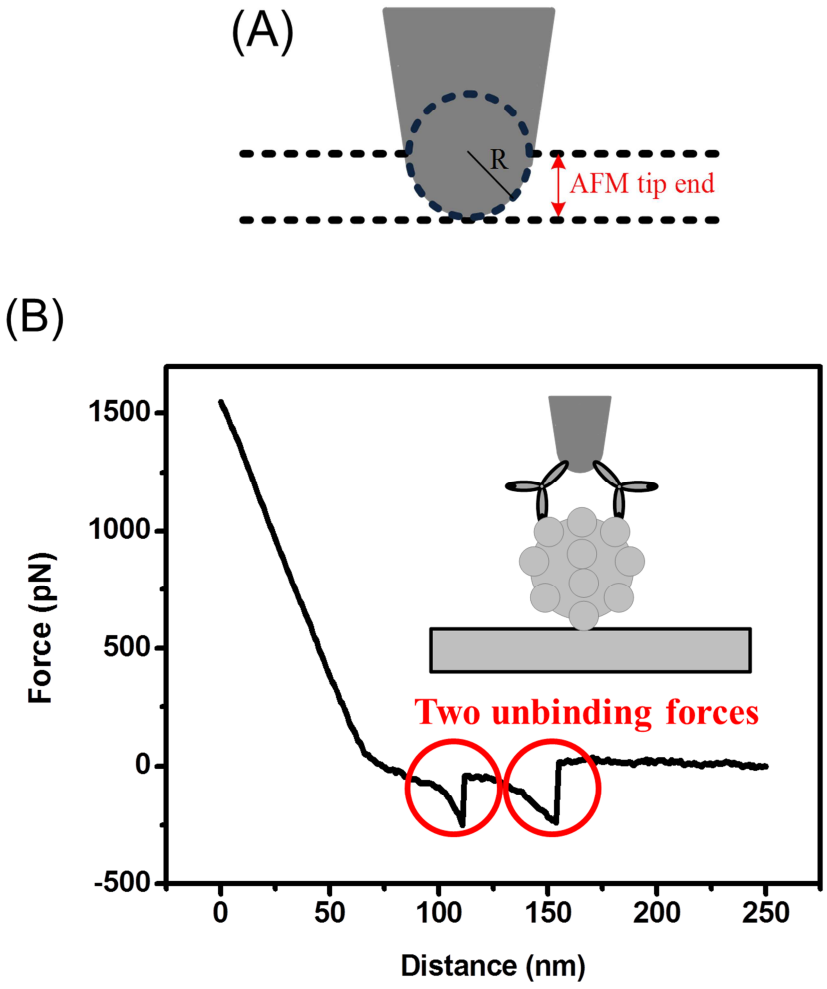


Fig. 6. Two unbinding events. (A) A schematic diagram of AFM tip end, and the letter 'R' represents the radius of curvature of AFM tip end. (B) AFM force measurement shows two unbinding forces. Such results indicate the unbinding events of two interactions between EV71 particle and antibodies (anti-EV71).

