Measuring the cell-cell adhesion force exerted by a cell adhesion molecule

Kate Bowman & Jane Saffell, **Division of Cell & Molecular Biology, Imperial College London**

Background

Cell-cell adhesion is of fundamental importance to multicellular organisms, controlling processes such as mammalian development, infection, wound healing and tumour cell metastatis. Differential cell-cell adhesion sorts cell types into tissues from the earliest stages of development, and specialised tight junctions, gap junctions and immune synapses between cells depend on cell-cell adhesion. Switching from strong cell-cell adhesion to weaker association allows cells to break free from their neighbours and migrate away, which is important during development but undesirable in cancer. In the fight against infection, the strength of adhesion between an immune cell and a potentially infected target cell will determine whether that cell is destroyed or not.

To investigate the function of different cell-cell adhesion molecules (CAMs) in tissues, or the significance of alterations in the type of CAM expressed on the surface of cells, it is useful to be able to determine the strength of cell-cell adhesion they mediate. Techniques such as surface plasmon resonance and ELISA can determine affinities of protein interactions, and interaction forces between immobilised CAMs can be investigated at the single-molecule level using atomic force microscopy (AFM). A limitation of these approaches it that they do not take into account differences the cellular environment could make to the strength of cell-cell adhesion, such as oligomerisation at the cell surface or interaction with the cytoskeleton, both of which greatly influence the strength of cell-cell adhesion. However, the development of an AFM derivative technology, single cell force spectroscopy, has made possible the measurement of adhesion forces between cells.

Here, we used the JPK NanoWizard II CellHesion® 200 single cell force spectroscopy module to investigate the homophilic cell-cell adhesion force mediated by neural cell adhesion molecule (NCAM) after different cell-cell contact times. To do this, we determined the force required to separate two NIH3T3 cells engineered to express human neural cell adhesion molecule (NCAM) on the cell surface, compared to the adhesion force of control non-engineered cells. Rounded cells in suspension were attached to a fibronectin-coated cantilever and brought into apposition with cells that had been cultured overnight on tissue culture plastic as an 'island' of substratum cells in the centre of the dish. After variable contact times, the cantilever with attached cell was raised, and the force required to detach the cells from one another was measured. The long pulling range of the CellHesion 200 (100 µm piezo movement) makes complete detachment of the cells possible over extended cell-cell contact times.

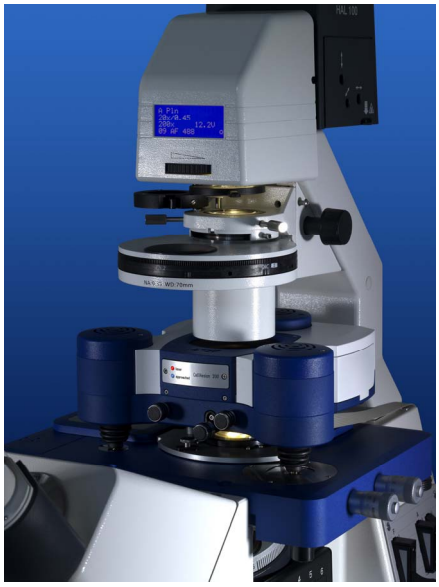


Fig. 1: CellHesion 200 head and Life Science stage mounted on Zeiss Axiovert 200 with light transmission condenser for Phase contrast and DIC illumination.

Methods

Preparation of dishes containing 'substratum' cell islands

Substratum cells were established by plating 200 cells in a 100 µl droplet of DMEM/10%FCS in the centre of a 35 mm tissue culture plastic dish, and culturing them overnight at 37°C in a humid atmosphere. 5 min before the experiment

the following day, 2 ml serum-free DMEM-Hepes (25 mM) was added to the dish. This resulted in a discrete, low density 'island' of the required cell type in the centre of the dish, the rest of which remained as underivatised tissue culture plastic. The 2 ml volume is sufficient to ensure that the cantilever remains below the fluid surface for all cell manipulations and force measurements. The JPK PetriDishHeater™ on the stage ensured that medium was maintained at 37°C for all force-distance measurements.

