Is there a predictable relationship between surface physical-chemical properties and cell behaviour at the interface?

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IS THERE A PREDICTABLE RELATIONSHIP BETWEEN SURFACE PHYSICAL-CHEMICAL PROPERTIES AND CELL BEHAVIOUR AT THE INTERFACE?

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Abstract

There is much interest in predicting and controlling the outcome of interaction between artificial surfaces and living cells. However, although there is an impressive amount of information on the behaviour of many cell populations deposited on a variety of surfaces, there is presently no available theory to explain or even summarize these data. Indeed, it is not even obvious that such a theory may exist. The aim of the present review is to emphasize the problems encountered when one attempts to build such a theory. Three sequential steps of cell surface interactions are considered: 1) protein adsorption is a preliminary step liable to involve irreversible interaction between the surface and several hundreds of molecular species occurring in blood or plasma. 2) the second step is the formation of adhesive bonds. Several theoretical frameworks were suggested to account for this step, including DLVO theory, physical chemistry of surfaces, and formation of specific ligand-receptor bonds. It is concluded that present evidence supports the latter approach, although this involves serious difficulties. 3) The last step is the triggering of a specific cell program such as apoptosis, proliferation, migration, differentiation or activation. Recent evidence suggests that in addition to the nature and amount of stimulated surface receptors, additional cues such as substratum mechanical or topographical properties may significantly affect cell behaviour.

Key words: Protein adsorption, DLVO theory, interfacial energy, ligand-receptor bonds, cell adhesion, cell signalling.

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Introduction

There is no need to emphasize the potential interest of controlling or even predicting the outcome of encounters between cells and artificial surfaces. Indeed, such knowledge would greatly facilitate the production and use of biomaterials. However, there is no evidence that a suitable theoretical framework might exist. It is not even obvious that this will impede future progress in producing biomaterials. Indeed, many examples such as vaccination or the development of antibiotics show that powerful procedures may be developed long before the theoretical basis required to understand them.

Despite these limitations, many authors have looked for basic laws of cell-surface interaction. This might be useful not only to explain available data, but perhaps also to summarize them, or to suggest new experiments that might provide unforeseen knowledge. The aim of the present review is to discuss previous work in the light of recent evidence in order to facilitate future progress.
In order to increase clarity, it appeared appropriate to split cell-surface interaction in three roughly sequential steps, although this is only an approximation:

First, it is well known that when an artificial surface is exposed to biological fluids, it becomes coated with proteins within seconds or less (Baier and Weiss, 1975). Hence, what cells see are only modified surfaces. Thus, an essential point is to predict and control the structure of the adsorbed layer formed on any given biomaterial. However, this is a quite complex phenomenon due to the multiplicity of proteins occurring in biological media, interaction between these proteins, and importance of time-dependent conformational changes.

Second, a critical step is the formation of adhesive bonds between cells and surface. Cell adhesion has been a field of intense activity during the last three decades, and an enormous amount of information has been obtained. It remains to organize this information in order to make it tractable.

Third, when a cell has adhered to a surface, it may have to chose an appropriate developmental line: indeed, it may undergo apoptosis and die or on the contrary survive and proliferate, it may remain on the site of adhesion or start migrating, it may undergo some kind of differentiation, finally, it may stay in a resting state or on the contrary trigger active processes such as synthesis and/or secretion of active mediators. The basis of the decision of the cell is a problem of the highest interest for the biological community. Much progress has been made in dissecting signalling cascades and developmental mechanisms. However, integrating available information is much

more difficult than in the prediction of adhesion, which is a much shorter and simpler process. Thus, we shall only discuss a few recent ideas that are currently explored.

Surface modification by soluble factors: is there a theoretical framework allowing to predict the structure of modified surfaces ?

As recently pointed out by Norde (2000) "Knowledge of the adsorption behaviour of proteins has largely progressed in the past few decades, but a unified predictive theory is still lacking". Thus, we shall only emphasize some points that appear particularly important for our purpose.

Macromolecule adsorption is a complex phenomenon
As was suggested in considering cell-surface interaction, it seems appropriate to split the adsorption process in several sequential steps:

1) first, soluble molecules will have to encounter the surface. The order of encounters is determined in particular by diffusion constants and concentrations of different species.

2) The second step is a reversible binding of molecules to surfaces. "Reversible" means that bound molecules may be detached within a time scale shorter than that of experiments. In this case, denaturation is not expected.

3) The third step, which in fact proceeds concomitantly with the second one, is a progressive modification of the composition of adsorbed layers: the most rapid and concentrated species may be expected to be partially replaced with more adhesive ones. This is the basis of the so-called Vroman effect.

4) Then, adsorbed proteins will undergo progressive conformation changes. This usually strengthens adhesion. Also, as will be emphasized below, this will expose new interaction sites to cells.

5) For the sake of completeness, we may consider the possibility of continuous adsorption with formation of multiple protein layers.

Why is there no general rule to relate the structure of native and biomolecule-coated surfaces ?
The following six points may be emphasized.
Low selectivity of adsorption. Most individual proteins can get adsorbed on a variety of hydrophobic or hydrophilic, neutral or charged surfaces. Thus, there is no selection rule allowing to restrict the potential number of molecule species liable to be bound by a bare surface exposed to biological environment. The only general way to prevent adsorption may be to coat surfaces with flexible hydrophilic polymers such as polyethylene glycol, a general mechanism for steric stabilization (Napper, 1977).
Nonadditive behaviour of different components. When a surface is exposed to a mixture of macromolecules, there is a competition between multiple adsorption processes. Thus, the behaviour of a mixture may not be predicted after determining the adsorption of individual components. As an example, Lassen and Malmsten (1997) spent much effort to study the interaction of a ternary mixture of fibrinogen, albumin and immunoglobulin G on different surfaces.
Typical biological fluids are highly complex. Indeed, while we have just emphasized the complexity of a ternary mixture, plasma probably contains hundreds of molecular species. Even if we follow Andrade and Hlady (1987) who suggested considering only a dozen molecular species likely to dominate adsorption (including albumin, immunoglobulin G, A and M, C3 complement component, fibrinogen, haptoglobin, α1-antitrypsin, α2-macroglobulin, low and high density lipoproteins), biologically relevant phenomena seem quite difficult to model.
We are dealing with irreversible processes. It has long been reported that protein adsorption may result in progressive conformational changes and denaturation, thus preventing efficient exchange between adsorbed and soluble phase after a few hours. Cell surface attachment indeed involves a variety of reactions beginning as soon as a few milliseconds after contact (Heinrich *et al*., 1999). Thus, the structure of a surface exposed to several molecular species is dependent on the whole history of the adsorption process. As an example, Pitt *et al*. (1986) sequentially exposed polyvinylchloride, polyethyleneglycol or silicone elastomers to albumin and fibrinogen: they concluded that the first adsorbed protein dominated further interaction of treated surfaces and platelets.
It is not sufficient to know the nature and density of adsorbed molecular species to understand interface structure. Indeed, as mentioned above, macromolecule adsorption may result in extensive conformational changes. It has long been demonstrated that these phenomena had high physiological relevance. Thus, it was reported that hydrophobic surfaces adsorbed higher amounts of fibronectin than hydrophilic ones, but the latter surfaces were more efficient in binding selected anti-fibronectin antibodies and supporting cell adhesion (Grinnell and Feld, 1982). The concept that cell behaviour at interfaces is dependent on underlying substrata as well as adsorbed molecule layers was indeed confirmed by more recent studies (Koenig *et al*., 2003). This finding illustrates the complexity of protein adsorption, but also suggests the possibility that cell behaviour at interfaces might somewhat reflect some features of underlying substrata independently of adsorbed molecules. It might be interesting to subject this concept to experimental test.
Adsorption energies are relatively low. Lastly, a general reason for the difficulty to predict the behaviour of macromolecules at interfaces is that ligand-receptor interaction energies amount to only a few percent of the folding energy of a molecule such as a protein. Thus, even modest conformational changes may strongly affect molecule-to-surface interaction. This is a general problem when one tries to derive the behaviour of proteins from *ab initio* principles.

