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CELL FITTING TO ADHESIVE SURFACES: A PREREQUISITE TO FIRM ATTACHMENT AND SUBSEQUENT EVENTS

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Abstract

Cell adhesion usually involves extensive shape reorganization. This process is important because i) it is required for efficient cross-linking of interacting surfaces by adhesion receptors the length of which does not exceed several tens of nanometers and ii) it influences subsequent cell differentiation and activation. This review focuses on the initial phase of cell deformation, preceding the extensive reorganization process known as spreading. This first phase includes local flattening at the micrometer scale and membrane alignment at the nanometer level, resulting in fitting of the cell to an adhesive surface. Three main points are considered. First, experimental methods available to study cell apposition to a surface are described, with an emphasis on interference reflection microscopy. Second, selected experimental evidence is presented to show that there is a quantitative relationship between "adhesiveness" and "contact extension", and some theoretical models aimed at relating these parameters are briefly sketched. Third, experimental data on the kinetics of initial contact extension are described and possible mechanisms for driving this extension are discussed, including nonspecific forces, receptor-mediated interactions, active cell movements or passive membrane fluctuations. It is concluded that both passive physical phenomena and random active cell movements are possible candidates for the initial triggering of contact extension.

Key Words : Alignment, cell mechanics, cytoskeleton, interference-reflection microscopy, spreading.

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Introduction

Importance of the formation of an extensive contact area between cells and adhesive surfaces

Cell adhesion to other cells or surfaces is a key regulator of prominent biological processes (Pierres *et al*., 2000), including survival, proliferation, differentiation, activation or migration. Also, the adhesive interactions between cells and biomaterials may determine the fate of implanted prostheses by regulating inflammatory reactions and strength of connection to surrounding tissues. It is now widely recognized that the control of biological adhesion is dominated by specific interactions beween cell surface receptors and their ligands, although cell attachment may also be influenced by nonspecific forces (Bongrand, 1998).

Cell survival and proliferation. It has long been demonstrated that many cells need to be bound to surfaces in order to survive and proliferate, a requirement that is well known as *anchorage dependence*. The simplest interpretation of this phenomenon might be that cell behaviour is controlled by biochemical signals generated by adhesion receptors, particularly integrins, when they meet their ligands. However, several reports clearly demonstrated that this is not the whole story. Thus, Re *et al*. (1994) found that the survival of endothelial cells required some spreading on surfaces coated with extracellular matrix components, and this signal could not be replaced by allowing round cells to bind microbeads coated with integrin ligands. Zhu and Assoian (1995) reported that NIH3T3 cells needed to spread on a surface in order to survive and proliferate, and adhesion was not sufficient for survival. Also, Chen *et al*. (1997) studied the fate of endothelial cells deposited on engineered surfaces consisting of adhesive islands surrounded by anti-adhesive areas. When these islands were separated by a distance sufficient that a given cell be confined within a single adhesive area, cell survival was positively correlated to island area. However, when multiple islands of low area were located in close proximity, a given cell might acquire a high projected area by interacting with many adhesive regions, and both proliferation rate and survival were correlated to the projected area rather than to the actual adhesion area.

The cell cytoskeleton might link cell shape to survival and progression through the cell cycle: thus, cytochalasin D prevented the proliferation of adherent fibroblasts exposed to suitable mitogens (Bohmer *et al*., 1996).

Differentiation and activation. Several authors reported that gene expression was at least partly regulated by cell shape. Thus, Hohn and Denker (1994) studied the production of chorionic gonadotrophic hormone by choriocarcinoma cells that were deposited on surfaces of similar composition but different mechanical properties. Hormone

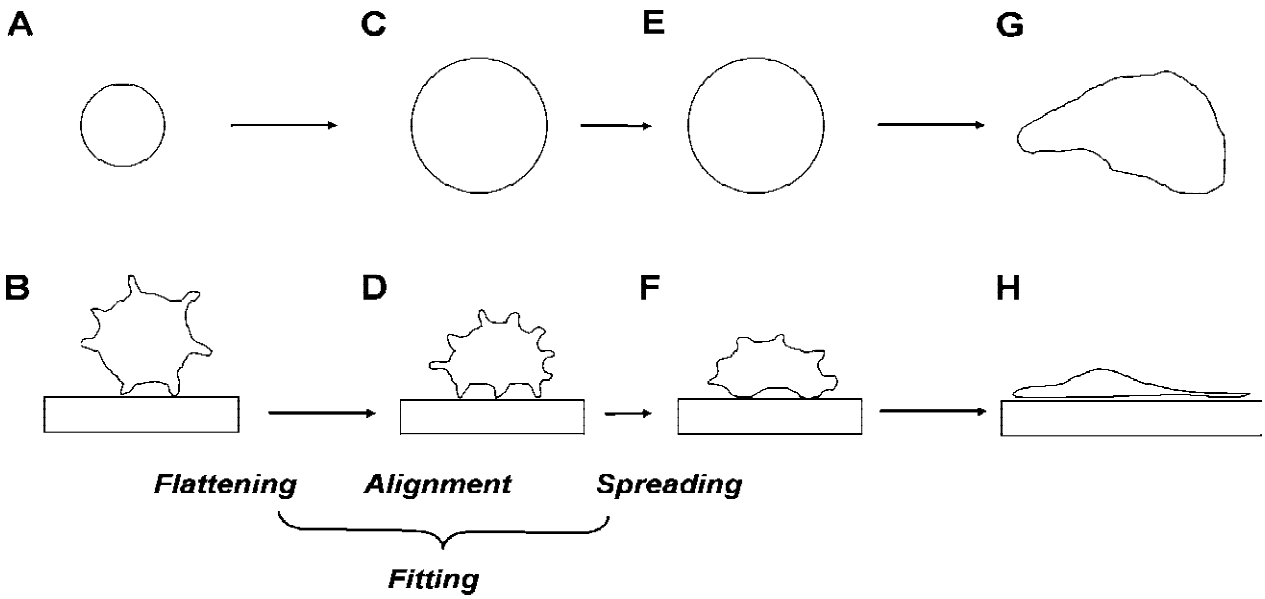

Figure 1: Sequential phases of contact extension. The sequential phases of contact formation between a cell and a planar adhesive surfaces are depicted. Figures 1 A, C, E and F represent conventional microscopic images drawn with low resolution (in the order of 1 μm). Figures 1 B, D, F and H represent vertical planes and submicrometer details are shown. In most cases, contact first involves a limited area corresponding to the tip of microvilli (B) and cell remains rounded (A). Contact extension then requires cell flattening at the micrometer level (D), with a slight increase of apparent diameter (C). Contact may thus extend through alignment of the cell membrane with the surface at the nanometer scale (F) without any change of cell aspect when observed with optical microscopy (E). Both flattening and alignment are necessary for membrane fitting to the surface. Finally, cell may display extensive morphological changes, with polarization and sending of lamellipodia, thus resulting in height increase of the contact area.

production was higher on soft gels than on more rigid substrates, and cells were more rounded on the former surfaces. Similarly, Roskelley *et al.* (1994) reported that casein expression by mammary gland cells required both integrin receptor occupancy and cell rounding. Finally, Geginat *et al.* (1999) found that interleukin-2 expression by T lymphocytes activated through T cell receptors required prolonged spreading.

