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Mechanistic Insights into the Physiological Functions of Cell Adhesion Proteins Using Single Molecule Force Spectroscopy

Vedula S.R.K.*, Lim T.S.†, Hunziker W.‡ and Lim C.T.§

Abstract: Intercellular adhesion molecules play an important role in regulating several cellular processes such as a proliferation, migration and differentiation. They also play an important role in regulating solute diffusion across monolayers of cells. The adhesion characteristics of several intercellular adhesion molecules have been studied using various biochemical assays. However, the advent of single molecule force spectroscopy as a powerful tool to analyze the kinetics and strength of protein interactions has provided us with an opportunity to investigate these interactions at the level of a single molecule. The study of interactions involving intercellular adhesion molecules has gained importance because of the fact that qualitative and quantitative changes in these proteins are associated with several disease processes. In this review, we focus on the basic principles, data acquisition and analysis in single molecule force spectroscopy experiments. Furthermore, we discuss the correlation between results obtained using single molecule force experiments and the physiological functions of the proteins in the context of intercellular adhesion molecules. Finally, we summarize some of the diseases associated with changes in intercellular adhesion molecules.

* Nano-Biomechanics Lab, Division of Bioengineering and Department of Mechanical Engineering, 9 Engineering Drive 1, National University of Singapore, Singapore 117576.

† Bioinformatics Institute, A*STAR (Agency for Science, Technology and Research), 30 Biopolis Street, Matrix, Singapore 138671.

‡ Institute of Molecular and Cell Biology, A*STAR (Agency for Science, Technology and Research), 61 Biopolis Drive, Proteos, Singapore 138673.

§ Corresponding author. Nano-Biomechanics Lab, Division of Bioengineering and Department of Mechanical Engineering, 9 Engineering Drive 1, National University of Singapore, Singapore 117576. email: ctlim@nus.edu.sg

1 Introduction

Cell adhesion is one of the most basic biological phenomenon that plays an extremely important role in cellular processes such as inflammation, migration, proliferation and differentiation. It is mediated by a complex interaction between several types of proteins. In adherent cell types such as epithelial cells, cell adhesion can be broadly divided into two categories: cell-cell adhesion (1) and cell-extracellular matrix (ECM) adhesion (2). While cell-ECM adhesion is primarily mediated by integrins present on the cell surface, cell-cell adhesion is mediated by homotypic or heterotypic interactions between different transmembrane proteins. Among these are E-cadherins and nectins at the adherens junctions; occludin, claudins and junctional adhesion molecules (JAMs) at the tight junctions and desmosomal cadherins at the desmosomes. Each of these proteins contributes in a different way to the process of cell adhesion. For example nectins initiate cell adhesion, E-cadherins and desmosomes contribute to mechanical stability, tight junctions form paracellular channels to facilitate the movement of solutes across cell layers, and integrins anchor the cell to the ECM. In suspended cells such as neutrophils, cell adhesion molecules called selectins (P-, E- and L- selectin), specific integrins (e.g. LFA-1) and intercellular adhesion molecules (e.g. ICAM-1) play the most important role in attaching these cells to endothelial cells during inflammation.

Quantitative and/or qualitative changes in cell adhesion proteins are associated with several diseased states. For example, mutations in claudins are associated with hereditary deafness (3) and

hypomagnesaemia (4, 5). Disruption in the integrity of the tight junction barrier is associated with diseases like asthma (6, 7), ventilation induced lung injury and several gastrointestinal diseases (8). Mutations in nectins are associated with some forms of cleft lip and cleft palate (9) while changes in E-cadherin expression are associated with several epithelial cancers (10). Furthermore, several cell adhesion molecules also act as receptors for certain viruses, e.g. nectins act as receptors for the herpes group of viruses (11), JAM-A acts as a receptor for reovirus (12) and certain claudins are necessary for entry of hepatitis C virus (13).

In view of this, study of interactions involving cell adhesion proteins can provide us with a better understanding of not only normal physiological functions but also pathogenesis of several diseased states. In this article, the basic principle, analysis and interpretation of single molecule force spectroscopy experiments using atomic force microscopy is reviewed. The article also focuses on how single molecule force spectroscopy has been applied to study and better understand the interactions involving cell adhesion proteins. Finally, the role of some of the cell adhesion molecules in various diseased states is briefly elaborated upon.

2 Principle and methods in single molecule force spectroscopy

The atomic force microscope has emerged as a powerful tool to analyze and interpret protein-protein interactions at the level of a single molecule. This method, also called single molecule force spectroscopy (SMFS) or dynamic force spectroscopy, has helped in better understanding the kinetics of protein interactions. Atomic force microscopy was primarily developed as a tool for imaging non-conductive surfaces e.g. polymer surfaces where scanning tunneling microscopy could not be used (14). It uses a highly flexible cantilever with a sharp tip to scan surfaces (Fig. 1). A laser reflecting off the surface of the cantilever onto a photodiode monitors the deflections of the cantilever as it scans the surface of interest. A piezoelectric scanner allows the relative distance between the tip and the surface to be controlled with nanometer resolution. Data obtained from the movement of the piezoelectric scanner and the cantilever deflection is used for reconstructing the topography of the scanned surface with high resolution. In this section we review the basic steps for obtaining and analyzing data in single molecule force spectroscopy experiments.