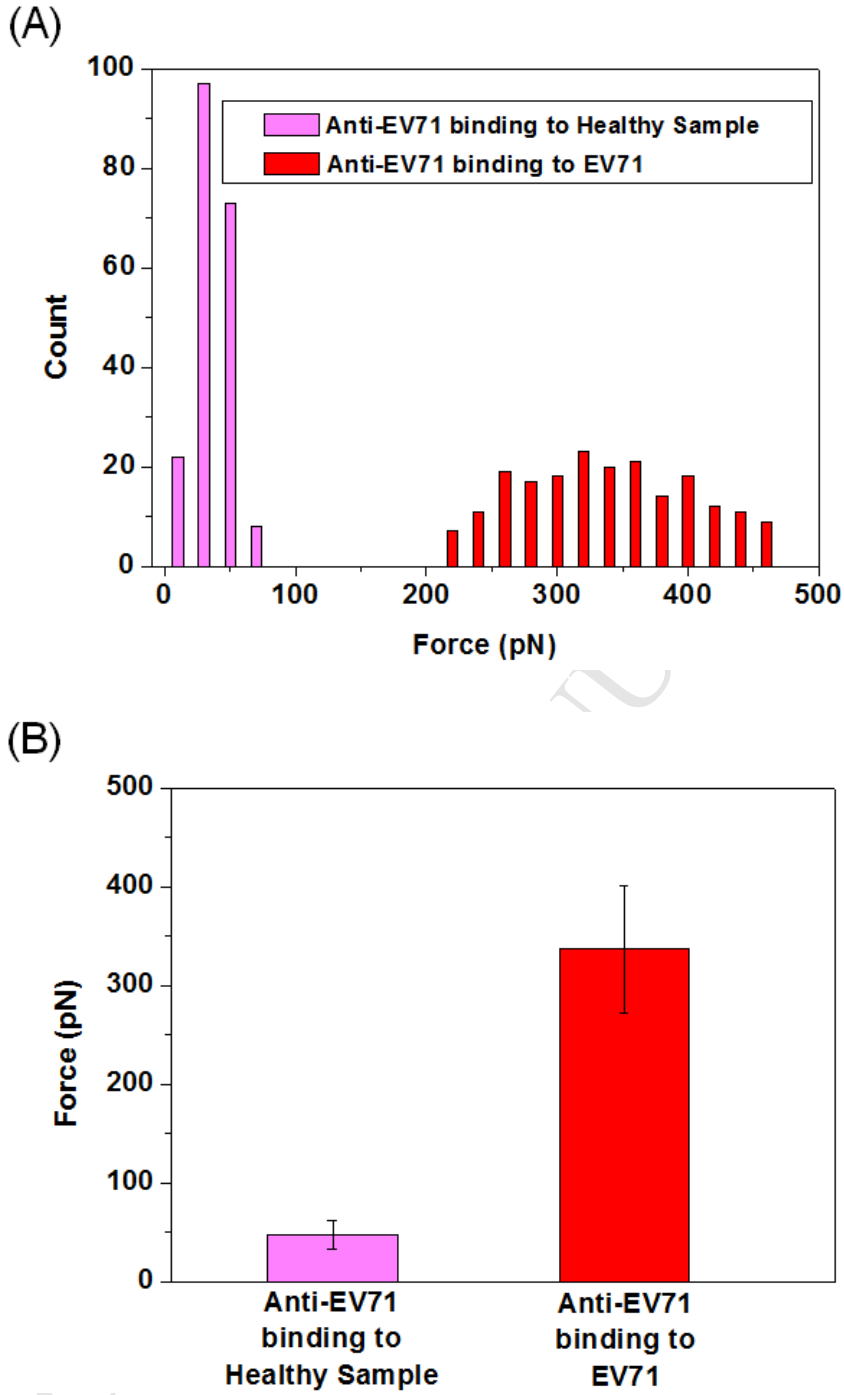


Fig. 7. (A) The distribution shows the difference of the forces between the EV71/anti-EV71 complex and the healthy specimen/anti-EV71, and the counts were 200 and 200, respectively; (B) the histogram shows the force between anti-EV71 and EV71 (mean±SD) is 336.9±64.7 pN, and the force between anti-EV71 and the healthy specimen (mean±SD) is 47.1±15.1 pN. The statistical results show the significant difference between the two groups ($P<0.05$).

Reference

[1] A. Seiff, Cambodia unravels cause of mystery illness, Lancet 380 (7) (2012) 205.

[2] Z.C. Zhuang, Z.Q. Kou, Y.J. Bai, X. Cong, L.H. Wang, C. Li, L. Zhao, X.J. Yu, Z.Y. Wang, H.L. Wen, Epidemiological research on hand, foot, and mouth disease in mainland China, Viruses 7 (12) (2015) 6400-6411.

[3] P. F. Horwood, A. Andronico, A. Tarantola, H. Salje, V. Duong, C. Mey, S. Ly, P. Dussart, S. Cauchemez, P. Buchy, Seroepidemiology of Human Enterovirus 71 Infection among Children, Cambodia, Emerg. Infect. Dis. 22 (1) (2016) 92.

[4] N.J. Schmidt, E.H. Lennette, H.H. Ho, An apparently new enterovirus isolated from patients with disease of the central nervous system, J. Infect. Dis. 129 (3) (1974) 304-309.