In view of the aforementioned remarks, it may seem a hopeless task to try and predict the detailed structural properties of an artificial surface exposed to a biological environment. It is therefore an essential point to determine to what extent we need to know these detailed properties to predict the outcome of cell-surface interactions. In order to address this question, we shall briefly review the main theoretical frameworks that were used to predict cell-sur-

face adhesion. Then, we shall briefly discuss the parameters that are likely to affect further evolution of an adherent cell.

Which theoretical framework is best suited to predict the occurrence of cell-surface adhesion?

Three main theoretical frameworks remain implicitly or explicitly used to discuss experimental data on cell surface interactions: DLVO theory, physical chemistry of surfaces and identification of specific molecular interactions. It is interesting to ask first whether they remain valid, and second whether they may prove fruitful with respect to our purpose.

DLVO theory: is it relevant to biological systems?
The DLVO theory was developed separately by Derjaguin and Landau, in Russia, and Verwey and Overbeek, in the Netherlands, during the 1940s. The theoretical basis and relevance to biological systems may be found in textbooks or review articles (Curtis, 1967; Bongrand *et al.*, 1982; Bongrand and Bell, 1984; Israelachvili, 1991). Therefore, we shall only mention essential features.

The DLVO theory was developed to account for the behaviour of colloid suspensions. The interaction energy between micrometer-sized particles was calculated as the sum of two terms:
Electrostatic repulsion between surface charges. In biological media, this interaction is strongly screened by water (the relative dielectric constant is about 78) and surrounding ions. The latter effect results in an exponential decrease of the interaction with a characteristic length of about 0.8 nm (the so-called Debye-Hückel length).
Electrodynamic attraction. The free energy of interaction between two semi-infinite media 1 and 2 with parallel surfaces separated by a distance d is $- A_{12}/12\pi d^2$ in vacuum. In aqueous environment, the Hamaker constant A_{12} is replaced with a combination

$$A^3_{12} = [A_{12} - (A_{13} + A_{23})/2] \qquad (1)$$

where 3 stands for the medium. Usually, two similar bodies will attract each other in water.

When both forces are added, the predicted interaction is shown in Figure 1: at large distances, exponential repulsion vanishes more rapidly than power-law attraction, resulting in overall attraction with a shallow minimum called the secondary minimum. When the distance is decreased, a repulsive barrier must be overcome to reach the so-called primary minimum, which is considered to result in irreversible adhesion.
Relevance of DLVO theory to cell/surface adhesion. Several features of the DLVO theory were a strong incentive for physically oriented biologists to try and apply this framework to biological systems. First, this theory met with definite success in elucidating the behaviour of artificial suspensions of charged particles. Second, DLVO theory is well suited to living cells, since they have a typical diameter of several micrometers, they are surrounded with a hydrophobic plasma membrane that is expected to generate electrodynamic attraction, and they bear a net negative surface charge (Mehrishi, 1972 ; Sherbet, 1978). Third, DLVO

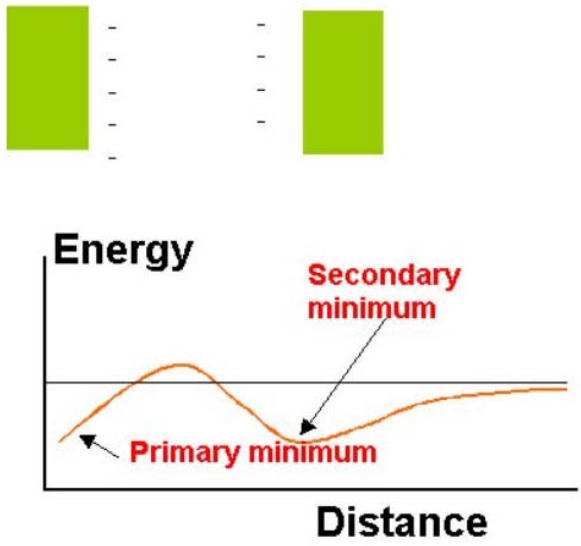

Figure 1. DLVO theory. The interaction between two similar charge bodies in an ionic solution (top) is calculated as the sum of electrostatic repulsion and electrodynamic attraction. A typical energy/distance curve is shown (bottom)

theory may explain weak and strong adhesion, which is indeed described with different cellular models. Fourth, much experimental evidence has shown that cell-cell adhesion could be markedly enhanced by decreasing the net surface charge of the cell (see e.g., Capo *et al.* 1981; Mège *et al.*, 1987;), and van der Waals attraction between biomimetic membranes has been demonstrated experimentally (Yu *et al.*, 1998). Thus, there is little doubt that the basic principles of DLVO theory should apply to cells.
Difficulty of predicting cell adhesive behavior with the DLVO framework. Despite the above arguments, it appeared that the DLVO framework could not be used to provide an accurate description of cell adhesive behaviour. A major problem is that cells cannot be considered as smooth structures when they are separated by a distance less than 10 or 20 nm, corresponding to the primary and even secondary minimum. Indeed, intrinsic membrane proteins may in principle raise several tens of nanometers above the lipid bilayer since the length of integrins is about 20 nm, and molecules such as the P-selectin adhesion molecule or possibly repulsive CD43/leukosialin (Cyster *et al.*, 1991) are more than 40 nm long. Thus, cell-cell or cell-material interactions are dependent on the precise distribution of charges on cell membrane molecules as well as the shape of the cell surface at the submicrometer level. Further, while electrostatic interaction seems involved in generating some kind of repulsion, this may be an indirect consequence of the influence of negative charges on the conformation of molecules liable to generate steric repulsion.

As a consequence, to our knowledge, no experimental work resulted in numerical determination of Hamaker constants and surface charge densities accurately accounting for the adhesive behaviour of precise cells and surfaces.

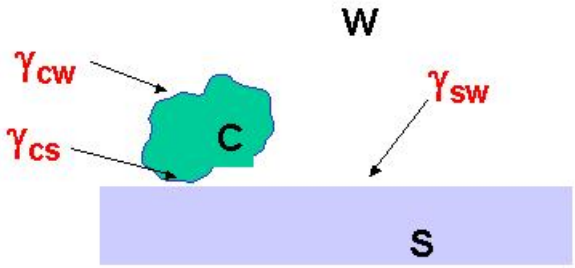

Figure 2. Surface physics and adhesion. The adhesion of a cell (C) to a surface (S) in aqueous medium (W) amounts to replacing CW and SW interfaces by a CS interface.

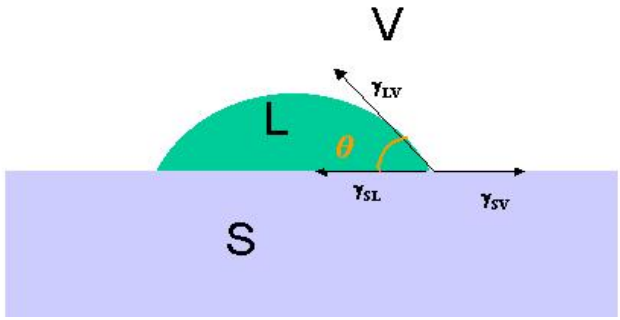

Figure 3. Contact angles. The determination of the contact angle of a liquid droplet (L) deposited on a surface (S) in a medium (V) provides a relationship between three interfacial energy parameters.