Geometrical parameters were also shown to affect the activation of some cell functions. Mescher (1992) studied the interaction of cytotoxic T lymphocytes and latex particles of various diameter (ranging between 1 and 5 μm) coated with ligands recognized by T cell receptors. An inverse relationship was found between particle diameter and capacity to activate lymphocytes, and it was not possible to compensate insufficient particle size by increasing the number of particles interacting with a given cells, thus suggesting that a large contact area was essential to the stimulation process. Similarly, the exocytotic capacity of RBL cells was increased when these cells were allowed to spread on large fibronectin-coated surfaces. However, when fibronectin was presented as adsorbed on microbeads, no spreading nor enhanced exocytosis were reported (Apgar, 1997). Cell cytoskeletal elements were presumably involved in these regulatory events. Indeed, microtubule depolymerisation was found to activate the transcription factor NFkB in HeLa cells (Rosette and Karin, 1995), and Swiss 3T3 cells displayed increased activation of Rho GTPase when microfilaments or microtubules were depolymerized with pharmacological agents (Ren *et al.*, 1999).

Interestingly, even nanometer-scale features may strongly influence cell behaviour (Curtis and Wilkinson, 1997). Thus, when fibroblasts were cultured on surfaces studded with nanoscale islands of 13-nm height, they displayed marked morphological changes as well as different gene expression patterns, as demonstrated with microarray technology (Dalby *et al.*, 2002)

Defining the different steps of contact formation and extension between cells and adhesive surfaces

As summarized in Figure 1, a typical binding event between a cell and a surface will include the following steps:

Formation of the first few bonds (or even a single one). Since suspended cells are often fairly rounded and their surface is studded with microvilli (Fig. 1 A,B), the first interaction will usually be restricted to a surface the area of which may be as small as 0.01 μm^2, corresponding to the tip of microvilli (Grinnell *et al.*, 1976; Ben Shaul and Moscona, 1975; Choi and Siu, 1987)

Attachment strengthening must then occur rapidly. Indeed, the noncovalent bonds mediating adhesion are disrupted by forces as low as a few tens of piconewtons and their natural lifetime is often shorter than a few seconds, as shown on selectins (Alon *et al.*, 1995), members of the immuglobulin superfamily (Pierres *et al.*, 1996), cadherins (Perret *et al.*, 2002), or even integrins before they are fully activated (Masson-Gadais *et al.*, 1999). In contrast cell adhesion may last hours or days, and forces within the

nanonewton range were often needed to separate bound cells from other cells (Bongrand *et al*., 1979; Bongrand and Golstein, 1983) or surfaces (Palecek *et al*., 1997). The formation of additional bonds will require an extension of the contact area, which may in principle involve two complementary processes: i) cell flattening at the micrometer level, resembling the flattening of a liquid droplet on a wettable surface, a process that is called spreading by physical-chemists, but that is quite different from cell spreading as defined by cell biologists (Fig. 2 C, D). This process will generate additional contact zones. And ii) enlargement of initial contact areas at the submicrometer level (Fig. 2 E, F) through alignment (Dustin *et al*., 1997) of the cell membrane to neighbouring surface as required to allow the formation of molecular bonds by adhesion receptors whose typical length ranges between 10 and 40 nm (Springer, 1990). Attachment strengthening will be completed by a concentration of adhesion molecules in contact areas (Kupfer and Singer, 1989; André *et al*., 1990), and a reinforcement of cytoskeletal elements below the plasma membrane, thus increasing membrane rigidity which may be an important determinant of binding strength (Rees *et al*., 1977).

Extensive cell topological reorganization may occur as a facultative consequence of adhesion, during the tens of minutes, hours or even days following contact. This phenomenon is defined as spreading by cell biologists, and includes the sending of lamellipodia and acquisition of polarized shape by cells (Fig 2 G, H).

In the present review, "spreading" will refer to active cell spreading, whereas "flattening" will refer to aforementioned micrometer-scale deformation, and membrane "alignment" will be used to designate subnanometer-scale apposition of cell membranes to adhesive surfaces. Both "flattening" and "alignment" are part of the "fitting" process (Fig. 2).

Many experiments have demonstrated the feasibility of discriminating between adhesion and spreading, by manipulating cell metabolism or adhesive surfaces: Thus, galactosyl groups were required for spreading, not adhesion of melanoma cells deposited on laminin-coated surfaces (Runyan *et al*., 1988). Chelating intracellular calcium inhibited spreading, not adhesion of human monocytes (Lefkowitz *et al*., 1992), while an artificial rise of intracellular calcium triggered the spreading of adherent neutrophils (Pettit and Hallett, 1998). The spreading of Hela cells adhering to collagen-coated surfaces was prevented by inhibiting a rise of intracellular pH that was involved in phospholipase activation (Chun, 1995). Also, adhesion and spreading display different kinetics, since cell adhesion may happen within a fraction of a second (Lawrence and Springer, 1991) while spreading often requires tens of minutes (Grinnell *et al*., 1976), hours or days.

It is therefore warranted to discriminate between fitting (a prerequisite for strong adhesion) and spreading, and there is no substantial reason for hypothesizing that similar mechanisms are involved in both phenomena.

Aim and scope of the review

The aim of the present review is to present current knowledge concerning the regulation of cell fitting to adhesive surfaces. First, we shall briefly discuss experimental approaches that are currently available to quantify cell-surface contacts. Second, we shall review our present understanding of the parameters responsible for the equilibrium topography of these contacts. Third, we shall discuss the kinetic mechanisms of contact extension. Indeed, although the discrimination between static and dynamic aspects is not fully warranted on theoretical grounds, this discrimination was felt to clarify data presentation.

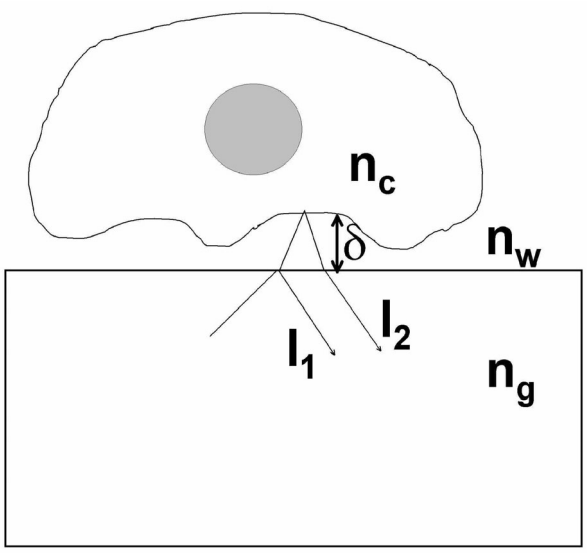

Figure 2: Simple interpretation of interference reflection contrast. Contrast results from the interference between I1 and I2.

Methods for Studying Adhesion-Related Cell Deformations

Conventional optical microscopy

Cell morphological changes involved in spreading are so clearcut that the detection of spread cells was found obvious by many authors: thus, spreading was defined as a loss of rounded aspect (Runyan *et al*., 1988; Yoshimura *et al*., 1995; Yu *et al*., 1998). In many cases, spreading was defined more quantitatively by measuring the projected area of adherent cells (Li *et al*., 1996; Cox *et al*., 1999; Watson *et al*., 2001): spreading resulted in 3-4 fold increase of this area. Additional properties reported to define spread cells are particular patterns of microfilaments revealed by labeling with fluorescent phalloidin derivatives (Masiero *et al*., 1999) or dull aspect on observation with phase contrast microscopy (Yu *et al*., 1998).

Electron microscopy

Electron microscopy has long been used to observe the region of interaction between biological surfaces. This allowed the delineation of regions of apparent contact where plasma membranes were separated by an electron-light gap of constant width, on the order of 20 nanometers (Easty and Mercer, 1962; Heaysman and Pegrum, 1973). This was indeed the basis of the definition by Bennett (1963) of the so-called glycocalyx, i.e. a polysaccharide-rich structure coating the lipoprotein core of plasma membranes and that was supposed to impede molecular contact between ap-

proaching lipid bilayers of plasma membranes.