[5] G.M. Samuda, W.K. Chang, C.Y. Yeung, P.S. Tang, Monoplegia caused by Enterovirus 71: an outbreak in Hong Kong, Pediatr. Infect. Dis. J. 6 (2) (1987) 206-208.

[6] K.B. Chua, A.R. Kasri, Hand foot and mouth disease due to enterovirus 71 in Malaysia, Virol. Sin. 26 (4) (2011) 221-228.

[7] M. Ho, E.R. Chen, K.H. Hsu, S.J. Twu, K.T. Chen, S.F. Tsai, J.R. Wang, S.R. Shih, An epidemic of enterovirus 71 infection in Taiwan, N. Engl. J. Med. 341 (13) (1999) 929-935.

[8] K.P. Chan, K.T. Goh, C.Y. Chong, E.S. Teo, G. Lau, A.E. Ling, Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore, Emerg. Infect. Dis. 9 (1) (2003) 78-85.

[9] D.S. Lee, Y.I. Lee, J.B. Ahn, M.J. Kim, J.H. Kim, N.H. Kim, J.H. Hwang, D.W. Kim, C.G. Lee, T.W. Song, Massive pulmonary hemorrhage in enterovirus 71-infected hand, foot, and mouth disease, Korean J. Pediatr. 58 (3) (2015) 112-115.

[10] M.H. Ooi, S.C. Wong, P. Lewthwaite, M.J. Cardosa, T. Solomon, Clinical features, diagnosis, and management of enterovirus 71, Lancet Neurol. 9 (11) (2010) 1097-1105.

[11] W. Liu, S. Wu, Y. Xiong, T. Li, Z. Wen, M. Yan, K. Qin, Y. Liu, J. Wu, Co-circulation and genomic recombination of coxsackievirus A16 and enterovirus 71 during a large outbreak of hand, foot, and mouth disease in Central China, PLoS One 9 (4) (2014) e96051.

[12] J.D. Tsai, H. J. Tsai, T.H. Lin, Y.Y. Chang, S.H. Yang, H.T. Kuo, Comparison of the detection rates of RT-PCR and virus culture using a combination of specimens from multiple sites for enterovirus-associated encephalomyelitis during enterovirus 71 epidemic, Jpn. J. Infect. Dis. 67 (5) (2014) 333-338.

[13] J. Boman, B. Nilsson, P. Juto, Serum IgA, IgG, and IgM responses to different enteroviruses as measured by a coxsackie B5□based indirect ELISA, J. Med. Virol. 38 (1) (1992) 32-35.

[14] G. Binnig, C.F. Quate, C. Gerber, Atomic force microscope, Phys. Rev. Lett. 56 (9) (1986) 930-933.

[15] R.A. Oliver, Advances in AFM for the electrical characterization of semiconductors, Rep. Prog. Phys. 71 (7) (2008) 076501.

[16] A. Yersin, H. Hirling, P. Steiner, S. Magnin, R. Regazzi, B. Hüni, P. Huguenot, P. De los Rios, G. Dietler, S. Catsicas, Interactions between synaptic vesicle fusion proteins explored by atomic force microscopy, Proc. Natl. Acad. Sci. U. S. A. 100 (15) (2003) 8736-8741.

[17] Z. Liu, R. Liu, Z. Zhou, Y. Zu, F. Xu, Structural changes of linear DNA molecules induced by cisplatin, Biochem. Biophys. Res. Commun. 457 (4) (2015) 688-692.

[18] J.L. Giongo, R. de Almeida Vaucher, V.P. Fausto, P.M. Quatrin, L.Q.S. Lopes,

R.C.V. Santos, A. Gündel, P. Gomes, M. Steppe, Anti-Candida activity assessment of Pelargonium graveolens oil free and nanoemulsion in biofilm formation in hospital medical supplies, Microb. Pathog. 100 (2016) 170-178.

[19] S. Casalini, A.C. Dumitru, F. Leonardi, C.A. Bortolotti, E.T. Herruzo, A. Campana, R.F. de Oliveira, T. Cramer, R. Garcia, F. Biscarini, Multiscale sensing of antibody–antigen interactions by organic transistors and single-molecule force spectroscopy, ACS Nano. 9 (5) (2015) 5051-5062.