Functionalisation of the cantilever

Cantilevers were functionalised by coating with fibronectin, to which fibroblasts readily adhere. First, since we re-used cantilevers, these were first cleaned with Piranha solution (3:1 sulphuric acid: 30% hydrogen peroxide) and rinsed in dH_2O. Next, they were coated with poly-L-lysine (16.6 µg/ml in water, RT) and then fibronectin (20 µg/ml in DMEM, 2h, 37°C). Cantilevers were left in fibronectin solution until used.

Cell attachment to functionalised cantilever

Rounded cells to be captured by a functionalised cantilever were introduced into the same plate as 'substratum' cells before each force measurement experiment. To achieve this, confluent cells in a separate dish were scraped into serum-free DMEM-Hepes (no trypsin), and triturated using a 19G needle into a single-cell suspension of rounded cells. A small volume was then injected into the dish containing the substratum cells, at the edge, as far as possible from the cellular island. Cells were allowed to settle for no more than 30 s before a functionalised cantilever was lowered over a cell in cell-contact-mode until contact was made, raised 100 µm after a few seconds, and the attached cell allowed to rest for 5 min before being brought into apposition with an adherent 'substratum' cell.

Calibration

Tipless arrow TL1 (Nanoworld) or CSC12 (MikroMash) cantilevers were used, with a nominal spring constant of 0.03 N/m. To calibrate them under the temperature and fluid conditions to be used in experiments, they were mounted onto the CellHesion 200 unit over dishes containing 2 ml of serum-free DMEM-Hepes at 37C, set up as for an experiment, and volt-distance curves for sensitivity calculation were run over areas of the dish where there were no cells. The actual spring constant was assigned by the JPK integrated thermal noise method, using the second resonance peak, which converted the cantilever deflection units into Newtons (N) using a correction factor of 0.251.

CellHesion 200 microscope setup

The microscope on which the CellHesion 200 was installed was a Zeiss 200M (see Fig. 1) with AxioCam camera driven by Axiovision software, and images were captured using a x20 magnification lens. The AFM was driven by CellHesion 200 software (JPK).

Cell adhesion measurements

The cell attached to a functionalised cantilever was moved above the substratum cell island and positioned such that contact with a substratum cell would be above the nucleus (see Fig. 2). To ensure contact was maintained between the cells during force-distance measurements, an applied set-point force of 0.5 nN was used, in constant-height and closed-loop mode, and the approach-retract velocity was also constant at 5 µm/s. The cell-cell contact time before retraction was varied: 5, 60, 120, 300, 600 s. Substrate cells were probed five times at each contact time, with 20 s pauses between force-distance curves and 120 s rests between contact time changes. A cantilever cell was used for up to 40 force-distance curves.

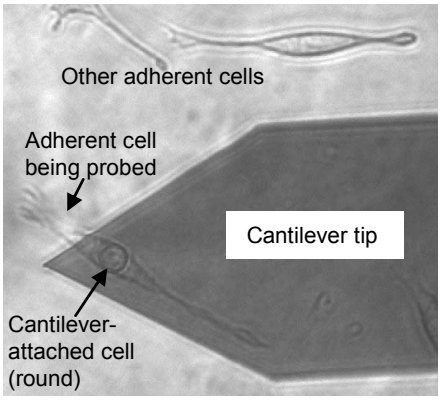


Fig. 2: Phase image of cantilever-captured cell apposed to an adherent cell. A rounded cell is captured by the cantilever and lowered up to 100 µm onto the region above the nucleus of an adherent cell. After variable contact times (5 – 600 s), the cantilever will be retracted 100 µm to separate the cantilever cell from the adherent (substrate) cell.

Results

Control or NCAM-expressing cells were each in turn attached to a functionalised cantilever and lowered 100 µm into contact with 'substratum' control or NCAM-expressing cells for variable contact times, before being returned to the starting position. Homophilic binding of CAMs at sites of cell-cell contact results in their accumulation (patching), resulting from further CAM molecules diffusing laterally into the contact site. To determine NCAM adhesion strength without and with the opportunity for patching and cytoskeleton reorganisation, we used contact times ranging from very short (5 s) to long (600 s). Cantilever deflection during approach and retraction was recorded and force-distance curves constructed by JPK image processing software.