Surface thermodynamics

The apparent discrepancy between the existence of physical interactions between cell surfaces and the incapacity of DLVO framework to yield useful prediction is a strong incentive to look for a better suited theoretical model to deal with this interactions. The concepts and models developed by surface physical chemists seem worthy of interest, since they were used to deal with actual surfaces of incompletely known molecular structure. First, we shall define basic parameters, then, we shall try to identify basic postulates required to apply these concepts to cellular models. Finally, we shall discuss their usefulness on the basis of several examples. Note that this approach was recently discussed in an insightful review from Morra and Cassinelli (1997).

Basic principles. The starting point is the concept of interfacial energy (Adamson, 1976). As shown in Figure 2, making a cell (C) adhere to a substratum (S) in aqueous environment (W) amounts to replacing cell-water and substratum-water interfaces with a cell-substratum interface. Using obvious notations, basic thermodynamic principles impose that cell-substratum adhesion will occur if this involves a decrease of the system free energy, i.e.

$$\gamma_{CS} < \gamma_{CW} + \gamma_{SW} \qquad (2)$$

Although this approach seems perfectly rigorous, it must be emphasized that its use relies on two essential postulates:

1) interfacial energy should exist! This means that while it is well known and accepted that actual solid surfaces may not be homogeneous at the submicrometer level, it would be difficult to apply surfaces physical chemistry to cells without assuming that membrane composition is sufficiently uniform to allow for the definition of some average cell-water interfacial energy.

2) *There must exist a combining rule* to relate the three parameters of equation (2). Otherwise, we may not hope to predict the outcome of interaction between two surfaces that have been studied individually. Further, which is worse, in absence of a reliable combining rule, it is quite difficult to determine the free energy of a solid-liquid interface.

Indeed, while it is fairly easy to determine the surface tension of a liquid (Adamson, 1976), solid surfaces are usually studied by measuring the contact angle of sessile droplets deposited on the surface and using standard Young-Dupré equation. Thus, denominating liquid, solid and vapor phases as L, S and V respectively (Fig. 3) :

$$\gamma_{LV} \cos\theta = \gamma_{SV} - \gamma_{SL} \qquad (3)$$

Thus, experimental determination of θ and γ_{LV} only yields a relationship between two unknown parameters, i.e. γ_{SL} and γ_{SV}.

Despite general thermodynamic reasoning (Neumann *et al*., 1974) suggested the possibility that there might exist a fairly universal combining rule between aforementioned parameters, compelling experimental evidence shows that the interfacial energy γ_{12} between two liquids is not a simple function of their surface tensions γ_1 and γ_2 (Bongrand *et al*., 1988). An example is shown in Table 1.

However, there remains a possibility that the interfacial energy between two media might be derived from material parameters. Thus, the work of adhesion per unit area of interaction between two media 1 and 2 might be tentatively written as a product :

$$W_{12} = \alpha_1 \alpha_2 \qquad (4)$$

where α_1 and α_2 are intrinsic material parameters specific for medium 1 and 2 respectively. This approximation is not entirely unreasonable in view of molecular theories of intermolecular forces (Margenau and Kestner, 1969; Israelachvili, 1991). An immediate consequence would be

Table 1. There is no universal combining rule for interfacial energies

Liquid 1	Liquid 2	γ_1 (mJ/m^2)	γ_2 (mJ/m^2)	γ_{12} (mJ/m^2)
water	n-octanol	72.8	27.5	8.5
water	CCl_4	72.8	27.0	45.0

The above example shows that interfacial energies between water and two liquids of similar surface tension can be quite different (adapted from Bongrand *et al*., 1988)

that the work of adhesion to medium 1 and 2 embedded in medium 3 would be:

$$W^3_{12} = - (\alpha_1 - \alpha_3)(\alpha_2 - \alpha_3) \qquad (5)$$

According to equation (3), two bodies exerting mutual attraction in vacuum might repel each other in a medium of parameter α_3 comprised between α_1 and α_2. Several points may be emphasized:

- First, equation (4) is only the simplest of a series of formulae that were suggested. Indeed, the simple product (4) was replaced with more complicated expressions such as a harmonic mean. Also, it appeared reasonable to split the material properties of a given medium into separate parameters accounting for different interaction such as electrodynamic attraction or polar interactions. The latter view was developed into a workable scheme by Van Oss *et al.* (1987) who suggested to split parameter α into three separate constants, respectively accounting for apolar, electron donor and electron acceptor component. These components could be obtained by performing contact angle measurements with three selected liquids.

- Second, an essential assumption is that two interacting surfaces will adapt their distance and conformation to reach some "equilibrium state". This requires a minimum amount of flexibility to allow the existence of an intrinsic interfacial energy parameter.

- Third, the experimental check of these concepts is made difficult by the excess of unknown parameters. This explains why this remains debated (Morra and Cassinelli, 1997).

- Fourth, the application of surface thermodynamics to cells is made still more difficult by specific problems. Thus, it is not possible to obtain cell surfaces as planar structures extended enough to allow contact angle measurements. Therefore, no accepted method presently allows quantitative determination of cell-medium and cell-substratum interfacial energy, thus precluding experimental check of any combining rule.

Despite these obvious gaps in our knowledge, it remains of interest to know whether the surface thermodynamic approach is in principle an acceptable way of interpreting cell-surface adhesion. We shall now describe a few experimental data to illustrate this point.

Experimental test of surface thermodynamics relevance to cell adhesion. It was the merit of Van Oss *et al.* (1975) to devise practical ways of testing the relevance of surface thermodynamics to biological phenomena. These authors were able to prepare layers of desiccated bacteria for determination of contact angle with water. A striking correlation was found between the contact angle measured on 21 bacterial species and the capacity of human neutrophils to ingest them (Fig. 4).

A problem with these experiments is that it is difficult to assess the significance of contact angles measured on dried structures. Indeed, the drying procedure may affect experimental data (Mège *et al.*, 1984). However, when surface energies were measured in aqueous environment by depositing dense fluorocarbon droplets on immersed biological surfaces, results confirmed the correlation between surface energy and adhesion (Gerson *et al.*, 1982).

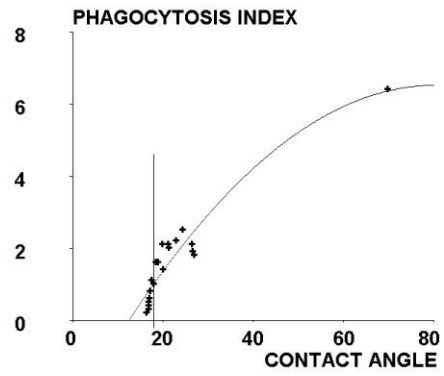

Figure 4. Relationship between bacterial hydrophobicity and uptake by phagocytic cells. Results reported by Van Oss *et al.* (1975) were plotted to provide an intuitive grasp of the relationship between phagocytosis of different bacterial species and uptake by phagocytic cells. The vertical straight line represents the contact angle measured on phagocytes with the same method.

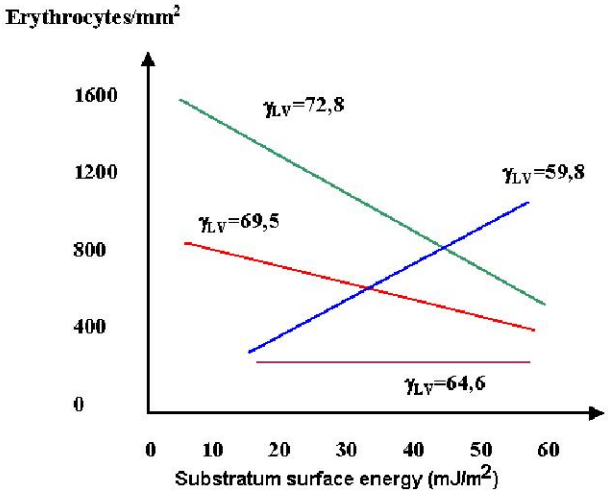

Figure 5. Effect of substratum and medium interfacial energy on erythrocyte adhesion. This figure is a sketch of results obtained by Absolom *et al.* (1985) in studying erythrocyte-substratum adhesion in protein-free environment.