[20] A.C. Dumitru, E.T. Herruzo, E. Rausell, V. Ceña, R. Garcia, Unbinding forces and energies between a siRNA molecule and a dendrimer measured by force spectroscopy, Nanoscale 7 (47) (2015) 20267-20276.

[21] M. Sorci, B. Dassa, H. Liu, G. Anand, A.K. Dutta, S. Pietrokovski, M. Belfort, G. Belfort, Oriented covalent immobilization of antibodies for measurement of intermolecular binding forces between zipper-like contact surfaces of split inteins, Anal. Chem. 85 (12) (2013) 6080-6088.

[22] S. Manna, S. Senapati, S. Lindsay, P. Zhang, A Three-Arm Scaffold Carrying Affinity Molecules for Multiplex Recognition Imaging by Atomic Force Microscopy: The Synthesis, Attachment to Silicon Tips, and Detection of Proteins, J. Am. Chem. Soc. 137 (23) (2015) 7415-7423.

[23] M. Saito, T. Watanabe-Nakayama, S. Machida, T. Osada, R. Afrin, A. Ikai, Spectrin–ankyrin interaction mechanics: A key force balance factor in the red blood cell membrane skeleton, Biophys. Chem. 200 (2015) 1-8.

[24] M. Bezanilla, S. Manne, D.E. Laney, Y.L. Lyubchenko, H.G. Hansma, Adsorption of DNA to mica, silylated mica, and minerals: characterization by atomic force microscopy, Langmuir 11 (2) (1995) 655-659.

[25] D. Rugar, P. Hansma, Atomic force microscopy, Phys. Today 43 (10) (1990) 23-30.

[26] Y. Martin, C.C. Williams, H.K. Wickramasinghe, Atomic force microscope–force mapping and profiling on a sub 100□Å scale, J. Appl. Phys. 61 (10) (1987) 4723-4729.

[27] R. Garcia, A. San Paulo, Attractive and repulsive tip-sample interaction regimes in tapping-mode atomic force microscopy, Phys. Rev. B 60 (7) (1999) 4961.

[28] R.W. Stark, Bistability, higher harmonics, and chaos in AFM, Mater. Today 13 (9) (2010) 24-32.

[29] C. Möller, M. Allen, V. Elings, A. Engel, D.J. Müller, Tapping-mode atomic force microscopy produces faithful high-resolution images of protein surfaces, Biophys. J. 77 (2) (1999) 1150-1158.

[30] Z. Liang, Q. Mao, F. Gao, J. Wang, Progress on the research and development of human enterovirus 71 (EV71) vaccines, Front. Med. 7 (1) (2013) 111-121.

[31] Y.H. Chiu, Y.L. Chan, L.W. Tsai, T.L. Li, C.J. Wu, Prevention of human enterovirus 71 infection by kappa carrageenan, Antiviral Res. 95 (2) (2012) 128-134.

[32] C.A. Peng, C.H. Wang, Anti-enteroviral Activity of Microalgal Extracts Probed by Bionanohybrids of Quantum Dots and Viruses, BioNanoScience 1 (4) (2011) 144-152.

[33] T.C. Chen, S.C. Liu, P.N. Huang, H.Y. Chang, J.H. Chern, S.R. Shih, Antiviral activity of pyridyl imidazolidinones against enterovirus 71 variants, J. Biomed. Sci. 15 (3) (2008) 291-300.

[34] C.C. Liu, M.S. Guo, F.H.Y. Lin, K.N. Hsiao, K.H.W. Chang, A.H. Chou, Y.C. Wang, Y.C. Chen, C.S. Yang, P.C.S. Chong, Purification and characterization of enterovirus 71 viral particles produced from vero cells grown in a serum-free microcarrier bioreactor system, PLoS One 6 (5) (2011) e20005.

Highlights

- A rapid, quantitative, and direct approach for enterovirus 71 (EV71) detection was developed.
- The unbinding force between EV71 antigen and its antibody was directly observed by the chemically modified AFM tip.
- The appearance of EV71 particle was directly observed by AFM tapping mode.