There are two problems with this technique: first, sample processing is rather long and tedious, and it precludes real time observation of cell surface reorganization. Second, this processing may result in significant variation of cell volume [see King (1991) for a quantitative study], which might hamper the significance of quantitative measurements. However, there are two arguments supporting the hypothesis that intermembrane distances are not dramatically altered by sample processing: i) a quantitative agreement was reported between estimates of membrane-substrate separation obtained by electron microscopy and interference reflectionn microscopy (Heath, 1982). ii) The range of experimental estimates of intermembrane distance, usually comprised between 20 nm and 40 nm, is compatible with the known length of adhesion receptors. Note that the significance of this agreement is somewhat weakened by two factors of uncertainty: it is not known whether adhesion receptors are perpendicular to the plasma membrane during cell adhesion. Also, the angle between the section plane and the membrane is not known. This may result in substantial alteration of distance estimates: indeed, assuming random orientation of the sample relatively to the section plane, the apparent membrane distance <dA> was shown to be related to the actual distance d through the simple formula (Foa *et al*., 1988):

$$<dA> = 2\ d \qquad (1)$$

Scanning electron microscopy was also shown to yield valuable information on cell surface contacts. Thus, in association with immunogold labelling it was used to image the distribution of vinculin, a protein often associated to focal contacts, in fibroblasts deposited on artifical surfaces (Richards *et al*., 2001).

Evanescent waves

Total internal reflection fluorescence (TIRF) was first applied to cellular microscopy by Axelrod (1981). This method consists of illuminating the region of contact between cells and a surface with an evanescent wave obtained with a light beam obliquely incident on the surface-liquid interface at an angle greater than the critical angle of refraction. The light intensity thus decays exponentially with perpendicular distance to the surface with a characteristic distance on the order of the light wavelength. This approach was cleverly used by Gingell *et al*. (1985) to study the interaction of chick heart fibroblasts and glass coverslips. The authors used as extracellular medium solutions of fluoresceinated dextrans of various molecular weight (ranging between 4,000 and 157,000). They found that only limited regions of the cell-glass interaction zone excluded the lower molecular weight marker, whereas larger molecules were essentially excluded from the whole interface. Images were fairly similar to those obtained with interference reflection microscopy (IRM). More recently, Truskey *et al*. (1992) used TIRF to quantitate the distance between glass microscopic slides and bovine aortic endothelial cells whose membranes were labeled with a fluorescent dye.

However, this approach was less frequently used than IRM during the previous years, probably due to the need for custom-made apparatus as well as technical difficulties.

Fluorescence resonance energy transfer (FRET)

Fluorescence energy transfer (Stryer and Haugland, 1967) seems ideally suited to image short-distance interactions between suitably labeled structures. The principle is to use two fluorescent species: a donor and an acceptor whose excitation spectrum overlaps the emission spectrum of the donor. When the distance between an excited donor molecule and an acceptor is less than a few nanometers, the acceptor may be excited through a non-radiative process (this means that transfer is different from mere absorption by the acceptor of a photon emitted by the donor – indeed, transfer results in a decrease of the lifetime of the excited form of the donor). This phenomenon was used by Niles *et al*. (1996) to image the regions of contact between large lipid vesicles labeled with coumarin acting as donor, and planar phospholipid membranes labeled with rhodamine acting as acceptor. However, this approach has not been widely used to study cell-surface contacts.

Direct demonstration of receptor-ligand association

While aforementioned methods yielded much information on cell contact areas, they did not allow any monitoring of molecular interactions responsible for adhesion. This was achieved with a clever technique by Dustin *et al*. (1997): these authors deposited cells expressing CD2 adhesion molecules on glass-supported bilayers containing fluorescent extracellular domains of CD48 or CD58 molecules, acting as ligands for CD2. Since these molecules were able to diffuse in planar bilayers, cell contact resulted in rapid increase of fluorescence in contact regions, due to the trapping of their ligand by cell-associated CD2 molecules. The authors simultaneously performed interference microscopic studies, and they readily demonstrated a coincidence between black contact regions (as detected with IRM) and fluorescence concentration.

Interference reflection microscopy (IRM) or Reflection interference contrast microscopy (RICM)

This methodology was introduced in biological laboratories by Curtis (1964). As shown in Figure 2, the basic principle is fairly simple. Cells are observed under epillumination conditions, like in standard fluorescence microscopy (only, the same wavelength is selected for illumination and observation, by replacing the dichroic mirror with an half-reflecting mirror, and adding suitable bandpath filters). Since the refraction index of aqueous medium (n_w) is lower than the refraction index of the cell membrane n_c and that of the glass coverslip n_g, there is is a phase shift π between the light rays reflected by the glass/water and water/cell interfaces. Using the normal incidence approximation of standard interference theory, the light intensity reflected in a region where the distance between the cell surface and the membrane is δ is given by:

$$I = I_1 + I_2 + 2\sqrt{(I_1 I_2)}\cos(4\pi\delta/\lambda) \qquad (2)$$

where l is the light wavelength and I_1 and I_2 are respectively the intensities of light rays reflected by the glass/water and the water/cell interfaces. Since l is of order of 400 nm (which is close to the wavelength of green light in water), formula (2) predicts that the illumination will be minimum at contact and a first maximum will be obtained

for a separation distance of 100 nm. Thus, a semi-quantitative treatment of images should be sufficient to reveal potential interaction areas where the cell/surface distance is lower than about 40 nm, corresponding to twice the length of the extracellular domains of typical adhesion receptors such as integrin molecules.

However, equation (2) is only a crude approximation, and there are some problems with the quantitative processing of IRM images (see Vince and Gingell, 1980; Curtis, 1994; Verschueren, 1995): i) due to multiple spurious reflections, the contrast of IRM images is usually fairly poor. This situation may be improved by using suitable microscope lenses that are now commercially available (e.g., Zeiss™ Antiflex objective), and by minimal digital image processing consisting of histogram expansion. This may require that images be acquired with a CCD camera yielding a sufficient number of grey levels. ii) There is a special problem when a very thin lamellipodium is observed, due to the influence of the reflection by the upper side. iii) There is a possibility that reflection by intracellular organelles might yield substantial artefacts. iv) It must be kept in mind that there is a basic accuracy limitation in defining the cell boundary: indeed, the occurrence of membrane folds and presence of membrane-associated molecules may make it unwarranted to consider the cell/medium interface as defined with nanometer accuracy. Despite these limitations, IRM proved a valuable tool to study contacts between cells and surfaces. Indeed, the unsuitability of the normal incidence approximation may prove an advantage, since close contacts may be detected with simple thresholding, despite the intrinsic ambiguity of equation 1. This is exemplified on Fig. 3 where a typical fringe pattern is shown, revealing rapid variation of cell-surface distance (arrow). As shown on Fig. 3B, the illumination intensity in the contact area is much lower that in regions were the membrane to surface distance is a multiple of l/2. The contact region of this typical cell is shown on Fig. 3C. The validity of contact definition with IRM was recently checked in our laboratory (Pierres *et al*., submitted): monocytic THP-1 cells were deposited on a polylysine-coated surface in a flow chamber and examined with IRM. They were then subjected to a distractive force of order of 100 piconewtons with hydrodynamic flow and examined again : in a given microscope field, cells that did not display dark contacts on IRM images were readily removed by the flow. On the contrary, cells that displayed these contacts resisted the distractive forces in most cases; further, when they were removed, they left dark patches suggesting that torn membrane fragments remained bound to the substrate, thus supporting the hypothesis that IRM revealed bona fide adhesion areas. In a series of experiments performed with this model, it appeared suitable to define cell-surface contact on the basis of equation 2. This was rewritten as :

$$I = (I_M+I_m)/2 - [(I_M-I_m)/2] \cos (4\pi\delta/\lambda) \qquad (3)$$

where I_m and I_M are respectively the maximum and minimum intensity level in the image. The basic assumption is that parameter δ spans all values ranging between about 0 and l/4. In an experimental study made of the alignment of THP-1 cells to polylysine-coated surfaces (Pierres *et al*.,