In another series of experiments, Absolom *et al.* (1985) studied the adhesion of erythrocytes to artificial surfaces in water/DMSO mixtures of varying surface energy. As shown on Figure 5, when the medium surface energy was higher than 64.6 mJ/m^2, erythrocyte adhesion decreased when the substratum free energy increased from about 20 mJ/m^2 to about 50 mJ/m^2. when the medium surface energy was 64.6 mJ/m^2, erythrocyte adhesion seemed independent of substratum surface energy. Finally, when the medium surface energy was lower than 64.6 mJ/m^2, erythrocyte adhesion increased when medium surface energy increased. The authors pointed out that this result was consistent with prediction from surface thermodynamics, assuming that erythrocyte surface energy was 64.6 mJ/m^2. Indeed, the basic idea was that erythrocyte adhesion to the substratum was favoured when these surfaces were closer to each other than to the medium. However, since adhe-

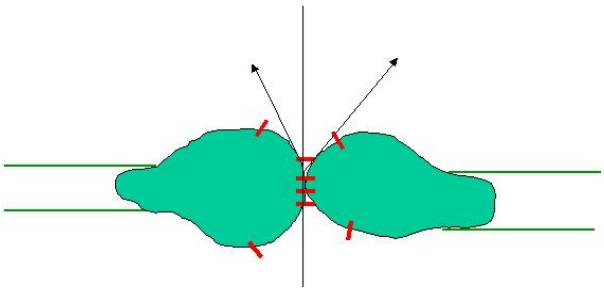

Figure 6. Direct determination of the work of separation of bound cells. Cells are brought into adhesive contact, then separated by pulling pipettes. The determination of the tangents to the membranes on the separation line allows the calculation of the adhesive force at the separation line provided membrane tension is known. Cell mechanical properties are determined by standard aspiration techniques.

sion occurred in all situations, the authors concluded that some other mechanisms must be involved in addition to surface energy effects.

Other experiments allowed direct estimate of adhesion energy (Tözeren *et al.*, 1989; Tözeren, 1990). Individual cytotoxic T lymphocytes and target cells were manoeuvred into contact with micropipettes (Fig. 6) and gradually separated. Determination of the angle between membranes allowed direct determination of 2-dimensional adhesion energy, provided membrane tension was first determined by standard micropipette aspiration techniques. It was concluded that the adhesion energy increased when the contact area decreased, in accordance with a theoretical model assuming that binding was mediated by individual bonds (Bell *et al.*, 1984).

Thus, the aforementioned experiments, as well as other experiments, strongly suggest that surface energy effects can indeed influence biological adhesion, but it was not possible to demonstrate that cell adhesion to surfaces could be quantitatively predicted with a well-defined function of physical chemical properties of interacting surfaces. A major problem is that even potentially useful physical chemical properties of cell surface cannot always be measured.

Can cell adhesion be entirely accounted for by specific bonds?

While the above two theoretical approaches were inspired by results from physics and chemistry, the description of cell adhesion as a consequence of specific ligand-receptor interactions is more akin to current biological way of thinking. The basic idea is that cell adhesion cannot be predicted from general principles, but requires a detailed knowledge of peculiar properties of studied systems.

Cell adhesion as determined by specific ligand-receptor interactions. Basic postulates. The specific view of cell adhesion relies on the following two simple ideas:

- First, it is assumed that most interactions between cells and surfaces are essentially determined by specific associations between well-defined receptors and ligands that could in principle be properly identified.

- Second, an implicit consequence is that cell-substratum interaction involves a small fraction of cell molecular area (Pierres *et al.*, 1998). Indeed, it is likely that 1,000 molecular bonds involving a molecular contact of 10 nm^2 each are sufficient to maintain a cell of 1,000 μm^2 area tightly bound to a flat surface. Clearly, if only 0.001 % of total cell area is involved in adhesion, it is unreasonable to expect that bulk surface properties will be related to adhesive behaviour.

It must be emphasized that the use of this framework will prompt particular experimental ways of studying adhesion. Thus, instead of assaying the hydrophobicity or charge density of a surface, in order to predict its capacity to bind cells, we shall look for known ligands of cell receptors, such as the well-known RGD sequence that was found to interact with many integrin receptors. As a consequence, testing a surface may rely on the quantification of specific sites, e.g. with labelled antibodies and techniques such as enzyme-linked immunoassay (ELISA) rather than contact angle determination.

What are specific interactions? Clearly, the above definitions are based on the concept of specific interaction. It may be useful to emphasize that this is not as straightforward as it might first seem. Two alternative definitions of specificity may be considered:

- First, focussing on function, an interaction might be considered to be specific if it is lost when the ligand is changed. Thus, an immunoglobulin binding group A antigen on blood cells is said to be specific if it does not interact with group B antigen. However, there is a problem with this definition, since more and more cell receptors are recognized to be “promiscuous”, which means that they can bind a variety of different ligands. A prominent example is represented by so-called scavenger receptors, which are thought to play an important role in natural immunity and were shown to recognize a variety of ligands including bacterial structures or altered lipids (Pearson, 1996). Interestingly, these receptors seem to be involved in the recognition of plastic surfaces by macrophages, an interaction that was long considered as nonspecific.

- Second, focussing on structure, an interaction may be defined as specific if it is dependent on detailed topographic features of interacting molecular surfaces (Fig. 7). Thus, attraction between surfaces bearing respectively a

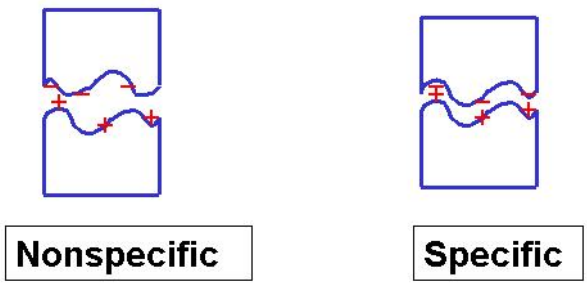

Figure 7. Specific and non specific interactions. The interaction between two surfaces of opposite charge (left) may be considered as non-specific. The interaction between two surfaces with fitting shape and matching opposite charges may be considered as specific.

net positive and negative charge may be considered as non specific, while an interaction involving accurate matching of charges and topographical features on apposed surfaces will be considered as specific. Note that it may not be easy to assess quantitatively the role of individual molecular groups in an interaction (Clackson and Wells, 1995).

Is it tenable to assume that most interactions between cells and natural or artificial surfaces are accounted for by the involvement of a limited number of well-defined receptor species? In our view, more and more recent evidence suggests that the answer to this question may be positive. **Firstly**, it is now well demonstrated that each cell species is endowed with a high number of surface receptors. Thus, it was recently emphasized (Barclay, 1998) that about 250 protein species were essentially specific for leukocytes, and about half of these molecule might act as adhesion receptors. **Secondly**, it is more and more widely recognized that many receptors are *multispecific* or *promiscuous* and may be involved in interactions that were previously held as nonspecific. Thus, the recognition of plastic surfaces by macrophages may well be due to a particular class of scavenger receptors (Fraser *et al.*, 1993), and a molecule such as $\alpha_M\beta_2$ integrin was reported to recognize more than 30 protein and non protein molecules including adhesion molecules, extracellular matrix components or bacterial structures (Yakubenko *et al.*, 2002). Finally, more and more specific interactions were reported between pathogens and cellular constituents such as e.g. integrins (Watarai *et al.*, 1996; Takeshita *et al.*, 1998), extracellular matrix components (Bisognano *et al.*, 1997 ; Giordano *et al.*, 1999) or blood group antigens (Geisel *et al.*, 1995).