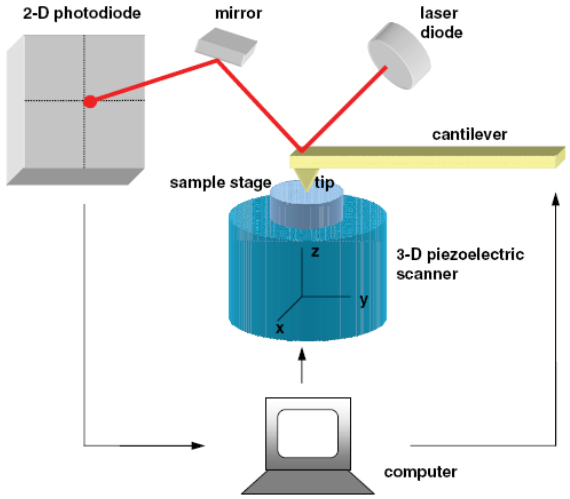

Figure 1: Schematic of atomic force microscopy. A laser reflecting from the cantilever surface onto a photodiode is used to monitor the defections of the cantilever. The feedback from the photodiode is used by the controller to regulate the movement of the piezoelectric scanner (55). Reprinted with permission from Elsevier.

2.1 Data acquisition and tip functionalization

In SMFS, a protein or cell of interest is coupled to the cantilever tip using different strategies. The cantilever is then made to approach a surface containing the interacting protein or cell. After allowing contact for a defined time period at a defined contact force, the cantilever is retracted at a particular pulling velocity. During retraction of the cantilever, bonds formed between the proteins or cells, rupture. The deflections of the cantilever and the movement of the piezoelectric scanner during one cycle of approach, contact and retraction constitute a single force-displacement curve

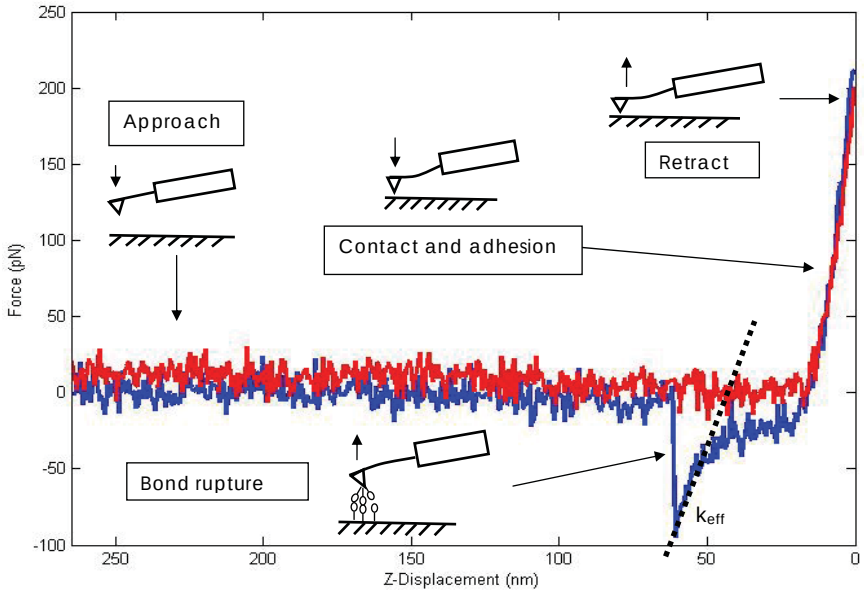

Figure 2: A typical force distance curve showing the approach (red) and reproach curve (blue). Bond ruptures are observed as sharp jumps in the reproach curve. The effective spring constant of the system (k_{eff}) is computed as the slope of the reproach curve prior to the rupture of the bond (dashed line). The loading rate is obtained by multiplying the effective spring constant of the system by the reproach velocity of the cantilever.

(Fig. 2). The force displacement curve constitutes the basic data unit in SMFS experiments. Several hundreds of force curves are obtained at different reproach velocities for analysis.

There are several methods available for functionalizing AFM tips with the proteins of interest or attaching cells to the cantilevers (15-17). To ensure that single bond rupture events and not multiple bond rupture events are probed, the cantilever has to be functionalized using a dilute solution of protein. Furthermore, the contact force and contact time have to be controlled so that the adhesion frequency is low. When force distance curves are obtained at adhesion frequency of less than 30%, Poisson's statistics predicts that more than 85% of the detected ruptures are due to single bond rupture events (18).

2.2 Analysis of force curves and Bell-Evans model parameter extraction

The strength of an interaction not only depends on the type of interacting molecules and temperature but also on the rate at which the bond is stressed. According to the model proposed by Bell and Evans (19, 20), the strength of an interaction (i.e. the most probable rupture force) increases linearly with the logarithm of the loading rate i.e. the rate at which the bond is stressed and is given by the following equation:

$$f = \frac{k_B T}{x_\beta} \ln\left(\frac{x_\beta}{k^0_{off} k_B T}\right) + \frac{k_B T}{x_\beta} \ln(r_f) \qquad (1)$$

where f = most probable rupture force; r_f = loading rate; x_β = reactive compliance; k_B = Boltzmann constant; T = Temperature; k^0_{off} = unstressed off rate.