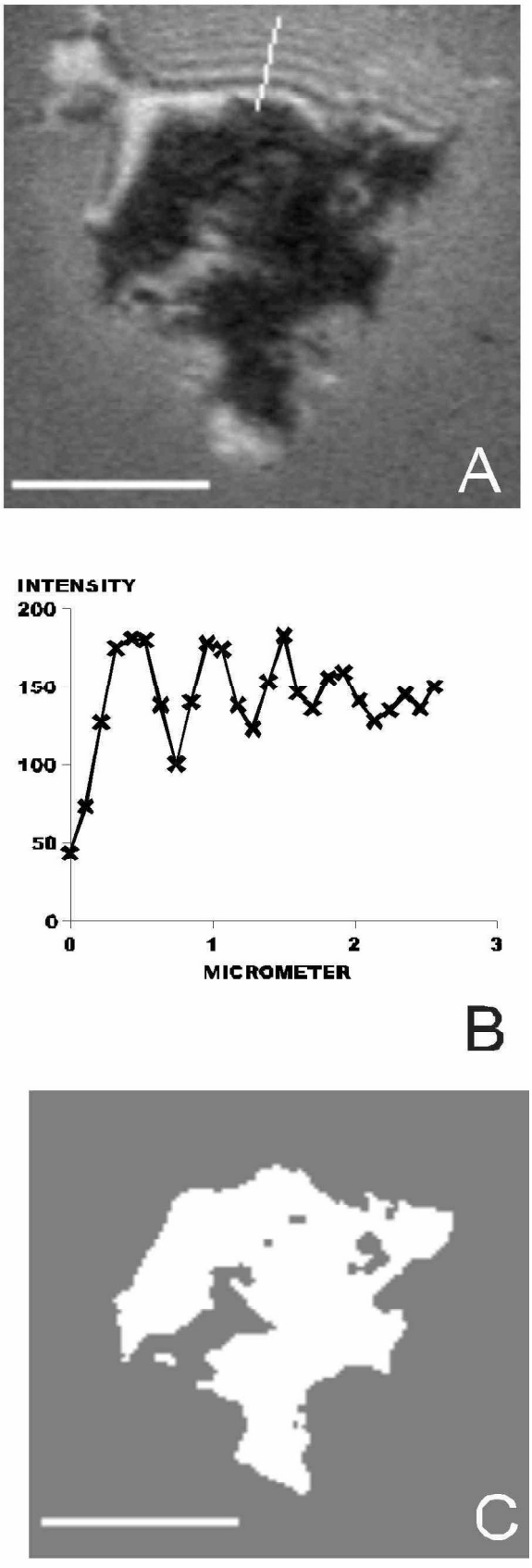

Figure 3: Derivation of contact boundaries from irm images. A typical IRM image of a THP-1 cell deposited on a polylysine-coated surface is displayed in Figure 3A (bar is 5 µm, contact time is about 2 minutes). The intensity profile along a line segment perpendicular to the cell edge (white line) is shown on B. Contact was determined by simple thresholding (C).

submitted), the optimal threshold for the definition of contact area was :

$$I \leq 0.305\ I_M + 0.695\ I_m \qquad (4)$$

with a light wavelength in vacuum of 546 nm. The corresponding threshold distance was 38 nm. Note that the interesting aspect of equation 3 is that it remains valid when all intensities are subjected to a linear transformation (corresponding to background subtraction or histogram expansion). A point of caution is that there is a fairly large statistical error in the determination of I_m and I_M: this may be reduced by subjecting images to median filtering, which results in substantial smoothing of intensity values.

It was interesting to determine the importance of the choice of the threshold distance for contact definition. Thus, a series of 19 sequential images were acquired when monitoring the first 5 minutes of contact between THP-1 cells and a polylysine-coated surface. Equation 3 was then used to calculate the contact areas with a threshold of 28 nm, 38 nm and 48 nm. The first two series of values were fairly proportional, with a ratio of 0.30 ± 0.06 (Standard Deviation), and a correlation coefficient of 0.89 while the contact area displayed about 5 fold increase. When the series of contact areas derived with threshold distances of 38 and 48 nm were compared, the correlation coefficient remained close to 0.90, but values were not proportional. The following regression coefficients were found :

$$A_{48} = 1.58\ A_{38} + 81.7\ \mu m^2 \qquad (5)$$

Where A_{48} and A_{38} are the contact areas calculated with equation 3, using distance thresholds of 48 nm and 38 nm respectively. Based on Fig. 3B, the simplest interpretation of this finding is that higher thresholding resulted in the addition of the fairly constant contribution of a fraction of noncontact regions, due to the oscillatory behaviour of the intensity/distance plot. It is concluded that the choice of the intensity threshold strongly influences the absolute estimate of contact areas, but our procedure is fairly robust regarding comparisons of sequences of images obtained after subjecting cells to different treatments.

Observations performed on numerous cellular systems led to the classical definition of *focal contacts* appearing as localized dark regions (e.g. short strips less than a micrometer wide), corresponding to a cell-substrate separation of order of 15 nm, while *close contacts* appeared as greyish areas where the cell-to-surface gap was about 30 nm wide (Izzard and Lochner, 1976; Burridge *et al*., 1988).

While "conventional" IRM is now used by many biological laboratories, some authors reported different modifications of this techniques. Thus, Davies *et al*. (1993) used so-called "tandem confocal scanning microscopy" to image the areas of contact beween endothelial cells and surfaces. Cell-surface distance in contact areas was usually less than 50 nm. Iwanaga *et al*. (2001) used so-called "fluorescence interference contrast" (FLIC) microscopy to measure with high accuracy the distance between fibroblasts and fibronectin-coated silicium surfaces acting as mirrors. They also found that the major part of the membrane was separated by 50 nm from the substrate. Interestingly, they found no correlation between the distribution of vinculin, an actin binding protein usually concentrated in focal contacts, and the areas where the membrane to surface distance was minimum. Further work is needed to assess the significance of this finding.

Conclusion

Several complementary methods yielded much information on interactions between a variety of surfaces and cell populations. We shall now discuss available results and models for contact extension.

A Static View of Cell-Surface Apposition

It would be of obvious interest to elaborate a means of deriving the extent of alignment or spreading of a cell on a surface from basic parameters such as density of adhesion receptors and cell mechanical properties. Two opposite views might be considered. On the one hand, cell deformation might be fully controlled by some internal cell machinery that would be triggered by specific ligand-receptor interactions. In this case, the nature of cell receptors involved in adhesion might be more important than the number of these receptors to determine final shape. On the other hand, cell shape might result from a balance between adhesive interactions and cell resistance to deformation, in analogy to the spreading of a droplet on a surface. In this case, active (and fairly random) cell deformation would essentially determine the kinetics of deformation. We shall now present some representative experimental data and theoretical models.