Thus, there is no compelling argument to disprove the assumption that most interactions between cells and surface are mediated by specific cell membrane structures.

Difficulty of using standard biochemical concepts to derive cell adhesion behaviour from receptor properties. Clearly, in order to test the validity of the above concepts, we need a theoretical framework allowing the derivation of cell-substratum adhesive phenomena from ligand-receptor properties. It would thus be possible to take advantage of the powerful immunological and genetic methodologies that allowed biologists to characterize hundreds of adhesion receptors and to prepare them in soluble form, thus making possible the determination of affinity constants or kinetic association and dissociation rates with a variety of tools based on e.g. surface plasmon resonance.

Unfortunately, as pointed out by Bell (1978), conventional biological or chemical-physical methodologies are insufficient to fulfil this program. These insufficiencies are illustrated by a model that attracted much interest during the last ten years, namely the mechanisms allowing activated endothelial cells to capture flowing leukocytes as a first step to inflammatory reactions (Springer, 1994). Under standard conditions, selectin molecules expressed by endothelial cells seem able to tether ligand-bearing leukocytes to the blood vessel surface, thus inducing a characteristic jerky motion called rolling, with a translation velocity of order of 5-10 µm/s, i.e. one hundredfold

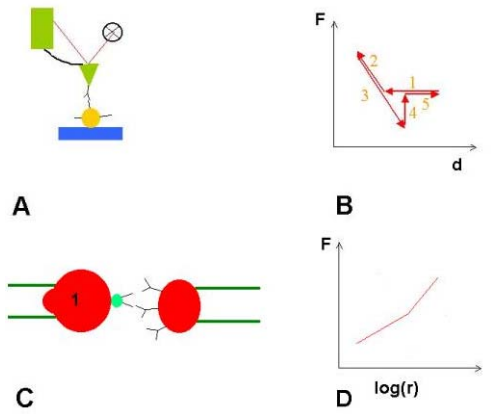

Figure 8. Determination of unbinding forces with an atomic force microscope or the biomembrane force probe. Using an atomic force microscope (**A**), a ligand-coated tip is brought into contact with a receptor-coated surface (**B** segment 1) and a positive force is applied for some time (2). Then the tip is progressively separated from the surface (3), thus imparting a pulling force on the bond. On rupture, a jump of the tip (4) is observed, which allows direct measurement of the unbinding force. The biomembrane force probe method (**C**) may be considered as improved atomic force microscopy: the cantilever is replaced with a soft vesicle such as a red blood cell (C-1) maintained with a micropipette, using variable pressure. The tip is replaced with a glass microbead glued to the biomembrane and coated with binding molecules (green). This device allows varying the rate of force increase (i.e. the loading rate, r, usually expressed in picoNewton/s) over several orders of magnitude. The unbinding force F is dependent on the loading rate. As shown in (**D**), when F is plotted versus the logarithm of r, the obtained curve may appear as several straight lines yielding quantitative information on the ligand-receptor energy-distance curve (Merkel *et al.*, 1999).

lower than that of freely flowing cells. However, ICAM-1 molecules bound to endothelial cells are not able to initiate rolling, even when their integrin receptors on leukocytes are properly activated. When this phenomenon was clearly demonstrated (Lawrence and Springer, 1991), it was not clear whether this difference was due to insufficient range, rate of bond formation or mechanical strength of the ICAM-1/integrin pair. Remarkably, no experimental tool was available to address this problem.

During the following years, it appeared that only experiments done at the *single molecule level* could yield reliable information on the rate of bond formation and dissociation between surface-attached molecules subjected to external forces. There are several explanations for this situation:

1) Interpreting experimental description of the separation of surface linked with multiple bonds requires a quantitative knowledge of the distribution of forces between molecules and possibility of rebinding (Seifert, 2000). This is usually lacking.

2) When multiple bonds are allowed to form between interacting surfaces, the rate of bond formation is usually dependent on the number and position of existing bonds. Also, molecule flexibility, environment and mode of connexion to underlying surface are important parameters.

We shall now present a brief description of recent results illustrating the potential of available tools to study receptor-mediated interactions between cells and surfaces. **Studying cell-substratum interaction at the single cell level**. During the last ten years, many authors studied the rupture of individual bonds (see Bongrand, 1999 for a review). Many important results were obtained with three methods (Figs. 8 and 9): atomic force microscopy (Florin *et al.*, 1994), the biomembrane force probe (Merkel *et al.*, 1999) and laminar chamber flow (Kaplanski *et al.*, 1993). The latter method probably yields most straightforward information. As summarized in Figure 9, when receptor-bearing cells or micrometer-size particles are driven along a ligand-coated surface in presence of a laminar shear flow with a typical wall shear rate of a few second^{-1}, they are subjected to a driving force of a few picoNewtons, thus allowing a single bond to provoke a detectable stop. Further, the translation velocity is of order of 10 µm/s, which makes the motion easy to monitor with high accuracy. It is thus possible to determine both the frequency and duration of individual arrests or *binding events*. Using a sufficient number of arrest durations allows straightforward determination of *unbinding plots*, i.e. dependence of the number of particles remaining arrested on time t after initial attachment. A typical curve is shown in Figure 9.

Now, we shall describe a study recently performed in our laboratory to illustrate the potential of the method and emphasize typical features of bond formation between surface-attached molecules (Vitte *et al.*, 2004). We studied the interaction of fibronectin-coated surfaces and human monocytic THP-1 cells under flow. Numerous binding events were observed and their frequency was drastically decreased by adding monoclonal antibodies known to block VLA-5 integrin (also denominated as CD24eCD29 or $\alpha_5\beta_1$), thus suggesting that adhesion was essentially mediated by specific bonds. Unbinding plot displayed the typical aspect displayed on Figure 9.

Now, the problem with unbinding plots is that multiple nonexclusive ways of interpreting curve shape must be considered: indeed, previous work done in our laboratory strongly supported the possibility that 1) delayed formation of additional bonds might occur after the initial

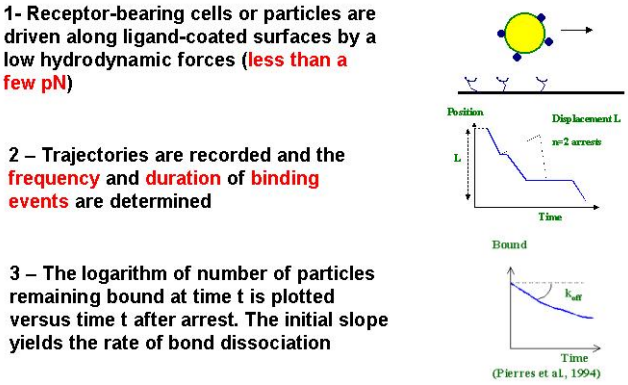

Figure 9. Studying individual ligand receptor bonds with a laminar flow chamber. The figure describes the use of a laminar flow chamber operated at very low shear rate.

binding event, 2) binding might involve the simultaneous formation of several bonds, and 3) ligand-receptor association might behave as a multiphasic phenomenon, with initial formation of a transient complex and subsequent dissociation or on the contrary transition towards a more stable state. We shall now show how we may deal with these difficulties with numerical data shown on Table 2.

These data may be interpreted as follows: binding events were probably mediated by multiple bonds with the highest fibronectin surface density, since ligand dilution to 3,850 sites/µm^2 resulted in marked shortening of arrest duration (see last column). However, when fibronectin surface density was further decreased to 1,436 sites/µm^2, the binding frequency was strongly decreased without any concomitant change of the initial detachment rate or fraction of cell bound 1s after arrest. This strongly suggests that binding events observed were essentially similar, corresponding to the *minimum detectable binding event*. It seemed reasonable to assume that single molecular interactions were indeed observed, since previous experiments supported the assumptions that single bonds could be detected with this methodology. However, as emphasized by Zhu *et al.* (2002), the single bond assumption is very difficult to prove formally.