Since both the loading rate and magnitude of rupture force can be obtained from the force-displacement curves, Bell-Evans model can be used to extract the unstressed off rate (k^o_{off}) and reactive compliance (x_β) for the interacting molecules. The most commonly used method for varying the loading rate on the bond is by varying the velocity at which the cantilever is retracted. As the spring constant of the cantilevers used is usually much higher than the spring constant of the interacting proteins, the effective or apparent spring constant of the system (k_{eff}) is obtained

by calculating the slope of the retrace curve in the force distance curve immediately before the rupture of a bond (dashed line, Fig. 2). The loading rate (r_f) is obtained by multiplying the effective spring constant of the system by the velocity of retraction of the cantilever (v) i.e.

$$r_f = (k_{eff}) \times (v) \tag{2}$$

After calculating the loading rate and the magnitude of rupture from the force curves, there are two methods by which they can be fit to the Bell-Evans model. In the velocity based method, the most probable loading rate and the most probable rupture force for all force-displacement curves obtained at a particular retraction velocity is computed. The most probable rupture force is then plotted against the logarithm of the corresponding most probable loading rate (Fig. 3a) (21). In the binning based method, each force displacement curve yields a single value for rupture force and loading rate. The rupture force is plotted against the logarithm of the corresponding loading rate for all the force curves obtained at different velocities. The data points are then grouped into different bins and the average rupture force for each bin is calculated (Fig. 3b) (18). When the binning method is used, we are using the average force rather than most probable rupture force. It can be derived from Bell-Evans model that the average unbinding force <f> is given by:

$$\langle f \rangle = \frac{k_B T}{x_\beta} \exp\left(\frac{k^0_{off} k_B T}{x_\beta r_f}\right) E_i\left(\frac{k^0_{off} k_B T}{x_\beta r_f}\right) \tag{3}$$

Here $E_i(z) = \int_z^\infty t^{-1} \exp(-t)\, dt$ represents the exponential integral (16, 18, 22-24).

In either case, once the loading rate curve is plotted, the data can be fitted using Eq.1 or Eq.3 to obtain the reactive compliance (x_β) and unstressed off rate (k^o_{off}). The unstressed off rate, in turn, provides an insight into the kinetic stability of the bond. A low k^o_{off} is usually associated with protein interactions which are typically very stable (e.g. antigen–antibody interactions) while a large k^o_{off} is usually associated with protein interactions which are highly dynamic (e.g. claudins). Certain protein interactions tend to show multiple values for k^o_{off} corresponding to different binding architecture or multiple energy barriers e.g. E-cadherins, ICAM-1/LFA-1 etc.

3 Force spectroscopy study of different cell adhesion proteins and correlation to their physiological functions

In this section, we will review single molecular force spectroscopy experiments performed on some of the important cell adhesion molecules along with a brief description of the structure and function of the corresponding protein. The final aim is to understand and correlate the kinetic parameters obtained in SMFS experiments with the physiological functions of the proteins (Table 1).

3.1 E-cadherin mediated interactions

E-cadherin belongs to the cadherin superfamily of proteins and is the prototype of classical cadherins localizing at epithelial cell junctions. It is considered probably the most important contributor to the mechanical stability of the intercellular junction in epithelial cells (25). Adhesion mediated by E-cadherins is Ca^{2+} dependent. Structurally, E-cadherins have been shown to possess five extracellular domains, a short trans-membrane region and a cytoplasmic region (Fig. 4a). There are pockets in between the domains that allow for binding of calcium. Extensive work has been done in understanding the adhesion forces mediated by E-cadherins both at the cellular level and at the level of single molecule. Transfection of E-cadherins has been shown to confer Ca^{2+} dependent adhesiveness to L-cells (26). Experiments done using the dual micropipette assay have shown that the adhesion mediated by E-cadherins is much stronger than that mediated by other cell adhesion proteins like nectins or claudins (27). Furthermore, the experiments also showed that the adhesion strengthened rapidly with time. However, in these experiments the kinetics of E-cadherin mediated adhesion remained elusive.

Single molecule force spectroscopy experiments performed on E-cadherins revealed a hierarchi-