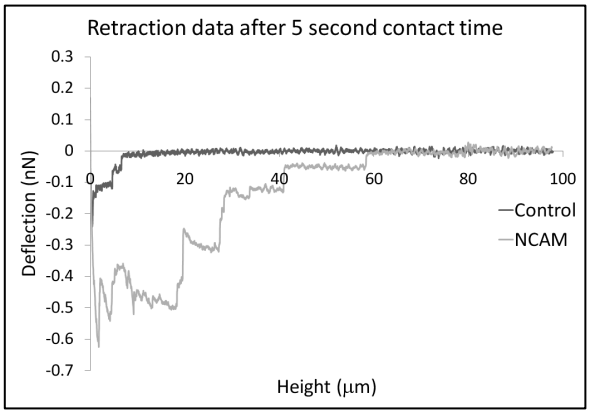


Fig. 3: Sample force-distance curves for control: control and NCAM: NCAM cell binding after 5s contact. Approach curves are omitted for ease of comparison. The vertical portions of the curve between plateaus indicate individual unbinding events where detachment of receptors or groups of receptors involved in cell-cell binding occurs. The peak deflection (~0.6 nN for NCAM here) provides a measure of the unbinding force required to fully detach the cells from one another. The area under the curve represents the work of detachment (J). Here the cell-cell separation at which the cells detached was 60µm.

Sample retraction curves for control: control cells and NCAM: NCAM cells after 5s contact time are shown in Fig. 3. The degree of cantilever deflection (nN) measures the adhesion force between the interacting cells, and the area under the curve indicates the work of detachment (J). The adhesion force and work of detachment are clearly greater for NCAM: NCAM interactions than for control: control.

Fig. 4A shows the mean adhesion force and mean work of detachment for control: control and NCAM: NCAM for 5s contact times, averaged from 30 force-distance curves. At this short contact time, NCAM: NCAM adhesion force was two-fold, and work of detachment was six-fold, greater than that for control: control interactions. However, at greater contact times (60, 120, 300 and 600s), NCAM-mediated adhesion force increased considerably, while adhesion force of control cells remained fairly constant (Fig. 4B).

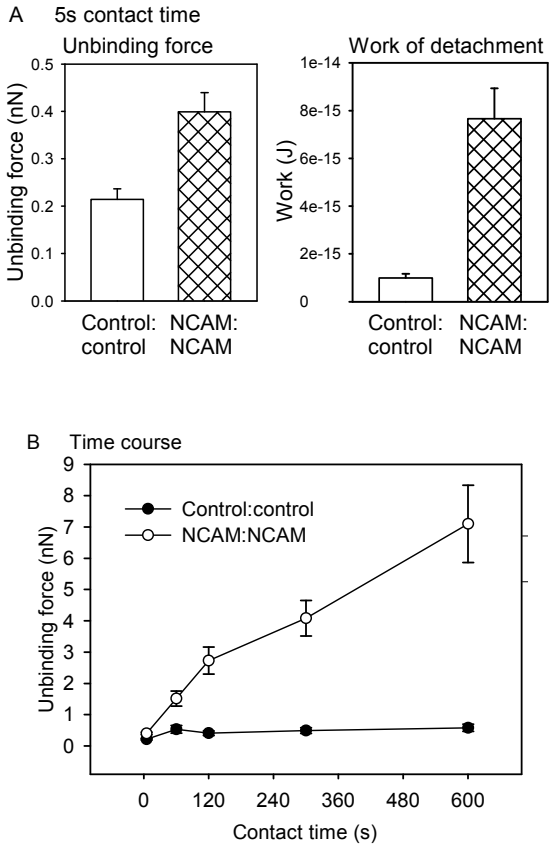


Fig. 4: Comparative total unbinding force (adhesion force) and work of detachment for interactions between Control: control, and NCAM: NCAM cells. Contact times were 5, 60, 120, 300 and 600s as indicated. Each data point is the mean of 30 force-distance curves; at least three different cantilever cells were used to probe at least six different 'substrate' cells.