Now, further experiments obtained on the same model will provide some support to the concept that bond topography is indeed an important determinant of cell adhesion. The surface distribution of fibronectin receptors on the

Table 2. Influence of fibronectin surface density on association and detachment rates

Fibronectin surface density (molecules/µm^2)	Binding frequency (mm^{-1})	Initial detachment rate (s^{-1})	Fraction of cells bound 1s after attachment
6,500	1.48 ± 0.07	0.96 ± 0.10	0.51 ±0.023
3,850	0.75 ± 0.08	2.26 ± 0.40	0.27 ± 0.046
1,436	0.21 ± 0.02	1.94 ± 0.27	0.32 ± 0.037

The motion of monocytic THP-1 cells along surfaces coated with various densities of fibronectin was studied. The frequency of binding events, slope of unbinding plots at time 0 (i.e. initial detachment rate) and fraction of cells bound 1 second after attachment are shown ± standard error (adapted from Vitte *et al.*, 2004)

Table 3. Influence of receptor aggregation on binding efficiency under flow

Cell treatment	Binding Frequency (mm^{-1})	Initial detachment rate (s^{-1})	Fraction of cells bound 1s after attachment
None (control)	0.75 ± 0.08	2.26 ± 0.40	0.27 ± 0.046
K20	0.45 ± 0.04	1.83 ± 0.29	0.36 ± 0.042
K20 + anti mouse	1.19 ± 0.08	1.02 ± 0.14	0.55 ± 0.030

The motion of monocytic THP-1 cells along surfaces coated with moderate fibronectin density (3,850 molecules/μm^2) was studied. Cells were treated with a neutral anti-beta 1 integrin monoclonal antibody (K20) with or without cross-linking polyclonal goat anti-mouse immunoglobulin $(Fab')_2$.The frequency of binding events, slope of unbinding plots at time 0 (i.e. initial detachment rate) and fraction of cells bound 1 second after attachment are shown ± standard error (adapted from Vitte *et al*., 2004)

surface of THP-1 cells was manipulated with monoclonal antibodies. First, cells were treated with K20 murine antibody, a beta 1 chain-specific monoclonal antibody considered as "neutral", i.e. without any effect on function. Second, anti-mouse immunoglobulin $(Fab')_2$ was added to cross-link VLA-5. This treatment induced a marked aggregation of surface receptors as shown with a semi-quantitative confocal microscopic study, suggesting that antibody treatment increased between 40 % and 100 % the average number of integrin receptors located in a volume of ca 0.045 μm^3 surrounding each integrin. Binding data are shown in Table 3.

Clearly, while K20 antibodies slightly decreased arrest frequency due to a probable decrease of accessibility, receptor aggregation increased binding frequency (from 0.45 to 1.19 mm^{-1} and decreased detachment rate. This suggests the influence of receptor topography on functional capacity.

Conclusion

While it is likely that nonspecific physical interactions such as electrostatic repulsion or steric stabilization may influence cell-substrate adhesion, no presently available theoretical framework can allow us to predict the outcome of interaction between a cell and an artificial surface of known physical-chemical properties. However, numerous reports suggest that in many different situations, cell-surface adhesion is essentially determined by a limited number of receptor species. Further, recently developed experimental methods allow precise determination of the properties of ligand-receptor interaction at the single molecule level. Thus, the most fruitful approach to understand cell-substratum interaction may consist of first identifying involved cell receptors. Indeed, even cell interaction with plastic surfaces (Fraser *et al*., 1993) or foreign structures such as microorganisms often involve a limited number of dominant molecular species.

Is it possible to predict the behaviour of a cell adhering to a surface through well-identified receptors?

Despite the complexity of aforementioned processes, they may be considered as remarkably simple as compared to the following issue: on which basis will a substratum-adhering cell chose its subsequent behaviour? This question will certainly initiate many lines of research during the following years, and it is certainly located at the frontier of current biological knowledge. While an in-depth discussion of this problem would not fit within the scope of this review, we shall emphasize some points that may be relevant.

The biochemical approach to cell activation

It has long been known that cell adhesion strongly influenced cell behaviour. The most straightforward interpretation for a cell biologist or biochemist would certainly consist of assuming that the dominant phenomenon is the stimulation of cell membrane receptors, resulting in the triggering of a cascade of biochemical events and second messenger generation. There is indeed much evidence showing that the nature of engaged membrane receptors will influence further events. Taking a simple example among many others, if a rat macrophage encounters an antibody-coated particle, it will engulf it as a consequence of proper stimulation of immunoglobulin receptors. However, if the same particle is bound through a lectin interacting with other membrane structures, no ingestion will follow (Capo *et al.*, 1978). Further, a quite detailed knowledge was obtained on the coupling between cell stimulation and simple response patterns. Thus, nearly complete reconstitution of the biochemical machinery involved in the generation of a simple process such as the phagocyte oxidative burst is conceivable (e.g. Price *et al.*, 2002). Therefore, it might be tempting to speculate that cell behaviour might be understood and even predicted through detailed identification of the nature and number of receptors engaged in a given interaction.

However, while enormous progress was done in the identification of activating pathways, biochemical studies revealed the existence of a complex network of triggers that cannot yet be understood with currently available biological tools, and many reports suggest that new concepts are required to integrate available information (see e.g. Charest and Pelech, 1998; Vilar *et al*., 2003). An important issue that might lead to huge simplification of this problem would be to know whether there is only a limited number of cell programs liable to stimulation at a given moment. A positive answer is indeed suggested by recent studies on cell transcriptome.

Importance of nonbiochemical signals in the determination of cell behaviour

While the biochemical view of cell guidance is certainly a dominant one, on the basis of the number of published papers, a steady flow of convincing reports strongly supports the view that cell responses are not only dependent on the nature of free or bound ligands detected in nearby environment. We shall briefly consider mechanical, physico-chemical and topographical cues.

Substratum mechanical properties. It has long been demonstrated that adherent cells exert forces on underlying surfaces (Harris *et al*., 1980). Further, cell behaviour was also shown to depend on substratum flexibility and some mechanistic information was recently reported (Wang *et al*., 2001).

Substratum nonspecific properties such as charge and hydrophobicity. The influence of these properties on cell adhesion has been discussed above. In addition, these features clearly influence many cell functions (Allen *et al*., 2003). The basic question is to know whether this action is mediated by a few well-defined receptor species, as previously argued.

Surface topography. There is ample evidence that the functions of an adherent cell are strongly influenced by the geometrical properties of contact areas (Pierres *et al.*, 2002). Thus, cell proliferation was demonstrated to be highly correlated to available adhesion area (Chen *et al.*, 1997). In addition to the total contact area, there is now compelling evidence that nanoscale surface features may strongly influence adherent cells (Dalby *et al.*, 2002 ; Schneider *et al*., 2003).

Conclusion. There is obviously a need to integrate the concepts that were only briefly sketched. Clearly, at least two pathways may be considered. **A first approach** might consist of trying to follow the lines of thought that were successful in the past. Thus, it might be argued that cell surface receptor stimulation is not only dependent on ligand recognition but also on topographical reorganization. Thus, a common mechanism of signal generation may consist of bringing a suitable kinase in close contact with a potential target, thus allowing tyrosine phosphorylation and generation of binding sites for scaffold proteins. Also, since it is well demonstrated that forces can change protein conformation, surface mechanical properties might affect the forces exerted on ligand proteins and influence the appearance of binding sites. According to this view, there might be a need to look for accurate relationships between ligand topography and receptor activation. A **second approach** might be to consider that biology must shift away from reductionism and aims at develop new methods to deal with biocomplexity. A notable example is the concept of tensegrity, suggested by Ingber (2003) as a means of overcoming difficulties presently met by biologists.