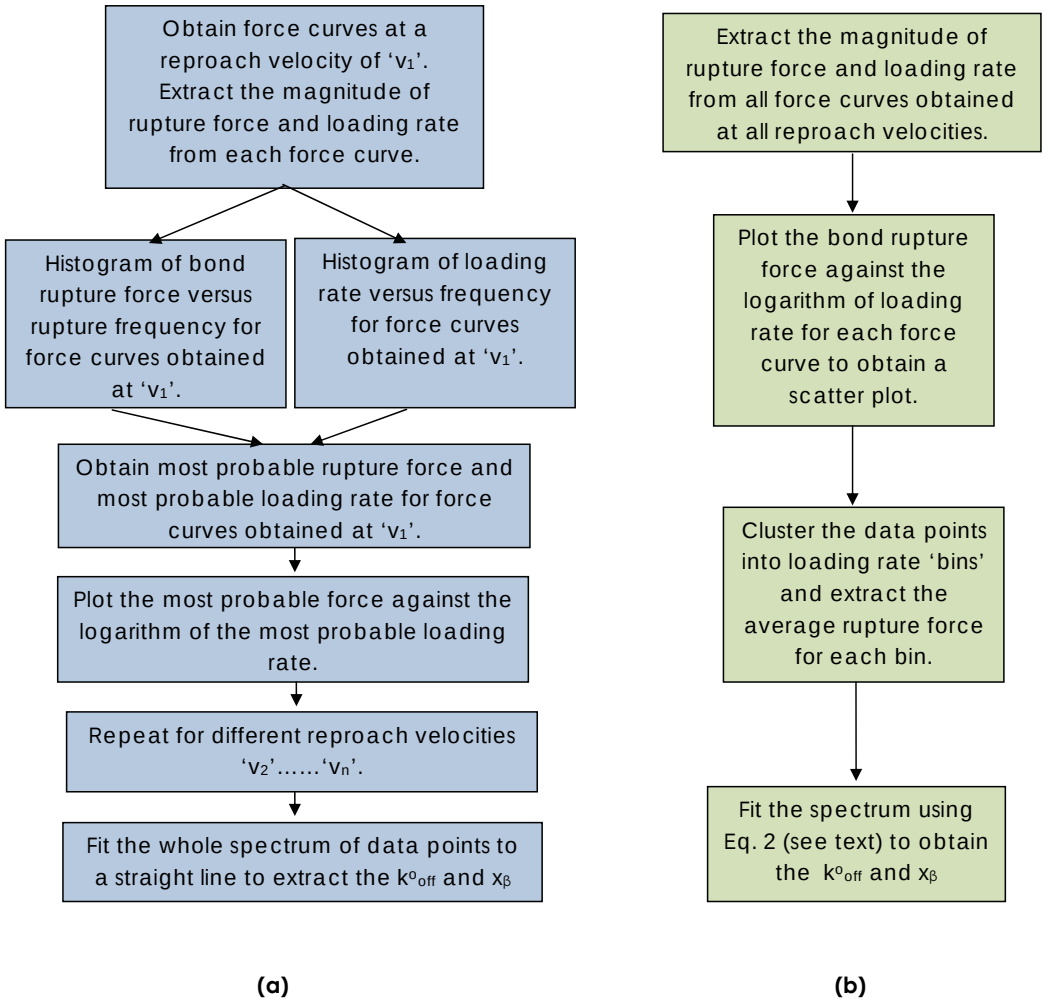

Figure 3: Flow charts depicting (a) velocity based method and (b) binning method for extracting kinetic parameters from force-distance curves obtained in SMFS experiments.

cal strengthening of adhesion with a co-operation between domains (28). Recombinant E-cadherin containing only the first two N-terminal domains showed a much higher k^o_{off} compared to E-cadherin that contained all the five domains. These results strongly suggest that E-cadherin mediated adhesion probably occurs in two steps. First step involves interactions occurring between the first two N-terminal domains. These interactions are unstable and have a low bond half life. However, following multiple domain overlap, the interaction gets greatly strengthened (Fig. 4a) (29). A very long bond half life (of the order of $10^2 - 10^5$ sec) observed for specific sub-states of E-cadherins expressing all five domains explains why E-cadherins are the major stabilizing components of intercellular adhesion.

3.2 Nectin mediated interactions

Nectins are a group of cell adhesion molecules that localize at adherens junctions along with E-cadherins (30). Structurally, nectins contain three extracellular immunoglobulin-like (Ig-like) loops, a short transmembrane region and a cytoplasmic tail. The cytoplasmic end is coupled to the actin cytoskeleton via a PDZ domain containing molecule called afadin. Nectins are important for initiating cell adhesion (31). This is attributed to their ability to form kinetically stable bonds rapidly even when they are present in low concentration (32, 33). Following this, E-cadherins

