van der Waals forces influencing adhesion of cells

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Adhesion molecules, often thought to be acting by a ‘lock and key’ mechanism, have been thought to control the adhesion of cells. While there is no doubt that a coating of adhesion molecules such as fibronectin on a surface affects cell adhesion, this paper aims to show that such surface contamination is only one factor in the equation. Starting from the baseline idea that van der Waals force is a ubiquitous attraction between all molecules, and thereby must contribute to cell adhesion, it is clear that effects from geometry, elasticity and surface molecules must all add on to the basic cell attractive force. These effects of geometry, elasticity and surface molecules are analysed. The adhesion force measured between macroscopic polymer spheres was found to be strongest when the surfaces were absolutely smooth and clean, with no projecting protruberances. Values of the measured surface energy were then about 35 mJ m$^{-2}$, as expected for van der Waals attractions between the non-polar molecules. Surface projections such as abrasion roughness or dust reduced the molecular adhesion substantially. Water cut the measured surface energy to 3.4 mJ m$^{-2}$. Surface active molecules lowered the adhesion still further to less than 0.3 mJ m$^{-2}$. These observations do not support the lock and key concept.

1. Introduction

van der Waals first postulated attractive forces acting between all atoms in 1873 to account for the incompressibility and condensation of gases. These ubiquitous attractions arise from instantaneous dipoles in one atom which induce a dipole in a neighbouring atom to give a weak attractive force. Bradley [1] found a way of adding these atomic attractions together as extended by Hamaker [2], while London [3] derived a quantum theory which fitted the earlier calculations of Lennard-Jones [4] to explain the properties of inert atoms like argon. Although these forces are far weaker than ionic or covalent bonds, they have the benefit that they are universal and can account for the adhesion of simple materials like paraffins or elastomers which do not have electrostatic, dipole or electron sharing capacity. Descriptions of these forces can be found in several papers and books [5–8]. As the van der Waals force must be acting in the adhesion of cells, it is necessary to consider its effect before adding in any other possibilities such as polar, dipole or covalent forces.

Cells which float freely around the body without adhering strongly to other structures, for example red blood cells (erythrocytes), may possibly be described through these weak van der Waals adhesive forces alone, because their attractions are comparable to Brownian impulses, very much weaker than those measured by atomic force microscopy (AFM) and other conventional adhesion measurements [9], also much weaker than chemical bonds.

The purpose of this paper is to review the early measurements of van der Waals adhesion from the early 1970s, based on the Young–Laplace concept of short-range attractions giving a surface energy $\gamma$ in a quasi-static system. By considering the contact of elastic bodies, it was evident that three parameters generally enter the equation for adhesive force $F$, as indicated in the equation [10]

$$F = K \left[ \frac{W E g^3}{(1 - \nu^2)} \right]^{1/2}$$

(1.1)
where \( K \) is a constant, \( W \) (equivalent to \( 2\gamma \) for identical material surfaces in contact) the work of adhesion in J m\(^{-2}\), \( E \) the elastic modulus in Pa, \( \nu \) the Poisson’s ratio and \( d \) the dimension in metres. From this model, it is clear that the adhesion molecules have an effect on \( W \), but elasticity \( E \) is equally influential and the geometry \( d \) is much more important. In this paper, the effects of geometry, elasticity and surface molecules are analysed. Finally, it is necessary to consider the range of the attractions close to the contact point in a dynamic situation where cells are attaching and detaching under Brownian conditions.

2. Adhesion theory based on surface energy

During the late 1960s, Roberts [11] found a way of moulding a rubber sphere with a very smooth surface by pressing and cross-linking the hot rubber material into a concave glass lens. When such a smooth, spherical rubber moulding was pressed into contact with another smooth sphere or flat surface, a black contact spot was formed as illustrated in figure 1, when viewed in reflected light using the microscope. Typically, a 1 mm radius black spot was seen at zero load and this could be increased by applying a normal force to press the surfaces further together. A few imperfections in the black spot due to dust particles were visible but the soft rubber deformed around them, so the defects had negligible influence.

It was interesting that, under clean dry conditions, the black spot was larger than expected from the well-established Hertz theory of pure elastic contact, indicating there was an adhesion force pulling the surfaces together. Additionally, it was clear that a tension force was necessary to pull the rubber surfaces apart to overcome this adhesion as shown in figure 2. Even when all precautions were taken to ensure that there was no electrostatic or dipole force present, the adhesion persisted.

The solution to this puzzle emerged when fracture mechanics theory was applied to the problem, balancing the surface energy released at the contact with the elastic energy stored in the deformed rubber, plus the potential energy of the applied force [12]. The diameter of the contact spot \( d \) (i.e. \( 2a \)) under a load \( F \) with work of adhesion \( W \) for two equal rubber spheres of diameter \( D \) was found to be given by the so-called JKR equation

\[
d^3 = \frac{3(1 - \nu^2)D[F + 3\pi WD/4 + [3\pi WD/2]^{1/2}]}{E} \]

The value of surface energy \( \gamma \) needed to fit the experimental results was 35 mJ m\(^{-2}\) which was the value expected for van der Waals forces acting between CH\(_2\) groups on the elastomer surfaces.

