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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 γ 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]

$$F = K\left[\frac{WEd^3}{(1-\nu^2)}\right]^{1/2}, \tag{1.1}$$

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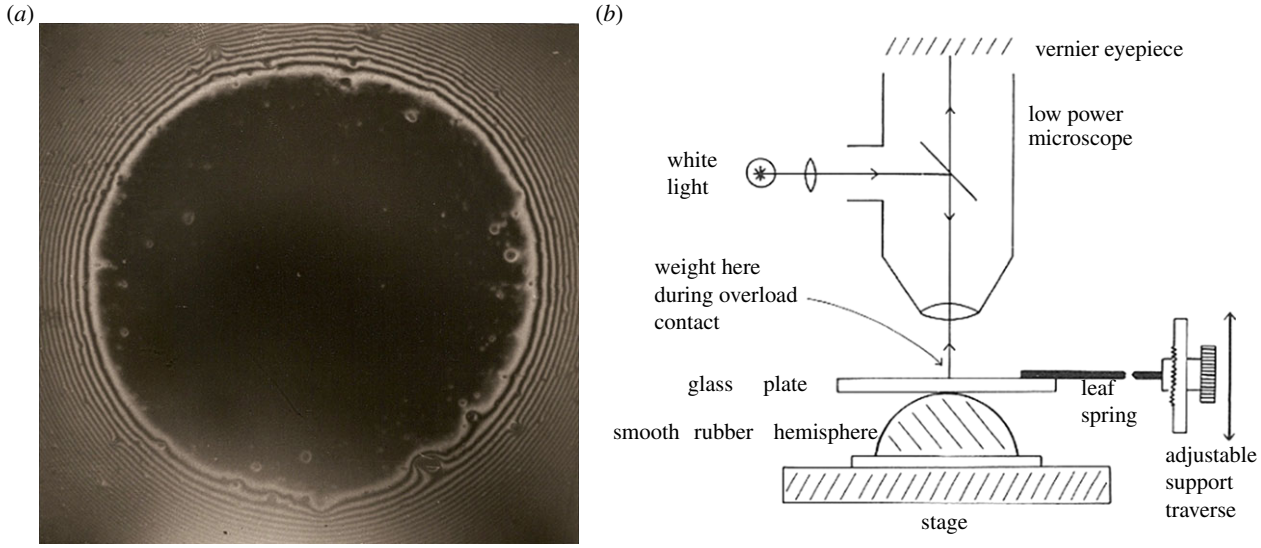

Figure 1. (*a*) Black contact spot of smooth rubber sphere viewed in microscope as shown in (*b*) [12]. (Online version in colour.)

where K is a constant, W (equivalent to 2γ for identical material surfaces in contact) the work of adhesion in $\mathrm{J\,m^{-2}}$, E the elastic modulus in Pa, ν 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 pushed 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

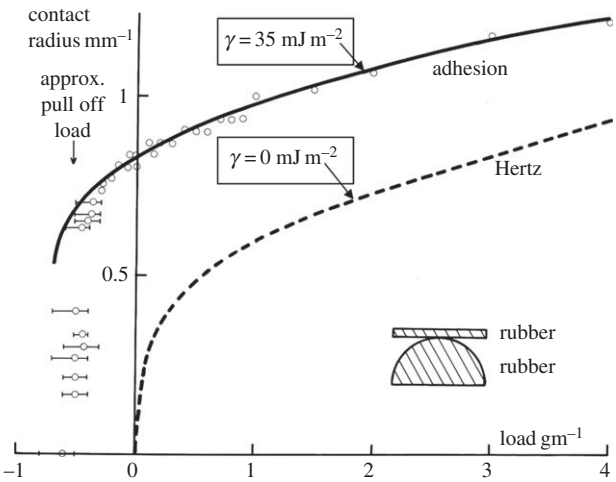

Figure 2. Smooth rubber adhesion results showing the contact circle radius as a function of normal applied force fitting JKR adhesion theory to compare with Hertz pure elastic contact. The adhesion pull-off force of −0.6 g was evident [12].