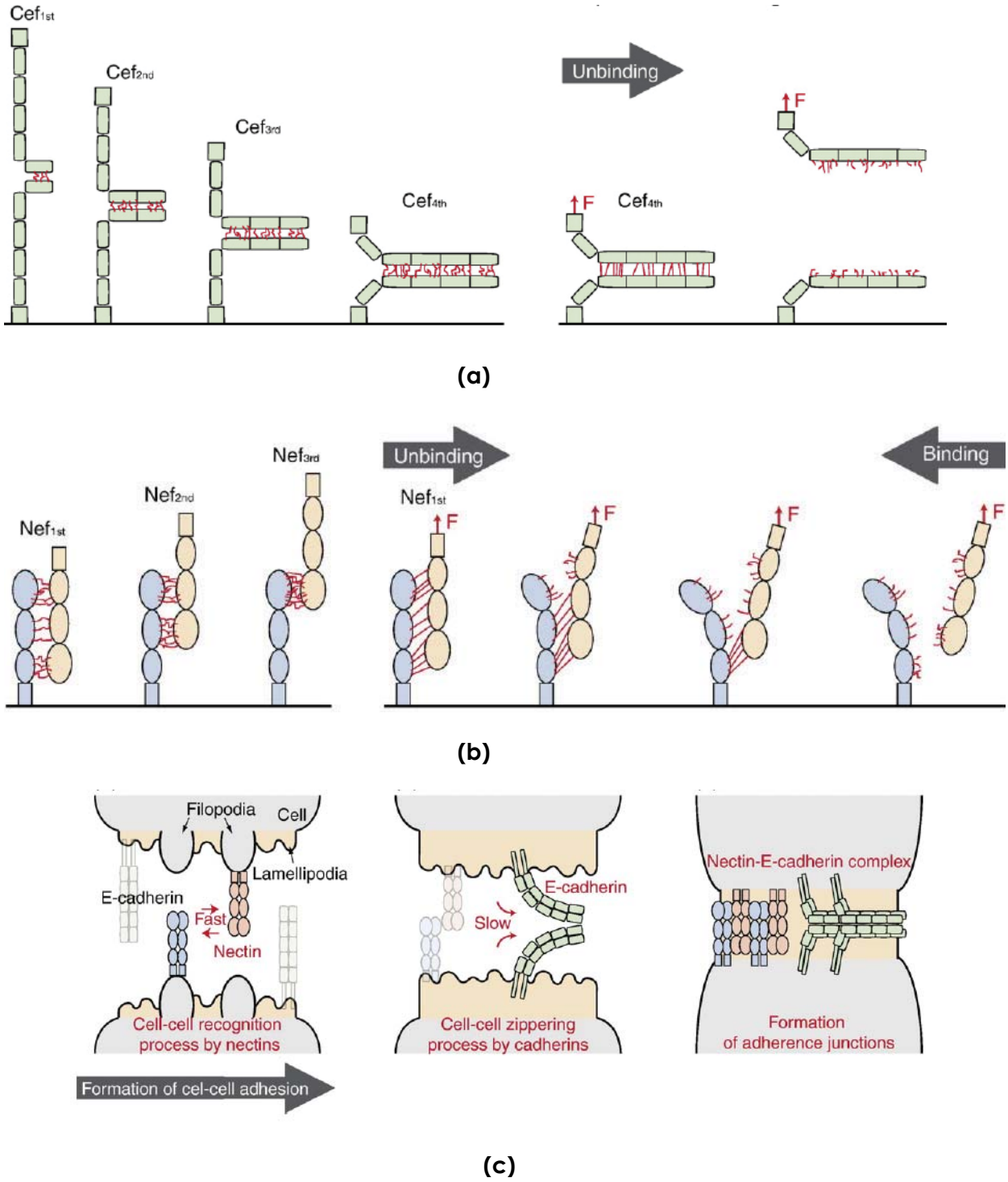

Figure 4: (a) Initial interactions that form between the extracellular N-terminal domains of E-cadherins are short lasting. Following multiple domain overlap, the adhesion gets significantly strengthened (b) the extracellular domains of nectins can interact in three different configurations. While E-cadherins show a co-operative and parallel like unbinding, nectins exhibit an uncooperative and zipper like unbinding (29) (c) Initial interactions between nectins form very rapidly and have a relatively long bond half life. These interactions can then recruit E-cadherins to the adhesion sites. Reprinted with permission from Elsevier.

Table 1: List of k^o_{off} values for some cell adhesion proteins obtained using SMFS experiments

Interacting molecular pairs	**Substrate**	**Tip**	**Dissociation rate, $k^o_{off}(s^{-1})$**	**Physiological functions**	**References**
E-cadherin/E-cadherin	E-cadherin	E-cadherin	$\sim 1.0*$ $\sim 10^{-5}$	Stabilizing intercellular junction	(28)
Nef-1/Nectin-1	L-cells	Fc-Nectin1	0.038* 1.155 1.465	Initiating cell adhesion	(34)
Claudin-1/Claudin-1	GST-Claudin1	GST-Claudin1	1.35	Paracellular gate regulation	(37)
JAM-A/JAM-A	Fc/mJAM-A	L-cells	0.688	Paracellular gate regulation	(56)
Desmoglein /Desmoglein	Fc-Desmoglein	Fc-Desmoglein	5.88	Stabilizing intercellular adhesion	(40)
Integrin/ligand	Type I collagen	$\alpha_2\beta_1$ integrin expressing CHO cell	1.3	Cell substrate adhesion	(21)
P-selectin/PSGL	PSGL expressing leukocytes	Fc-Pselectin	0.22	Leukocyte capture and rolling.	(18)
LFA-1/ICAM-1	ICAM-1	Leukocyte	4.0 and 57**§ 0.17 and 40**Ψ	Leukocyte adhesion, crawling and transmigration	(16)

*Corresponds to different binding configurations, **Corresponds to two different energy barriers, §Corresponds to high affinity LFA and Ψlow affinity LFA.

are recruited to these adhesion sites leading to the maturation and strengthening of the cell adhesion.