Experimental data

Positive corrrelation between the extent of cell deformation along a surface and intensity of the adhesive stimulus. Many reports support the view that cell-surface contact area is often quantitatively related to the intensity of the adhesive stimulus. Thus, Folkman and Moscona (1978) reported a decrease of spreading of endothelial cells on a culture surface coated with increasing amounts of poly hydroxyethyl methacrylate (polyHEMA, an anti-adhesive polymer). Conversely, the projected area of cells deposited on fibronectin-coated surfaces increased in proportion to the amount of deposited fibronectin (Ingber, 1990). These observations were performed with conventional optical microscopy, but they are fully consistent with results reported by Capo *et al*. (1982) who studied with electron microscopy the interaction between thymocytes agglutinated with increasing amounts of the lectin concanavalin A: contact area was defined as the region where electron dense bilayers appeared less than 50 nm apart. This area increased from about 0.3 μm^2 to 6.5 μm^2 when the amount of cell-bound lectin increased from 50,000 to 106 molecules per cell. In an other study that was also performed with electron microscopy, Mège *et al*. (1987) reported that the contact area between phagocytic cells and altered erythrocytes was strongly increased when the negative charges borne by red cells was partially removed with neuraminidase, or neutralized by adding positively charged polylysine molecules. Interestingly, both the apparent contact area (i.e. the area that appeared involved in contact when observation was performed with fairly low magnifi-

cation, using conventional optical microscopy) and the percent of apparent contact where bilayers were less than 50 nm apart were increased following decrease of repulsive interactions.

Specific interellular metabolic events may strongly influence spreading. It is also well established that cell spreading may be altered by suitable manipulation of cell signalling machinery. Thus, spreading was triggered by blockade of some kinases (Yoshimura *et al*., 1995; Haller *et al*., 1998) or altering intracellular pH (Demaurex *et al*., 1996). Interestingly, in many cases, a positive correlation was suggested between the increase of spreading and decrease of so-called cortical tension as a result of cell metabolic alteration. Thus, spreading was stimulated by activating the small G protein Rac without any detectable change of the density or activity of adhesion receptors (D'Souza-Schorey *et al*., 1998) and Rac might inhibit cell tension by inactivating Rho (Zhong *et al*., 1997; Ory *et al*., 2000). Spreading was also induced by stimulating intracellular cytosolic release of caged calcium (Pettit and Hallett, 1998), which is expected to cause marked changes of cell mechanical properties (Richelme *et al*., 2000). Treating murine macrophages with colchicin or nocodazole, two microtubule inhibitors, inhibited spreading (Cheung and Terry, 1980), and microtubule blockade was found to increase the cell cortical tension in another model (murine fibroblasts, Pletjushkina et al., 1998). Also, treating murine fibroblasts with deoxycholate increased spreading and decreased membrane tension as measured by pulling with optical tweezers at microspheres bound to the cell surface (Raucher and Sheetz, 2000). Finally, we recently observed that exposing monocytic THP-1 cells to hypotonic medium for 30 minutes increased both cell deformability, as measured with micropipette aspiration, and rate of alignment to a polylysine-coated surface, as measured with interference-reflection microscopy (Pierres *et al*., submitted).

Theoretical models for cell apposition to adhesive surfaces

Simple mechanical treatment. The simplest model of cell apposition to an adhesive surface may consist of assuming that deformation is entirely determined by the balance between adhesive forces et cell resistance to deformation. This concept is well illustrated by a clever set of experiments performed by Evans and Leung (1984): First, using micropipette aspiration to assay the mechanical properties of erythrocytes (Fig. 4A), they found that cell exposure to the lectin wheat germ agglutinin (WGA) dramatically increased rigidity. Second, when pushing a soft untreated red cell in contact with a WGA-coated erythrocyte, then pulling out (Fig. 4B) they observed a deformation that could be measured in order to derive a work of separation between membranes : values as large as 10^{-3} J/m^2 were obtained for erythrocytes equilibrated with 0.4 µg/ml WGA. Also, they found that flaccid cells did not spontaneously deform on the spherical test surface, indicating that the work of adhesion (or "affinity") was at least one thousandfold lower than the work of separation. Shortly thereafter, Evans (1985a,b) presented a mechanical analysis of membrane-membrane adhesion and separation. Interestingly, he showed that in contrast with the case of continuum molecular cross-bridge, the more realistic model of discrete

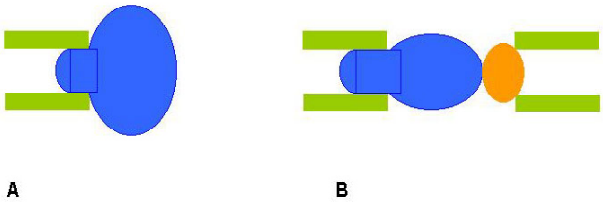

Figure 4: Determination of adhesive energy with micropipettes: In a first series of experiments, cells are sucked into a micropipette with controlled pressure and deformation is recorded in order to estimate the surface tension (A). The cell is then pushed into contact with a rigid adhesive particle (right) and pulled out after contact formation. The work of separation may be derived from the angle between particles and surface tension of the softer cell.

molecular bonds might account for a difference between the level of tension associated with adhesion and separation (i.e. hysteresis).

Accounting for cell surface roughness. There is a basic problem with the application of aforementioned concepts to nucleated cells: in contrast with erythrocytes, other cell types are studded with a variety of submicrometer-scale protrusions. There are two consequences: first, the molecular contact area cannot be measured on conventional optical micrographs. Second, there is no obvious relationship between the mechanical properties of the cell surface at the micrometer and submicrometer level. Mège *et al.* (1987) attempted to overcome this difficulty by taking advantage of a method allowing to quantitate cell surface roughness by computer-assisted analysis of electron micrographs (Mège *et al*., 1986). They demonstrated that highly villous macrophages displayed marked local smoothing when they bound smooth erythrocytes that had been rendered stiff and macrophage-adherent by glutaraldehyde treatment. Then, they used micropipette aspiration to derive a relationship between membrane tension and smoothing, assuming that this smoothing fully accounted for the apparent area increase of deforming cells. Finally, they assumed that there existed a functional relationship between local macrophage roughness and percent of membrane area in molecular contact with erythrocyte membranes in the areas of apparent contact. It was thus possible to estimate the molecular adhesion energy from morphometric analysis. They estimated at 8.4 x 10^{-5} J/m^2 the affinity between macrophages and glutaraldehyde-treated surfaces, and this affinity increased to 18 x 10^{-5} J/m^2 when negative electric charges of the erythrocyte surface were removed or masked.

More recently, Williams *et al.* (2001) used a clever micropipette assay to compare the binding efficiency of immunoglobulin coated red cells and immunoglobulin receptors (CD16b) coupled to erythrocyte surfaces or expressed by transfected CHO or K562 cells: they reported a 50-fold increase in effective affinity for receptors on erythrocytes over CHO and K562 cells, and electron microscopical observations showed that contact areas were

much more extended in erythrocytes. They suggested that cell surface roughness might strongly influence effective binding affinity.

Is it warranted to use the concept of adhesion energy? It is by no means obvious that intercellular adhesion might be correctly described by a single parameter representing a work of adhesion between interacting surfaces. Indeed, it has long been demonstrated that cell membrane receptors often get concentrated in adhesion zones (Singer, 1976; André *et al*., 1990) and the possibility of ligand redistribution was suspected by Evans and Leung (1984). This possibility is addressed by a model elaborated by Bell *et al*. (1984): These authors hypothesized that the equilibrium shape of a cell adhering to a surface was determined by the balance between adhesion energy resulting from interactions between freely diffusive surface receptors and repulsion generated by bulky surface structures composing the glycocalyx. This model is consistent with the finding that the work of separation is dependent on the contact area, as first reported by Evans and Leung (1984) and later confirmed by Tözeren *et al*. (1989) who found that the effective adhesive energy of interaction between cytotoxic T lymphocytes and target cells increased from about 2 x 10^{-4} J/m^2 to 5 x 10^{-4} J/m^2 when the contact area decreased from 6 μm^2 to 2 μm^2. Another noticeable feature of this model is that cell rigidity was not invoked to account for the limitation of contact extension.