W for two equal rubber spheres of diameter D was found to be given by the so-called JKR equation

$$d^3 = 3(1-\nu^2)D\{F + 3\pi WD/4 + [3\pi WDF/2 + (3\pi WD/4)^2]^{1/2}\}/E \tag{2.1}$$

The value of surface energy γ needed to fit the experimental results was 35 $\mathrm{mJ\,m^{-2}}$ 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

$$d^3 = 9\pi WD^2(1-\nu^2)/2E \tag{2.2}$$

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

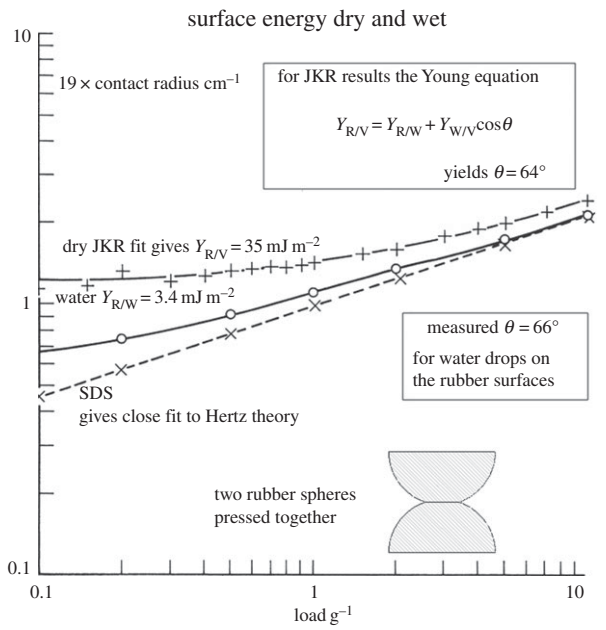

Figure 3. Log–log plot of the black spot contact radius against applied normal force for two smooth contacting elastomer spheres, compared to JKR theory (full lines) and Hertz elastic theory (broken line) [12].

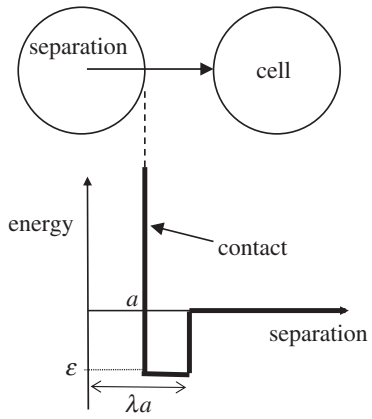

Figure 4. Potential energy versus separation of two spherical particles radius a, with a square well potential of depth ε and extent λa [28].

was that projections on the surface required by a 'lock and key' model would diminish van der Waals adhesion.

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 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 brought into focus by considering fracture mechanics analysis applied to a rigid cylindrical punch adhering to a soft elastic solid such as gelatin/water solution cooled to room temperature [10]. Equation (1.1) fitted the experimental results with a surface energy of 52 mJ m^{-2} and indicated that adhesive forces to cells measured with rigid probes, as in AFM experiments, depend not just on the molecules and probe diameter but also on the elastic properties of the cells. Of course, the problem with these equations is that they relate only to equilibrium conditions. Lossy circumstances, for example when cells are viscoelastic, cause large deviations from the theory that are still not fully explained.

Since these early results concerning van der Waals force influencing cell adhesion, many more recent papers have appeared discussing such effects [15–17].