Single molecule force spectroscopy experiments performed on nectin-1/nectin-3 heterophilic and nectin-1/nectin-1 homophilic interactions show that nectins can interact in different configurations(Fig. 4b) (29, 34). Molecular studies have previously shown that nectins can form bonds even when their concentration is very low. Combined with the fact that their bond life time is long, this makes nectins ideal for initiating cell adhesion. Based on single molecule force spectroscopy experiments and molecular biology experiments, there is a strong consensus for a model of cell adhesion in which nectins are important for initiating cell adhesion while E-cadherins are more important in stabilizing the adhesion. According to this model, at the sites where lamellipodia come in contact with one another, nectins rapidly interact with one another(Fig. 4 c) (29). This is followed by recruitment of E-cadherins to the adhesion sites. E-cadherin interactions are initiated once their concentration becomes sufficiently high. Though the initial E-cadherin interactions have a short half life, multivalent or hierarchical interactions involving multiple domain overlap soon ensue leading to a significant strengthening of the adhesion complex.

3.3 Tight junction protein mediated interactions

Tight junctions (TJs) are located at the apex of the intercellular adhesion complex and are largely responsible for regulating the diffusion of solutes across the paracellular route across epithelial monolayers (35). TJs are constituted by three dif-

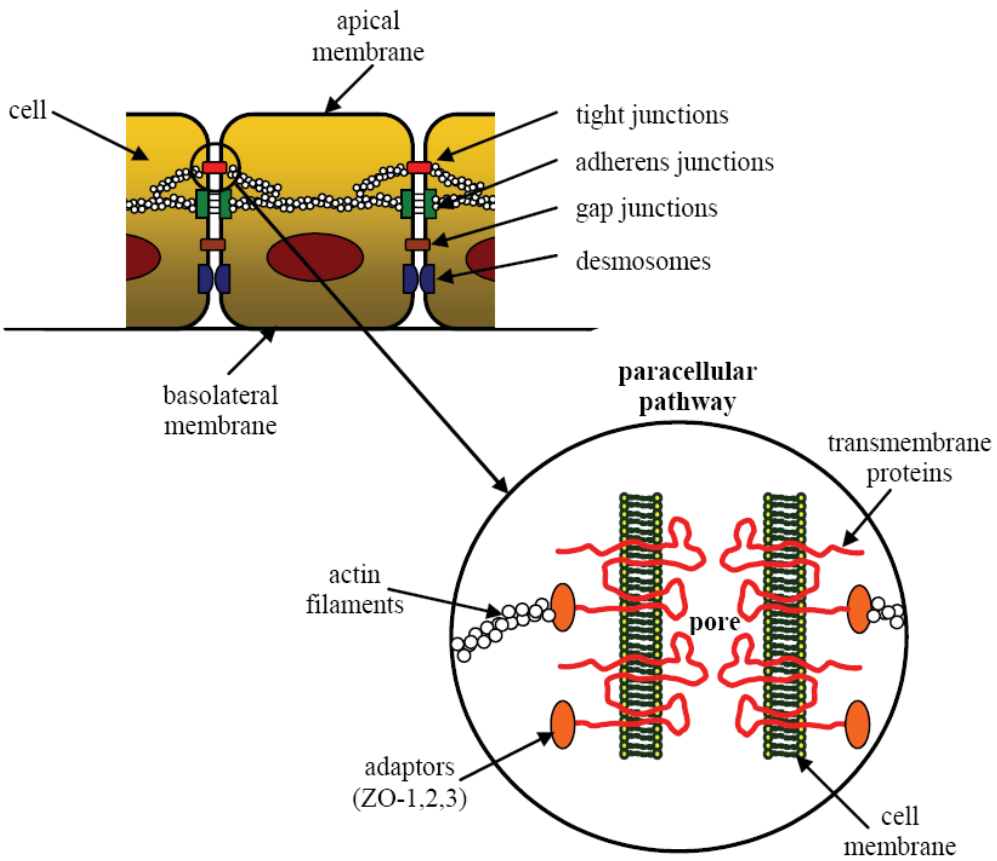

Figure 5: Schematic of tight junction organization in epithelial cells. The two extracellular loops of claudins of adjacent cells come in contact with one another to form the paracellular pores that regulate solute diffusion (49).

ferent groups of transmembrane proteins and their cytoplasmic adaptor molecules (Fig.5). These are occludin, claudins and junctional adhesion molecules (JAMs). They have been shown to play an important role not only in gating the paracellular barrier but also in regulating cellular processes such as proliferation and differentiation (36). It has been shown that transfection of claudins into mouse L-cells confers Ca^{2+} independent adhesiveness to the cells (26). Experiments using micropipette assays have shown that the adhesion forces conferred by claudins are very weak. However, these experiments could not provide any insight into the kinetics of the adhesion.

Single molecule force spectroscopy experiments performed on recombinant Claudin-1 and JAM-A as representative prototype of TJ proteins, on the other hand, show that these interactions have a short bond half life time (37). A short half bond life time imparts a highly dynamic nature to these interactions. Such a dynamic association and dissociation process has been demonstrated in cells transfected with GFP tagged claudin molecules (38). It is likely that the dynamic nature of claudin interactions provides the cell with a better platform for controlling the paracellular gate in response to external signals.