What is the reliability of aforementioned models? The problem with the assessment of aforementioned models is that they included many largely unknown parameters such as bond elasticity, 2-dimensional affinity constants, cell rheological properties at the micrometer and submicrometer scale, force/distance relationship for intercellular repulsion. Therefore, the finding of a semi-quantitative agreement between model predictions and experimental data is not a formal proof of their validity. It is thus important to achieve independent determination of the aforementioned parameters: the validity of a given model may then be assessed by determining whether fitted values of unknown parameters match estimates obtained with altogether different approaches. Although a detailed discussion of recent advances would not fall into the scope of this short review, it may be of order to list some points of interest.

First, modelling cell-surface interaction requires a quantitative knowledge of the rate of bond formation (k_{on}) and dissociation (k_{off}) between surface-attached receptors and ligands, or at least the 2-dimensional affinity constant ($K_{2D} = k_{on}/k_{off}$). During the last ten years, many authors used various techniques based on hydrodynamic flow, atomic force microscopy, soft membrane probes or optical tweezers to study bond rupture at the single molecule level (reviewed in Pierres *et al*., 1998a and Bongrand, 1999). Much less information is available on association rates (Chesla *et al*., 1998; Pierres *et al*., 1998b). Finally, only the aforementioned fluorescence-based methodology developed by M. Dustin yielded direct information on 2D affinity constants (Dustin *et al*., 1997, 2001)

Second, a new dimension in the complexity of cell-cell repulsive interactions appeared when these interactions were found to be time-dependent (Patel *et al*., 1995; Sabri *et al*., 1995).

Third, cell fitting to a surface is obviously dependent on the mechanisms of shape control. Basic reports on cell rheology are the study by Schmid-Schönbein *et al*. (1981) who modeled the small deformations of polymorphonuclear neutrophils with a three-parameter standard viscoelastic model, and Evans and Kukan (1984) who accounted for large deformations by modelling these cells are viscous liquid droplets surrounded by a membrane under constant tension: viscosity and surface tension were respectively 100 Pa.second and 0.035 millinewton/m (see Richelme *et al*., 1996, for additional references). These basic models were progressively refined, and recently Drury and Dembo (2001) elaborated a seven-parameter model to account for the deformation of cells subjected to micropipette aspiration under a wide range of time and aspiration pressure. The difficulty is to elaborate a model sufficiently simple to yield tractable equations and with a range of validity sufficient to account for small deformations (e.g. cell surface smoothing) and flattening on the scale of the whole cell. Thus, it is not surprising that some important problems remain unsolved at the present time. Indeed, depending on cell populations, leucocytes might be modeled as liquid structures surrounded with a membrane under constant tension (Evans and Kukan, 1984) or an elastic membrane with zero initial tension (Mège *et al*., 1987 ; Foa *et al*., 1988). This may be important to assess the relevance to biological cells of physical phenomena such as repulsive undulation forces (Helfrich, 1978) that are exquisitely sensitive to membrane tension (Servuss and Helfrich, 1989).

Fourth, a general problem is that it is difficult to discriminate between active and passive phenomena: indeed, subjecting cells to mechanical forces may suffice to induce definite activation, as revealed e.g. by marked rise of intracellular cytosolic calcium (Horoyan *et al*., 1990), and blocking these events may alter passive cell mechanical properties. Also, while passive mechanisms may account for lateral redistribution of receptors in contact areas, this redistribution is probably amplified by active cell mechanisms. This possibility is supported by an elegant report from Wülfing *et al*. (1998) who studied the interaction between T lymphocytes loaded with a fluorescent calcium probe and cells expressing a genetically engineered fluorescent form of the adhesion protein ICAM-1: T lymphocytes seemed to induce a redistribution of ICAM-1 by an active mobilisation of their ICAM-1 receptor (the LFA-1 molecule), concomitant to a rise of intracellular calcium. This intricacy between active and passive processes is so often encountered in cell biology that it might be hypothesized that active processes appeared during evolution to increase the efficiency of functions that were initially driven by purely physical phenomena.

In view of the aforementioned problems, it may be warranted to alleviate modelling problems by studying simplified systems retaining basic components of cell behaviour. Thus, Sackmann and colleagues prepared model vesicles where they incorporated both adhesion receptors and repulsive elements (Albersdörfer *et al*., 1997; Kloboucek *et al*., 1999; Boulbitch *et al*., 2001) and they studied their attachment to ligand-containing surfaces with interference reflection microscopy (IRM) also called reflection con-

trast interference microscopy (RICM). They were able to observe lateral phase separation between areas of weak and strong adhesion. They also developed a clever analysis technique for deriving the adhesion energy from the membrane contour near the boundary of contact zones: this varied between about 10^{-6} and 10^{-9} J/m^2 in a model where binding was mediated by an homophilic adhesion protein from the slime mold Dictyostelium discoideum.

Kinetics of Cell Fitting to an Adhesive Surface

Experimental results

Although the kinetics of contact formation may display wide variations between experimental models, it may be useful to give an order of magnitude of the time required for a cell to complete the different phases of attachment. Therefore, we shall recall some typical results:

Ben Shaul and Moscona (1975) studied the aggregation of neural retinal cells: after 30 minutes incubation, contact was mediated by filopodia, and this pattern was replaced with large contacts after 120 minute incubation.

Jones *et al*. (1976) performed a detailed electron microscopical study of the contact between Patella vulgata hemocytes: contacts first involved lamellipodia, and they were replaced by direct apposition of the cell body within 4 minutes.

Grinnell and colleagues (1976) used electron microscopy to study the contact of baby hamster kidney cells with planar surfaces: contacts involved the tip of microvilli after 15 minutes, and progressive enlargment proceeded during the following 45 minutes.

Foa *et al*. (1988) reported an electron microscopical study of the contact area formed by cytotoxic T lymphocytes and target cells. The area of the region where cell membranes were less than 50nm apart was nearly maximal after 1 minute contact.

The time required for cell spreading displayed wide variations: While vascular cells began to spread after 60 minute contact with an artificial surface (Haller *et al*., 1998), monocytic THP-1 or U937 cells began spreading only 24h after deposition (Aepfelbacher *et al*., 1994) and 3Y1 cells began spreading after only 10 minute incubation (Liu *et al*., 2001)

Wülfing *et al*. (1998) reported that lymphocytes could form a tight interface with antigen-presenting cells within 20 seconds after contact, while Bunnell *et al*. (2001) reported a progression of IRM-detected contacts between T lymphocytes and surfaces reacting with T lymphocyte antigen receptors during the first 10 minutes following contact.

Recently, Pierres *et al*. (submitted) used interference reflection microscopy to monitor the interaction between freshly deposited monocytic THP-1 cells and polylysine-coated surfaces: they observed a continuous increase of contact area during the first 3-4 minutes following contact, up to an area of order of 100 μm^2 per cell. The shape of the contact zone was very irregular, and the translation velocity of the contact margin ranged between 0.01 and 0.02 μm/s during the period of contact extension (Figs. 5 and 6). When cells were treated with cytochalasin D to block microfilament function, contact extension was reduced tenfold. However, even when the cell metabolism was fully blocked by paraformaldehyde fixation, some cells still displayed significant contact, thus suggesting that membrane-surface alignment could be driven by passive physical processes and it was strongly amplified by active cell reorganization.