General Conclusion

While the present review certainly illustrates the need for further work rather than provides answers to specific problems, we wish to suggest some conclusions.

First, although we may hope to be able to manipulate cell-surface interaction before we fully understand how new procedures work, it is certainly warranted to look for such an understanding, which may suggest new experiments and new questions, in addition to alleviate the burden imposed on scientists memory.

Second, among the many theoretical frameworks that were discussed, perhaps the relevant question is to know which one is most fruitful, rather than determining which is true. Indeed, many experiments have shown that different approaches may yield complementary information, and no basic principle was completely disproved.

Third, before new tools are developed to study biocomplexity, it is suggested that the "specific interaction approach" may still be used. This would consist of identifying cell surface receptors involved in the interaction between a given cell population and a particular surface, thus examining (possibly at the single molecule level) the mechanisms of interactions between cells and surfaces, and last identifying the initial triggering mechanisms. At this stage, probably new tools will be required to deal with biological complexity.

References

Absolom DR, Zingg W, Thomson C, Policova Z, Van Oss CJ, Neumann AW (1985) Erythrocyte adhesion to polymer surfaces. J. Colloid Interface Sci **104**: 51-59.

Adamson AW (1976) Physical Chemistry of Surfaces. Wiley, New York.

Allen LT, Fox EJP, Blute I, Kelly ZD, Rochev Y, Keenan AK, Dawson KA, Gallagher WM (2003) Interaction of soft condensed materials with living cells: phenotype/transcriptome correlations for the hydrophobic effect. Proc Natl Acad Sci USA **100**: 6331-6336.

Andrade JD, Hlady V (1987) Plasma protein adsorption: the big twelve. Ann NY Acad Sci **516**: 158-172.

Baier RE, Weiss L (1975) Demonstration of the involvement of adsorbed proteins in cell adhesion and cell growth on solid surfaces. Adv Chem Ser **145**: 300-307.

Barclay N (1998) Concluding remarks and the challenge from the immune system. Faraday Disc **111**: 345-350.

Bell GI. (1978) Models for the specific adhesion of cells to cells. Science **200**: 618-627.

Bell GI, Dembo M, Bongrand P (1984) Cell adhesion: competition between nonspecific repulsion and specific bonding. Biophys J **45**: 1051-1064.

Bisognano C, Vaudaux PE, Lew DP, Ng EYW, Hooper DC (1997) Increased expression of fibronectin-binding proteins by fluoroquinolone-resistant Staphylococcus aureus exposed to subinhibitory levels of ciprofloxacin. Antimicrob Agents Chemother **41**: 906-913.

Bongrand P (1999) Ligand-receptor interactions. Rep Prog Phys **62**: 921-968.

Bongrand P, Bell GI (1984) Cell-cell adhesion : parameters and possible mechanisms. In: Cell Surface Dynamics: Concepts and Models. Perelson AS, DeLisi C, Wiegel FW (eds). Marcel Dekker, New York, pp. 459-493.

Bongrand P, Capo C, Depieds R (1982) Physics of cell ddhesion. Progr Surface Sci **12**: 217-286.

Bongrand P, Capo C, Mège JL, Benoliel AM (1988)

Surface physics and cell adhesion. In: Physical Basis of Cell-Cell Adhesion. Bongrand P (ed). CRC Press, Boca Raton, FL. pp 91-123.

Capo C, Bongrand P, Benoliel AM, Depieds R (1978) Dependence of phagocytosis on strength of phagocyte-particle interaction. Immunology **35**: 177-182.

Capo C, Garrouste F, Benoliel AM, Bongrand P, Depieds R (1981) Nonspecific binding by macrophages : evaluation of the influence of medium-range electrostatic repulsion and short-range hydrophobic interaction. Immunol Commun **10** : 35-43.

Charest DL, Pelech SL (1998) Intracellular communication *via* protein kinase networks. In: Dynamical Networks in Physics and Biology - At the Frontier of Physics and Biology. Beysens D, Forgacs G (eds). EDP Sciences Paris/Springer Berlin, pp 189-200.

Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE (1997) Geometric control of cell life and death. Science **276**: 1425-1428.

Clackson T, Wells JA (1995) A hot spot of binding energy in a hormone-receptor interface. Science **267**: 383-386.

Curtis ASG (1967) The Cell Surface - Its Molecular Role in Morphogenesis. Logos Press - Academic Press, London, pp 105-110.

Cyster JC, Shotton DM, Williams AF (1991) The dimension s of the T lymphocyte glycoprotein leukosialin and identificaiton oflinear protein epitopes that can be modified by glycosylation. EMBO J **10**: 893-902.

Dalby MJ, Yarwood SJ, Riehle MO, Johnstone HJ, Affrossman S, Curtis AS (2002) Increasing fibroblast response to materials using nanotopography : morphological and genetic measurements of cell response to 13-nm-high polymer demixed islands. Exp Cell Res **276**: 1-9.

Florin EL, Moy VT, Gaub HE (1994) Adhesion forces between individual ligand-receptor pairs. Science **264**: 415-417.

Fraser I, Hughes D, Gordon S (1993) Divalent cation-independent macrophage adhesion inhibited by monoclonal antibody to murine scavenger receptor. Nature **364**: 343-346.

Geisel J, Steuer MK, Ko HL, Beuth J (1995) The role of ABO blood groups in infections induced by Staphylococcus saprophyticus and Pseudomonas aeruginosa. Int J Med Microbiol Virol Parasitol Infectious Dis **282**: 427-430.

Gerson DF, Capo C, Benoliel AM, Bongrand P (1982) Adhesion, phagocytosis and cell surface energy : the binding of fixed human erythrocytes to rat macrophages and polymethylpentene. Biochim Biophys Acta **692**: 147-156.

Giordano R, DL Fouts, Tewari D, Colli W, Manning JE, Alves MJM (1999) Cloning of a surface membrane glycoprotein specific for the infective form of Trypanosoma cruzi having adhesive properties to laminin. J Biol Chem **274**: 3461-3468.

Grinnell F, Feld MK (1982) Fibronectin adsorption on hydrophilic and hydrophobic surfaces detected by antibody binding and analyzed during cell adhesion in serum-containing medium. J. Biol. Chem. **257**: 4888-4893

Harris AK, Wild P, Stopak D (1980) Silicone rubber substrata: a new wrinkle in the study of cell locomotion. Science **208**: 177-179.

Heinrich L, Voegel JC, Schaaf P (1999) Direct observation of the anchoring process during the adsorption of fibrinogen on a solid surface by force-spectroscopy mode atomic force microscopy Proc Natl Acad Sci USA **96**: 6705-6710.

Ingber DE (2003) Tensegrity II. How structural networks influence cellular information processing networks. J Cell Sci **116**: 1397-1408.

Israelachvili JN (1991) Intermolecular and Surface Forces. Academic Press, New York, 2nd edition. pp 246-250.

Kaplanski G, Farnarier C, Tissot O, Pierres A, Benoliel AM, Alessi MC, Kaplanski S, Bongrand P (1993) Granulocyte-endothelium initial adhesion. Analysis of transient binding events mediated by E-selectin in a laminar shear flow. Biophys J **64**:1922-1933.

Koenig AL, Gambillara V, Grainger DW (2003) Correlating fibronectin adsorption with endothelial cell adhesion and signaling on polymer substrates. J Biomed Mater Res **64A**: 20-37.

Lassen B, Malmsten M (1997) Competitive protein adsorption at plasma polymer surfaces. J Colloid Interface Sci **186**: 9-16.