3.4 Desmosome mediated interactions

Desmosomes also belong to the superfamily of cadherins, and similar to E-cadherins, play an important role in providing mechanical stability to cell-cell junction in epithelial cell sheets (39). The importance of desmosomal proteins in maintaining intercellular adhesion is evident in several diseases like pemphigus, where auto antibodies against the desmosomal protein desmogelin-1 make the epidermis very fragile leading to the for-

mation of blisters (40).

Single molecule force spectroscopy experiments on desomglein-1 (Dsg-1), a representative component of desmosome, have shown that its adhesion kinetics share a lot of similarities with those of E-cadherins (40). Multivalent interactions have been shown to occur between Dsg-1 which has been attributed to multiple domain overlap and different interaction configurations. Based on this, it is highly probable that similar to E-cadherins, initial interactions between Dsg-1 might be vey short lasting, but development of multivalent interactions could significantly strengthen the interaction.

3.5 Gap junctions

Gap junctions are formed by tetraspan membrane proteins called connexins. Hexamers of connexins form hemi-channels on the cell surface that come in contact with adjacent hemi-channels from neighboring cells to form one complete communication channel. These channels act as a means for communicating ions and small chemical molecules (41). Apart from their role in acting as communication channels, it is recently becoming clear that their adhesion property also plays an important role in regulating several cellular functions (42). However, the adhesive properties and the adhesion kinetics of connexins remain as yet unexplored and provide a promising area for future research using SMFS.

3.6 Integrin mediated interactions

Integrins are heterodimeric transmembrane receptors that comprise one α and one β subunits (43, 44). Though their primary function is to anchor adherent cell types to the ECM, some integrins are also expressed on certain suspended cell types e.g. LFA-1 on leukocytes.

Experiments performed on CHO cells transfected with $\alpha_2\beta_1$ integrins show that the unstressed off rate (k^o_{off}) for initial interaction events ($t < 60$ sec) between $\alpha_2\beta_1$ and collagen I is about 1.3 s^{-1} (21). However, interaction events occurring after 60 seconds show significantly enhanced bond strengths. Together with existing data, these SMFS experiments strongly support that integrin interaction with ECM occurs in two steps. The first step predominantly comprises weak and kinetically unstable interactions mediated by single integrin molecules. In the second step, clustering of integrins occurs that causes a significant strengthening of the interaction. Furthermore, these interactions show a relatively low compliance length of 0.23 nm. Such a low compliance length can render the interaction highly resistant to externally applied forces. This is highly significant considering the fact that externally applied forces can in turn lead to further recruitment of integrins and maturation of the cell-ECM contact (45).

3.7 Cell adhesion molecules involved in inflammation

Selectins are a group of molecules expressed on the endothelial cells and leukocytes that play a central role in leukocyte adhesion, rolling and migration during the process of inflammation (46). P-selectin and E-selectin are expressed by endothelial cells during inflammation and interact with PSGL-1 (P-selectin glycoprotein ligand) present on the leukocytes leading to the capture of leukocytes at the site of inflammation. L-selectin, on the other hand, is expressed on leukocytes and helps in facilitating secondary recruitment of leukocytes. Integrins like LFA-1 (leukocyte function associated antigen) and VLA4 (very late antigen) interact with ICAM-1 expressed on endothelial cells (intercellular adhesion molecules) also play an important role in leukocyte rolling.

Single molecule force spectroscopy studies performed on P-selectin/PSGL interactions show a "catch bond to slip bond" transition behavior (47). Catch bonds are characterized by their ability to withstand large forces when subjected to high loading rates while they fail rapidly under low forces and low loading rates. This behavior is ideal for selectins because the P-selectin/PSGL "catch bonds" can help the endothelial cells in capturing a rapidly moving free leukocyte. Once leukocytes adhere to endothelial cells, the loading force on the P-selectin/PSGL interaction decreases and the bond fails. On the other hand, ICAM-1/LFA-1 interactions are also stable under

slow loading. Hence, while P-selectin/PSGL acts as a 'transient connector', ICAM-1/LFA-1 interaction acts a 'persistent connector' (48).

4 Cell adhesion proteins and diseases

As mentioned earlier, one of the primary reasons for studying cell adhesion proteins and their interactions is due to the role that these proteins play in several disease processes. In this section we briefly elaborate on the association of some of the cell adhesion molecules with different disease processes.

4.1 Tight Junctions

Qualitative and quantitative changes in tight junction proteins are associated with diseases involving almost all organ systems of the body (Table 2, (49)). For example, mutations of claudins are associated with several diseases like familial hypomagnesaemia and hypercalciuria syndrome and hereditary deafness. While JAM-A has been found to act as a receptor for reoviruses, claudins have been recently found to act as co-receptors for HCV virus. Altered expression of tight junction proteins is also associated with several carcinomas making them potential candidates for new tumor markers.

4.2 Adherens Junctions

Altered expression and mutations of E-cadherin and nectin are also associated with several diseases (Table 3, (50)). While mutations in nectins have been shown to be associated with infertility, cleft lip and cleft palate; altered expression of e-cadherins has been shown to be associated with several carcinomas. Nectins have also been shown to act as a receptor for herpes group of viruses.