To investigate the specificity of the NCAM-mediated adhesion force, we repeated the measurements (at 60s contact time) in the presence of (a) a peptide previously shown to inhibit NCAM homophilic adhesion, or (b) a scrambled version of the same peptide, to control for non-specific peptide effects. The inhibitory properties of this peptide were originally demonstrated using chicken NCAM and the chicken peptide sequence (KYSFNYDGSE). Here our cells express human NCAM, so we have synthesised a peptide corresponding to the human sequence in this region, KYIFSDDSSQ, and the scrambled version SDYIDFSSKQ. In the presence of the NCAM adhesion-blocking peptide (but not the scrambled control peptide), the adhesion force for NCAM-expressing cells was inhibited to that of control cells (Fig. 5).

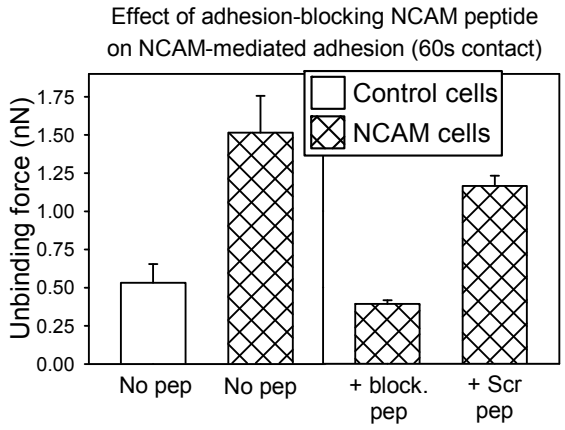
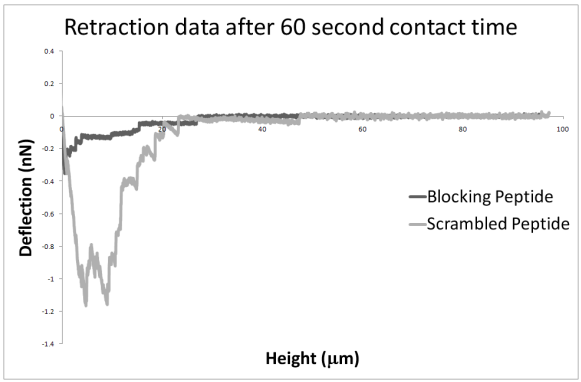


Fig. 5: Comparative unbinding force between NCAM: NCAM cells treated with a blocking peptide and a scrambled control. First, force-distance measurements were made in the absence of peptide, to ensure NCAM cells showed unbinding forces comparable to those previously recorded (i.e. mean from Fig. 3B). Next, 1 mM peptide in serum-free DMEM-Hepes was added to the cells, and was allowed to equilibrate for 20 mins before further measurements were taken. A contact time of 60 s was chosen. Each data point is from a single experiment and is the mean of 20 force-distance curves.

Sample force-distance curves recorded for 60s NCAM: NCAM interactions in the presence of either the blocking or scrambled peptide are shown in Fig. 6. These results confirms that the increased adhesion force measured for NCAM-expressing cells relative to control cells is indeed mediated by NCAM, as it is specifically abolished by a peptide known to block NCAM homophilic binding.

Fig. 6:



Sample retraction curves for NCAM: NCAM cell interaction following 60s contact time, in the presence of either the peptide blocker of NCAM binding or a scrambled control peptide (curves correspond to data shown in Fig. 5).

Conclusion

The CellHesion 200 AFM has allowed us to determine the adhesion force of NCAM: NCAM homophilic binding between cells, and to determine how the force changes with the length of time the cells are in apposition. Importantly, this technology has enabled us to study the adhesion force of NCAM within the cellular context, the normal physiological milieu in which the homophilic interaction takes place. Greatly increased NCAM adhesion force was evident at the greater contact times that allow for lateral diffusion of CAMs and cytoskeletal reorganisation.

Authors' address:

Kate A Bowman & Jane L Saffell
Division of Cell & Molecular Biology
Faculty of Natural Sciences
Imperial College London, South Kensington Campus, London SW7 2AZ.
Email: j.saffell@imperial.ac.uk