Lawrence, MB, Springer TA (1991) Leukocytes roll on a selectin at physiologic flow rates : distinction from and prerequisite for adhesion through integrins. Cell **65**: 859-873.

Margenau H, Kestner M (1969) Theory of Intermolecular Forces. Pergamon Press, Oxford.

Mège JL, Capo C, Benoliel AM, Bongrand P (1984) Nonspecific cell surface properties: contact angle of water on dried cell monolayers. Immunol Commun **13**: 211-217.

Mège JL, Capo C, Benoliel AM, Bongrand P (1987) Use of cell contour analysis to evaluate the affinity between macrophages and glutaraldehyde-treated erythrocytes. Biophys J **52**: 177-186.

Mehrishi JN (1972) Molecular aspects of the mammalian cell surface. Progr Biophys Mol Biol **25**: 3-70.

Merkel R, Nassoy P, Leung A, Ritchie K, Evans E (1999) Energy landscapes of receptor-ligand bonds explored with dynamic force spectroscopy. Nature **397**: 50-53.

Morra M, Cassinelli C (1997) Bacterial adhesion to polymer surfaces: a critical review of surface thermodynamic approaches. J Biomater Sci Polymer Edn **9**: 55-74.

Napper DH (1977) Steric stabilization. J. Colloid Interface Sci **58**: 390-407.

Neumann AW, Good RJ, Hope CJ, Sejpal M (1974) An equation-of-state approach to determine surface tensions of low energy solids from contact angles. J Colloid Interface Sci **49**: 291-305.

Norde W (2000) Proteins at solid surfaces. In: Physical Chemistry of Biological Interfaces. Baszkin A, Norde W (eds). Marcel Dekker, New York. p 115.

Pearson AM (1996) Scavenger receptors in innate immunity. Curr Opin Immunol **8**: 20-28.

Pierres A, Benoliel AM, Bongrand P (1998) Interactions between biological surfaces. Curr Opin Colloid Interface Sci **3**: 525-533.

Pierres A, Benoliel AM, Bongrand P (2002) Cell fitting to adhesive surfaces : a prerequisite to firm attachment and subsequent events. Eur Cells Mater **3**: 31-45.

Pitt WG, Park K, Cooper SL (1986) Sequential protein adsorption and thrombus deposition on polymeric biomaterials. J. Colloid Interface Sci **111**: 343-162.

Price MO, McPhail LC, Lambeth JD, Han CH, Knaus UG, Dinauer MC (2002) Creation of a genetic system for analysis of the phagocyte respiratory burst: high-level reconstitution of the NADPH oxidase in a nonhematopoietic system Blood **99**: 2653-2661.

Schneider GB, Perinpanayagam H, Clegg M, Zaharias R, Seabold D, Keller J, Stanford C (2003) Implant surface roughness affects osteoblast gene expression. J Dental Res **82**: 372-376.

Seifert U. (2000) Rupture of multiple parallel molecular bonds under dynamic loading. Phys Rev Lett **84**: 2750-2753.

Sherbet GV (1978) The biophysical characterization of the cell surface. Academic Press, London, pp 36-145.

Springer TA (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration : the multistep paradigm. Cell **76**: 301-314.

Takeshita A, Murakami Y, Yamashita Y, Ishida M, Fujisawa S, Kitano S, Hanazawa S (1998) Porphyromonas gingivalis fimbriae use beta(2) integrin (CD11/CD18) on mouse peritoneal macrophages as a cellular receptor, and the CD18 beta chain plays a functional role in fimbrial signaling. Infection Immunity **66**: 4056-4060.

Tözeren A (1990) Cell-cell conjugation. Transient analysis and experimental implications. Biophys J **58:** 641-652.

Tözeren A, Sung KLP, Chien S (1989) Theoretical and experimental study on cross-bridge migration during cell disaggregation. Biophys J **55**: 479-487.

Van Oss CJ, Gillman CF, Neumann AW (1975) Phagocytic engulfment and cell adhesiveness as cellular surface phenomena, Marcel Dekker, New York. pp 29-30.

Van Oss CJ, Chaudury MK, Good RJ (1987) Monopolar surfaces. Adv Colloid Surface Sci **28**: 35-64.

Vilar JMG, Guet CC, Leibler S (2003) Modeling network dynamics : the *lac* operon, a case study. J Cell Biol. **161**: 471-476.

Vitte J, Benoliel AM, Eymeric P, Bongrand P, Pierres A. (2004) Beta 1 integrin-mediated adhesion may be initiated by multiple incomplete bonds, thus accounting for the functional importance of receptor clustering. Biophys J. **86**: 4059-4074.

Wang HB, Dembo M, Hanks SK, Wang YL (2001) Focal adhesion kinase is involved in mechanosensing during fibroblast migration. Proc Natl Acad Sci USA **98**: 11295-11300.

Watarai M, Funato S, Sasakawa C (1996) Interaction of ipa proteins of Shigella flexneri with alpha(5)beta(1) integrin promotes entry of the bacteria into mammalian cells. J Exp Med **183**: 991-999.

Yakubenko VP, Lishko VK, Lam SCT, Ugarova TP (2002) A molecular basis for integrin $a_M b_2$ ligand binding promiscuity. J Biol Chem **277**:48635-48642.

Yu ZW, Calvert TL, Leckband D (1998) Molecular forces between membranes displaying neutral glycosphingolipids: Evidence for carbohydrate attraction. Biochemistry **37**: 1540-1550.

Zhu C, Long M, Chesla SE, Bongrand P (2002) Measuring receptor/ligand interaction at the single-bond level: experimental and interpretative issues. Ann. Biomed. Engineering **30**: 305-314.

Discussion with Reviewers

A.S.G. Curtis: Since nearly all macromolecules either aid adhesion or retard it there may be no such thing as a neutral molecule to use as a control in adhesion experiments. Comment please.

Authors: This question raises a very important and often overlooked problem: if you wish to test the hypothesis that a cell specifically binds to a surface coated with a given molecular species, say A, it is tempting to use as a control a so-called neutral molecule B. However, results may be difficult to interpret: if the cells stick to both A and B, it is difficult to rule out the possibility that the cell has receptors specific for B, in view of the variety and promiscuity of cell membrane receptors. Conversely, if the cell sticks to A, not to B, you may not exclude the possibility that B is an anti-adhesive molecule, and A generates nonspecific adhesion. Thus, another strategy is needed: You may try so-called blocking experiments. Antibodies may be quite useful, but they are not neutral molecules: coating a cell or a surface with antibodies may generate steric hindrance, preventing cell-to-surface approach, and what you test is then the cell ability to bind immunoglobulin. A more satisfactory way of studying specific cell-surface interactions is to alter selectively binding sites. Thus, if you are able to block interactions between cells or particles and avidin-coated surfaces by adding the small biotin molecule in the fluid phase, you may conclude that attachment was generated by bona fide avidin-biotin interactions. However, blocking is not always feasible, since it is difficult to block interactions between surface-bound molecules with soluble ligand, as a consequence of the importance of multivalency. Another control would consist of comparing a macromolecule with a similar molecule with a few mutations specifically altering binding sites. Clearly, this may be difficult to achieve. A reasonable procedure would consist of coating non-adhesive surfaces with the molecules you wish to test. Thus, if a cell does not adhere to PEG-coated surfaces, if you couple type A molecules to PEG (i.e. polyethyleneglycol) and you induce adhesion, it seems reasonable to conclude that cells can bind to A.

A.S.G. Curtis: How does my statement equate with the authors' view that there may be only a few cell membrane molecules involved in adhesion?

Authors: While many cell surface molecules have a potential to influence adhesion, it is often found that adhesion is dominated by one or a few molecular species under specific experimental conditions. In this case, the strategy we described may be used to analyze interactions.