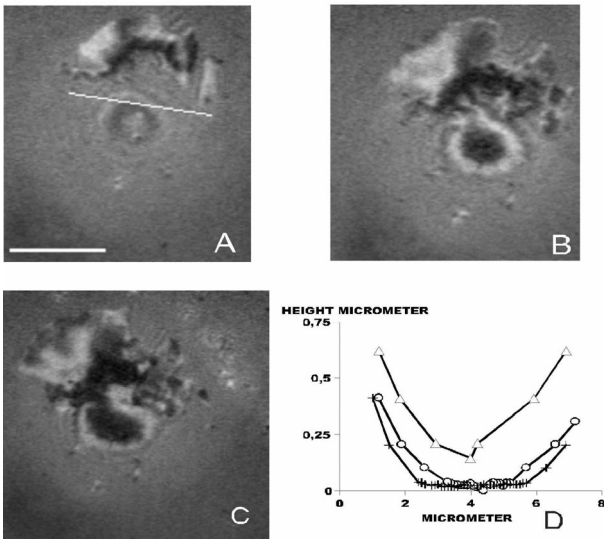

Figure 5: Contact extension. Three sequential IRM images were obtained 60 (A), 73 (B) and 83 (C) seconds after depositing THP-1 cells on polylysine-coated surfaces. Images were used to derive the membrane profile along a segment (white line on A), and profiles are shown on D. Bar length is 5 μm.

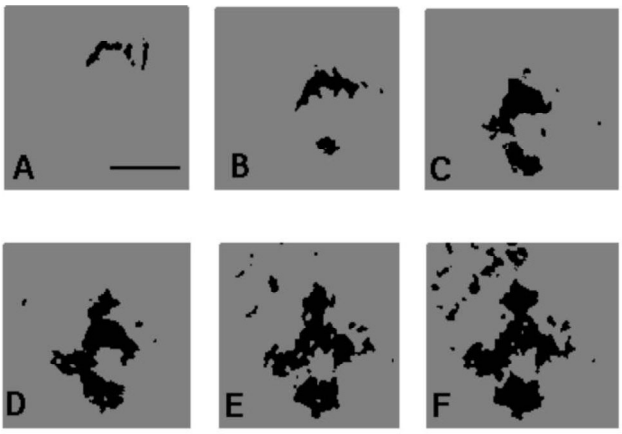

Figure 6: Kinetics of contact extension. Monocytic THP-1 cells were deposited on a polylysine-coated surface and they were monitored with interference-reflection microscopy to determine contact extension: this figure represent cell-surface contact in a selected molecular field observed 60 s (A), 73 s (B), 83 s (C), 95 s (D), 108 s (E) and 117 s (F) after the onset of the experiment. Clearly, the increase of cell-surface contact area is driven by both extension of existing contact and formation of additional contact zones. Bar length is 5 μm.

In conclusion. Cell interaction with an adhesive surface may involve three sequential phases:

1) *adhesion*: a cell may be considered as adherent to a surface if it cannot be displaced by a moderate force such

as hydrodynamic force generated by gentle washing. This may require the formation of only a few ligand-receptor bonds. Such attachment may be achieved within a fraction of a second, and this may not require active cell participation (Lawrence and Springer, 1991, 1993; Pierres *et al*., 1994).

2) *fitting*: this results in formation of a substantial contact area (≥ 1 μm^2) where surfaces are close enough to allow molecular interactions between adhesion receptors and their ligands. This phenomenon may occur in absence of active cell participation, but it is strongly accelerated by microfilament-dependent mechanisms. Its duration usually ranges between 1 and 10 minutes.

3) *spreading*: This involves active cell reorganization and it is much more dependent on metabolic inhibitors than the previous phase. This may result in marked changes of cell structural and functional properties.

Models

Despite some attempts at modeling the kinetics of bond formation and dissociation (Hammer and Lauffenburger, 1987; Qi *et al*., 2001), we are not aware of any specific model for cell-surface alignment. Five main processes might be considered as potential mechanisms for driving contact extension:

Nonspecific forces. Cell-surface apposition might be driven by short range interactions at the margin of the contact region (Fig. 7A). These interactions might result from the balance between van der Waals attraction and steric forces generated by mobile surface repellers. Random diffusion of these repellers might result in fluctuations of repulsive interaction, thus allowing contact extension during periods of attraction, and this extension might be stabilized by the formation of adhesive bonds.

Attraction generated by elongated receptors located out of the contact area. The possibility of such a mechanism is supported by a recent work performed on a model system. Wong *et al*. (1997) used the surface force apparatus to study the interaction between surfaces bearing long flexible polymer chains: they observed long distance attractive forces generated by binding events involving rare elongated conformations of these spacer polymers. Similarly, attractive forces might be generated by flexible receptors located near the boundary of contact zones (Fig. 7B)

Spreading pressure generated by ligand-receptor couples trapped in the contact zone. As illustrated in Figure 7C, if cell membrane receptors and surface ligands may diffuse freely in the surface where they are embedded, they may generate a 2-dimensional pressure that might enhance extension. In order two obtain a rough estimate for this pressure, we may calculate the 2D pressure generated by point-like particles – in analogy to perfect gases – with a realistic surface area of 100 nm^2 per complex. We obtain:

$$P = n\, k_B T = 4\ 10^{-5}\ J/m^2 \qquad (6)$$

(where n is the surface density of ligand receptor complexes, k_B is Boltzmann's constant and T is the absolute temperature). This estimate of P is quite comparable to the estimated surface tension of cell membranes (Evans and Kukan, 1984). Therefore, it might contribute contact extension. A thorough theoretical treatment of this mechanism was recently provided by Brochart-Wyart and de Gennes (2002).

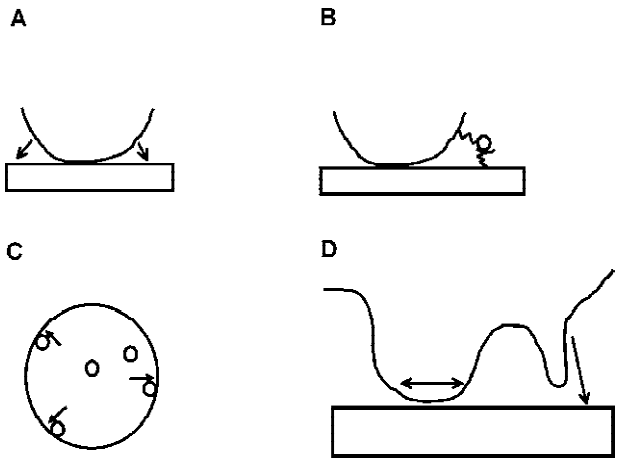

Figure 7: Possible mechanisms for contact extension. Four nonexclusive mechanisms might account for contact extension. Cells might be attracted to surfaces by nonspecific forces (A) or by ligand-receptor interactions involving flexible molecules (B). Ligand-receptor complexes trapped in the contact area might generate a substantial spreading pressure (C). Contact might also be driven by active membrane deformations parallel (double arrow) or perpendicular (arrow) to the adhesive surface.

Active extension of cell-surface contact. As depicted in Figure 7D, cell surface contact might be driven by active cell deformations that would be stabilized by receptor-ligand interactions. This mechanism would be somewhat similar to the ratchet mechanism suggested by Peskin *et al*. (1993) to account for lamellipodial extension. This mechanism is likely to play a role in the spreading phase of contact extension. Different processes might be involved, including actin-myosin interaction as shown by Cramer and Mitchison (1995) in a study of postmitotic kidney cells, and actin polymerization, as emphasized by Mallavarapu and Mitchison (1999) who studied the mechanisms of lamellipodial extension, Vasioukhin *et al*. (2000), who reported on cadherin-mediated adhesion of epithelial cells, and Bunnell *et al*. (2001) in a study of lymphocyte spreading. Also, Izzard (1988) showed that a microfilament-based structure was a precursor of focal contacts.