4.3 Desmosomes

Mutations in desmosomes are typically associated with several diseases of the skin and in some cases heart, emphasizing their importance in maintaining the integrity of cell-cell adhesion in tissues subjected to extensive mechanical strain (Table 4, (51)).

4.4 Gap junctions

Mutations in connexins most often manifest as skin disorders and/or deafness (Table 5, (52)). Peripheral neuropathy has also been observed in mutations involving connexin 32. Currently, over a hundred different types of mutations in connexin 32 have been identified and associated with the Charcot-Marie-Tooth syndrome.

5 Conclusions

Single molecule force spectroscopy of protein interactions is a useful tool to understand their physiological functions. It not only provides information about the interaction forces but also helps us in better understanding the adhesion kinetics. One of the biggest advantages of SMFS, compared to other biophysical methods like surface plasmon resonance, is that it allows us to investigate interactions of proteins which are present on the cell. This not only allows us to probe proteins in their natural conformation, but in case of several cell adhesion proteins (e.g. e-cadherins, integrins), also ensures that differences in binding activity due to association with cytoskeleton are accounted for.

Though several cell adhesion molecules (e.g. e-cadherins, nectins, claudins) have been studied extensively using SMFS experiments, there is still a large gap in our understanding of adhesion kinetics of several other cell adhesion molecules e.g. connexins. There is also a significant lack of studies involving comparative adhesion kinetics between normal cell adhesion molecule and between mutated cell adhesion molecules or between viral proteins and the corresponding receptor cell adhesion molecules. Such studies in future could provide a deeper understanding of functions of different cell adhesion molecules.

Despite several advantages, SMFS also has its share of drawbacks. While some errors and disadvantages arise from the experimental set up (e.g. inability to detect forces below the thermal noise of the cantilever), some limitations arise from the Bell-Evans model (e.g. it assumes a kinetic dissociation in one dimension only). Furthermore, systematic errors can also arise from the manner

Table 2: List of diseases in various organ systems involving qualitative and/or quantitative changes in tight junction proteins (49).

Organ system	**Associated diseases**
Central Nervous System	Multiple sclerosis, Alzheimer's disease, HIV encephalitis and dementia, auto immune encephalitis, astrocytomas and glioblastoma multiforme, hyperthermia , Duchene's muscular dystrophy, hypoxia.
Gastro-Intestinal System	Diarrhea induced by various bacterial toxins, inflammatory bowel diseases, colitis, Celiac disease, gastro-esophageal reflux disease (GERD), Menetrier's disease.
Hepato-biliary-pancreatic system	Cholestasis associated with common bile duct ligation, primary biliary cirrhosis and primary sclerosing cholangitis, cholelithiasis, acute pancreatitis
Respiratory system	Asthma, shock lungs, interstitial lung disease, ventilator induced lung injury.
Renal System	Familial hypomagnesaemia and hypercalciuria, pseudohypoaldosteronism.
Carcinomas	Hepatocellular carcinoma, endometrial carcinoma, GI tract carcinomas, pancreatic carcinoma, oral carcinomas, breast carcinoma
Other diseases	Autosomal recessive hearing loss, diabetic retinopathy, uveitis.

Table 3: List of diseases associated with altered expression and/or mutations in adherens junction proteins (50).

Type of protein	**Associated diseases**
Nectin-1	Receptor for herpes virus entry, mutation in Zlotogora-Ogur syndrome, microphthalmia in knockout mice.
Nectin-2	Receptor for herpes virus entry, male specific infertility in knockout mice.
Nectin-3	Receptor for herpes virus entry, male specific infertility and microphthalmia in knockout mice.
Nectin-4	Over expressed in breast carcinoma.
E-cadherin	Endometrial, gastric and breast carcinomas.

Table 4: List of diseases arising from altered/impaired function of desmosomal proteins (51).

Desmosome component	**Associated diseases**
Desmogelin-1	Pemphigus foliaceus, pemphigus vulgaris, Staphylococcal scalding skin syndrome, bullous impetigo, striate palmoplantar keratoderma
Desmogelin-3	Pemphigus vulgaris
Desmogelin-4	Inherited hypotrichosis
Plakophilin-1	Autosomal recessive ectodermal dysplasia and skin fragility syndrome
Plakophilin-2	Arrhythmogenic right-ventricular cardiomyopathy (ARVC)
Plakoglobin	Autosomal recessive Naxos disease
Desmoplakin	Striate palmoplantar keratoderma, acantholytic epidermolysis bullosa, Carvajal syndrome.

Table 5: List of diseases associated with mutations in different connexins that form gap junctions (52).

Gap junction protein type	Associated diseases
Connexin 26	Keratitis-ichthyosis-deafness and hystrix-like ichthyosis-deafness, Vohwinkel's syndrome, palmoplantar keratoderma
Connexin 30	Clouston's syndrome
Connexin 31	Erythrokeratodermia variabilis
Connexin 32	X-linked Charcot-Marie-Tooth syndrome
Connexin 43	Oculo-dento-digital dysplasia (ODDD)

in which data is analyzed. Advances in enhancing the sensitivity of the system (53) as well as in compensating for errors arising from data analysis (54) will hopefully provide us a better understanding of protein interactions at the single molecular level.

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