At zero applied force \( F = 0 \), this resolved into

\[
d^3 = \frac{9\pi WD^2(1 - \nu^2)/2E}{(2.1)}
\]

showing that the size of the black contact spot was a direct measure of the cube root of van der Waals surface energy but also depended on sphere radius and elastic modulus. Also apparent was the strong effect of surface roughness of the rubber sphere. Even slight scratches, abrasions or deliberate dust coverage reduced the adhesion strongly. The conclusion
The optical system developed later [13] allowed the black spot to be viewed under water, where the reflected interference fringes of the ordinary Newton’s rings pattern had previously lost intensity and visibility but still had been diminished. The black spot radius dropped under water (figure 3, middle curve) showing that the van der Waals adhesion was shielded by the water molecules as expected from theoretical analysis [7]. Addition of surface active molecules lowered the surface energy still further, as illustrated in figure 3, bottom curve, almost fitting Hertz elastic theory and suggesting a surface energy of less than 0.3 mJ m⁻².

The surface energy of the smooth rubber under water was 3.4 mJ m⁻² when fitted to the JKR equation. With SDS surfactant, the surface energy fell to less than 0.3 mJ m⁻². This result was somewhat unexpected because it is known that organisms like ants often exude a fluid such as water containing adhesion molecules to increase the adhesion of their feet to leaf surfaces [14]. The explanation of this effect is that liquids can extend the contact area between rough surfaces considerably. Thus, even though the attractive forces between the molecules on the ants’ feet diminish, the contact area between the rough surfaces rises so much due to the liquid that the overall force of adhesion is higher. Therefore, the liquid should be viewed as a sealant rather than an adhesive because it fills gaps while reducing molecular adhesion.

The effect of elastic modulus on the adhesion force was shown in figure 3. The JKR fit of the experimental results gives a surface energy of 35 mJ m⁻² for the rubber spheres pressed together. The Hertz model gives a lower estimate of 3.4 mJ m⁻². The difference in energy comes into play [6]. In general, the shape of the potential is not known but can be postulated for molecular modelling in order to predict the forces and the structures [18].

3. Influence of the range of van der Waals force

The theory based on the one parameter of surface energy is an approximation which gives a good fit to experiments at the macroscopic scale because the range of van der Waals force is so short. However, when considering adhesion at the nanometre scale of cell membranes and adhesion molecules, it is necessary to consider how the potential varies with distance, such that two parameter models are required and even more parameters may be needed as other bonding mechanisms come into play [6]. In general, the shape of the potential is not known but can be postulated for molecular modelling in order to predict the forces and the structures [18].

The most basic potential curve is the square well which was used in computer calculations for hard spheres in the 1950s by Alder & Wainwright [19]. This ideal concept has allowed a cell counting method for measuring and understanding cell adhesion to be devised. The object was to remove the need for probes because these damage the cells and change the conditions. Also, the idea was to produce an absolute measure of cell adhesion which did not demand new definitions of binding. It seemed logical to define adhesion of cells in terms of the two parameter model of adhesion interactions in which there is an adhesion energy ɛ and a range a as shown in figure 4.

A spherical particle approaches its neighbour at constant speed until, at a certain separation λa, the particles are attracted to each other with an energy ɛ. If this energy remains constant until the spheres touch rigidly at the point of contact, then the square well potential is revealed. The approaching sphere travels at constant speed, is accelerated into the potential well, reflects rigidly on contact, and then is decelerated as the particles move apart. Stainton [9,20] solved this problem to predict the number of doublets in a suspension. The result is that the ratio of doublets to singlets N₂/N₁ is proportional to the volume fraction ϕ of the cells and depends on the range λ and the energy ɛ of the well.
4. Experiment on erythrocyte adhesion

Red blood cells (erythrocytes) were used because of their low and reversible adhesion [22]. Experiments on aggregation of red cells have been fundamental to the understanding of blood types and many complex diseases including infections due to malaria and virus parasites [23–25]. But the problems of purifying and standardizing red cells remain significant [24].

In this study, cells were prepared from three species, human blood from North Staffordshire Hospital, fresh horse blood in EDTA and fresh rat blood from Central Animal Pathology Ltd. Each blood sample was washed six to seven times in phosphate buffered saline to remove the non-red cell components. The control sample of horse cells in isoton showed some weak adhesion, whereas glutaraldehyde, a cell fixative and preservative, reduced the adhesion by about 25%, whereas fibronectin, an adhesion molecule [28], increased the adhesion significantly, whereas glutaraldehyde, a cell fixative and preservative, reduced it modestly. Typical error bars were between 5 and 20%.

The control sample of horse cells in isoton showed somewhat weaker adhesion than the sample shown in table 1. Such variation was found to be common in different samples of horse blood. Differences between animals in type, age, etc., and also in blood cell conditioning had a distinct influence [23]. It is evident from the results that glutaraldehyde reduced the adhesion by about 25%, whereas fibronectin increased the adhesion by 10%, changes which were comparable with the effects seen on human red cells. The results indicate that the adhesion molecules were having only slight effects on the adhesion, suggesting that a slight perturbation of van der Waals attraction could explain the outcome.

5. Conclusion

The adhesion of cells may be compared with that measured on smooth elastomer or gelatin spheres. Theory and experiment showed that van der Waals forces were causing adhesion for these materials. The diameter of contact, the effect of elasticity and sphere radius were explained. The ‘lock and key’ model was not consistent with the results.
The adhesion was reduced by water and further diminished by surface active molecules and surface projections. Adhesion molecules reduced the adhesion; they did not cause adhesion.

Adhesion tests were developed for red blood cells, based on counting the doublets formed in competition with Brownian impacts. A square well potential was assumed, on counting the doublets formed in competition with

References