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 objective 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 ε and a range λ 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 ε. 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 N_2/N_1 is proportional to the volume fraction ϕ of the cells and depends on the range λ and the energy ε of the well

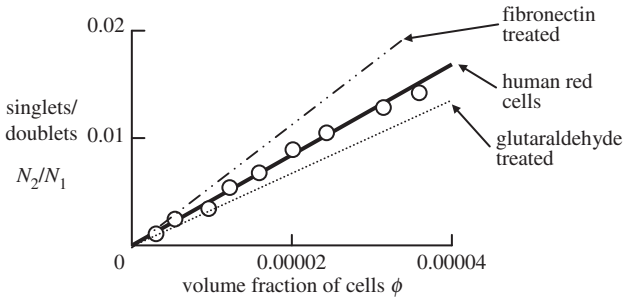

Figure 5. Ratio of doublet/singlet red cells versus volume fraction of cells [9].

according to the equation

$$N\frac{N_2}{N_1^2} = 4\phi(\lambda^3 - 1)\exp\left(\frac{\varepsilon}{kT}\right) \approx \frac{N_2}{N_1}, \tag{3.1}$$

where N is the total number of cells. The conclusion of this argument is that a plot of doublet to singlet ratio versus particle volume fraction should yield a straight line passing through the origin. The gradient of the line is a measure of the adhesion which depends on range and energy of the interactions [9]. Thus a high gradient signifies high adhesion and a low gradient low adhesion. The experimental objective was to define this gradient for three different species of red cells, horse, rat and human, approximating to spheres, and to explore its variation with the addition of adhesion molecules. The key advance was the most sensitive measure of adhesion ever achieved, with resolution of femtonewton forces [21].

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, before suspending in physiological saline solution, then examined by both optical and Coulter tests. Each species of cell was treated in three ways to judge the effect of surface adhesion molecules by adding glutaraldehyde, a preservative, or fibronectin, an adhesion molecule.

The cells were placed in an accurately defined 10 μm space within a glass apparatus which was imaged using a video microscope at 40× magnification. Each cell could then be clearly seen moving around with Brownian movement, while not overheating as occurred at 100× magnification. Pictures of the cells were taken at random locations in the space and the numbers of doublets and singlets were counted by image analysis software. Taking the ratio of doublets to singlets, the gradient $(N_2/N_1)\varphi$ was obtained. The effect of glutaraldehyde in reducing adhesion was noted, while fibronectin gave a slight increase in the gradient figure 5 [9].

Horse and rat erythrocytes were then tested in the same way and shown to give significantly higher adhesion. Baskurt *et al.* [26] have shown that the aggregation of such cells is increased over human cells, but volume fraction effects were not taken into account. Popel *et al.* [27] recognized that horse cells stick better and this was attributed to the athletic nature of the animal. Table 1 quantifies the difference of adhesion in terms of the gradient $(N_2/N_1)\phi$.

Table 1. Comparison between adhesion of various red cells.

animal	gradient $(N_2/N_1)\phi$
horse	1488 ± 200
rat	750 ± 4
human	420 ± 5

Table 2. Effect of surfactants on horse red cell adhesion.

horse cell treatment	adhesion number $(N_2/N_1)\phi$
isoton	1279 ± 203
isoton + glutaraldehyde	1020 ± 162
isoton + fibronectin	1399 ± 184

These results show conclusively that rat cells are almost twice as sticky as human red cells, while horse erythrocytes are almost twice as adhesive as rat cells. Whether this can be explained in terms of the higher energy of the bonds ε, as defined by equation (3.1), or in larger range λ of bonds remains to be determined. The important conclusion stemming from these results is that cell adhesion can be measured very sensitively, giving added impetus to the quest to resolve the theories of the different attractions observed. Understanding the attractive interaction potential curve remains a challenge for the future.

Addition of surface active molecules to the cell suspension was also studied. The results for horse cells are illustrated in table 2 which shows that 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, but the effects of adhesion energy and range could not be distinguished in this model. The number of doublets was found to be proportional to the red cell concentration as expected from the theory. Very sensitive measures of adhesion were obtained which could distinguish between horse, rat and human red cell adhesion. Adhesion molecules caused rather slight changes in the observed adhesion, suggesting that modest variations in the van der Waals shielding could be the cause.

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