It is not obvious that the same mechanisms are involved in contact extension during the "fitting" phase of adhesion. A common finding is the presence of a circular ring of microfilaments surrounding the IRM-detected contact zone during the first minutes following leukocyte adhesion (Boyles and Bainton, 1979; Bunnell *et al*., 2001; Pierres *et al*., submitted).

Formation of new contacts through random fluctuations of the cell membrane. It seems well demonstrated that contact extension can be driven by pure physcal phenomena. Interestingly, contact formation between soft vesicles and adhesive surfaces displayed some features resembling cell behaviour, such as a margin extension velocity ranging beween 0.01 μm/s and 0.2 μm/s (Feder *et al*., 1995; Boulbitch *et al*., 2001). In the latter study, the authors found

that the displacement of the adhesive front varied as the square root of time at low receptor concentration, suggesting that receptor diffusion was the rate limiting step. When high receptor concentrations were achieved, the displacement was a linear function of time, and the authors hypothesized that the kinetics of ligand-receptor association might be rate limiting. It would be important to determine whether these findings might be extended to cellular systems or altogether different mechanisms are involved in both situations. Indeed, a major difference between the surfaces of cells and lipid vesicles is related to membrane mobility. Further, cell alignment to surfaces might be strongly impaired by surface rigidity and viscosity. The possible consequences of these differences are not clear, since membrane fluctuations might decrease adhesion by generating repulsive forces (Helfrich, 1978; Servuss and Helfrich, 1989), and they might also accelerate contact extension.

It is thus of interest to ask whether living cells display membrane fluctuations comparable to those reported on artificial vesicles (Duwe and Sackmann, 1990). While fluctuations of erythrocyte membranes are well documented (Brochard and Lennon, 1975; Duwe and Sackmann, 1990), there is some evidence that the surfaces of nucleated cells display continuous vibrations: in addition to the continuous advance and retraction of lamellipodia (Rinnerthaler *et al*., 1991; Dunn *et al*., 1997) with a period on the order of a minute, Yu *et al*. (1990) reported on rapid fluctuations (up to 30 Hz) of monocyte or lymphocyte surfaces with an amplitude of 20-30 nm. Peak surface velocity would thus be of order of several tens of μm/s, which might be sufficient to account for the kinetics of contact extension reported in cellular systems (Pierres *et al*., submitted).

Concluding Remarks

Before concluding, we shall briefly discuss two important points that are certainly relevant to the fitting process.

First, although the possible influence of van der Waals attraction and electrostatic repulsion on initial cell-substrate adhesion was acknowledged in this review, the DLVO (Derjaguin Landau Verwey Overbeek) theory was not felt an adequate framework for modelling this process (see Bongrand *et al*., 1982; Bongrand and Bell, 1984; and Pierres *et al*., 2000 for further references). This opinion deserves some comments: applying the DLVO theory to cell interactions would consist of estimating cell-cell or cell-substrate interaction force as a sum of i) electrodynamic attraction, which is inversely proportional to a power of the distance and ii) electrostatic repulsion, which exhibits exponential decrease with respect to distance. In physiological media, the characteristic decay length (i.e. Debye-Hückel constant) is about 0.8 nm. A further points is that this theory does not account for molecular details and it needs a reasonably "smoothed" model to represent the cell surface. In a previous report (Bongrand and Bell, 1984), cells were modeled as spheres surrounded with an hydrophobic layer corresponding to the core of the plasma membrane and responsible for van der Waals attraction, coated with a charged zone corresponding to the glycocalyx. When realistic values were used for the Hamaker constant and surface charge, the following estimates were obtained for cell-cell interaction forces: i) when the intercellular distance is higher than a few tens of nanometers, electrodynamic attraction is dominant, however, this is probably insufficient to influence cell-cell approach since the force experienced by a surface corresponding to the tip of a microvillus is at most on the order of 0.1 piconewton. ii) electrostatic repulsion may play a significant role when distance is decreased. However, when the separation is a few tens of nanometers, the cell surface cannot be modeled as an homogeneous structure. Probably, repulsion is more realistically accounted for by models derived from polymer theory : cell surface polymers are responsible for "steric stabilization", as a consequence of structural properties that are determinated by hydrophilic groups, including those with electrostatic charge. Further, at this distance, electrodynamic attraction may be generated by localized short distance interactions between a variety of surface-attached molecules. The view that surface forces are dominated by short distance interactions between mobile individual encountering molecules may explain why cell-cell interactions are time-dependent, as previously emphasized (Sabri *et al*., 1995; Patel *et al*., 1995). Thus, it must be borne in mind that cell interactions are essentially mediated by mobile surface-attached molecules with an average length of 10-40 nm, and a few individual receptors may mediate substantial attachment. However, there is a wide range of affinities between cell-surface receptors, and numerous "nonspecific" interactions between a variety of surface molecules may play a role in adhesion when specific adhesion receptors display low density, low affinity and/or low accessibility due to their size.

Another important question is to know whether cell fitting is significantly influenced by medium viscosity, due to the requirement for fluid drainage between encountering surfaces. We are not aware of any detailed experimental study providing a clearcut answer to this question. However, some interesting reports concerning artificial "biomimetic" vesicles seem worthy being briefly mentioned. First, Bernard *et al*. (2000) presented a quantitative study of the spreading of giant vesicles on surfaces: they found that there was a delay of several tens of seconds between "apparent contact" between a cell and a vesicle, and the occurrence of strong adhesion. This seemed to be accounted by the well-known Reynolds formula relating the interaction force F between a disk of radius L and a planar surface separated by distance h and approach velocity:

$$dh/dt = (2/3\pi)\, F\, h^3 /\eta L^4 \qquad (7)$$

where η is the fluid viscosity. Applying this formula to the encounter between a disk of 0.1 μm radius (corresponding to the tip of a surface microvillus) and a surface 10 nm apart, a low force of 1 piconewton will be sufficient to achieve 2 μm/s velocity. This means that viscosity effects are unlikely to impair initial cell-surface contact. Further, Brochart-Wyart and de Gennes (2002) presented a theoretical study of the spontaneous growth of a contact patch between a vesicle and a surface. The hydrodynamic force F_h opposing the contact growth (per unit length) was estimated as:

$$F_h = 3\ L\ \eta\ \theta^{-1}\ dR/dt \qquad (8)$$

where R is the patch radius, θ is the contact angle and L is a logarithmic (i.e. slowly varying) factor of order 10. Tentatively estimating the contact angle at 0.1 rd and the advance velocity dR/dt at 0.1 μm/s, the force is 0.03 piconewton per micrometer, which is quite low as compared to forces generated by the cell cytoskeleton.

Taken at face value, the above estimates would suggest that medium viscosity is not a significant determinant of cell spreading. However, there are two problems with our estimates: i) since the force is depending on the fourth power of the contact radius in equation (7), quite different results might have obtained if contact was mediated by a smooth cell patch of 1 μm radius. ii) It was probably unwarranted to use bulk medium viscosity for parameter η, since fluid flow is expected to be markedly impaired by the presence of cell-attached molecules. Thus, more experimental data are needed to assess the possible importance of hydrodynamic forces in cell-surface attachment.

In conclusion, cell fitting to adhesive surfaces is an essential part of the attachment process and subsequent regulation of cell functions. While the details of the fitting mechanisms remain elusive, many recent experiments disclosed a variety of biological or physical processes that might play a role, isolated or in combination, in driving contact extension.

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