Johnson-Kendall-Roberts Theory Applied to Living Cells

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ABSTRACT

The Johnson-Kendall-Roberts (JKR) theory is an accurate model for of soft deformable materials with strong adhesion energies. Little is known about the validity of this theory on complex systems such as living cells. We have addressed this question using a depletion controlled cell adhesion and measured the force necessary to separate the cells with a micropipette technique. We show that the cytoskeleton can provide the cells with a 3D structure that is sufficiently elastic and has a sufficiently low deformability for JKR theory to be valid. When the cytoskeleton is disrupted, JKR theory is no longer applicable.

Keywords: cell adhesion, depletion forces, elastic spheres, elastic shells, cytoskeleton

1 INTRODUCTION

In living matter, interactions and bonds are unceasingly formed and broken between various molecules or organised aggregates and cells[1]. These interactions occur either spontaneously or after a signal which triggers production of the molecular species corresponding to the programmed biological phenomenon. Cell adhesion is involved in a large number of biological processes such as the defences of organism, transport, metastasis invasion[2]. A majority of cells can divide and multiply only when they adhere to other cells or tissues. The adhesion between cells if often a way to exchange signals, for instance mediated by a cross-talk between different families of cell adhesion molecules.

There is an increasing interest in understanding biological adhesion in views of designing new anti-inflammatory drugs, new biomaterials, understanding the processes linked to metastasis proliferation, and also control the properties of numerous materials nowadays designed to have increasing sophisticated functions. There is a large difference between the adhesion of inert materials and biological ones. In general, the latter is triggered by a signal while the former only depends on more static physico-chemical features. In contrast to inert materials, the adhesion of cells always relies on receptor/ligand (often named key/lock) interactions which consist in molecules from one cell that recognise those of another cell. This molecular recognition always results from known physico-chemical interactions (van der Waals forces, electrostatic forces, hydrophobic interactions, hydrogen bonds…) combined with a particular geometry of the molecules. In spite of these complications, several physical methods allow to investigate this biological adhesion either by studying simplified models with a limited number of parameters, or by studying a single bond between cell adhesion molecules, or by investigating realistic biological systems such as cells for which it is still possible to obtain some physical description.

This study deals with the adhesion of living cells and some theoretical description of adhering spheres and shells. An experimental procedure for the measurement of the adhesion strength is presented. This procedure is applied to cells subjected to an artificial adhesion, produced by deletion forces, and the results are compared to theory.

2 ADHESION OF LIVING CELLS

2.1 Living cells: an active material

A living cell can usually be schematized by a viscous medium wrapped in a membrane. Its usual size is about 10 µm. This medium has the specific feature of containing a cytoskeleton that provides its peculiar mechanical properties. It can reorganize with the various constraints applied to the cell. The characteristic time of reorganization is about a few minutes. Three main types of cytoskeleton are present in the cell : the cortical cytoskeleton (made of actin filaments), the microtubules (tubulin) and the intermediate filaments. Pictures of these types of cytoskeleton are shown in figure 1. The cortical cytoskeleton has a structure which is mainly bidimensional and is bound to the cell membrane. Red blood cells, for instance, have no cytoskeleton except this cortical one. Microtubules and intermediate filaments have a three-dimensional structure. Most of the time, there is no cytoskeleton in the nucleus of the cell.

When the forces are applied fast enough for the cytoskeleton not to reorganize, and when the resulting deformations are small compared to the cell size, it can be imagined that the cell will behave like a classical elastic material. The type of cytoskeleton (cortical ou three-dimensional) will then determine whether the cell can be seen as a spherical shell or as a full elastic sphere. In this

part, we will see how to determine which is the best adapted model for two adhering cells.

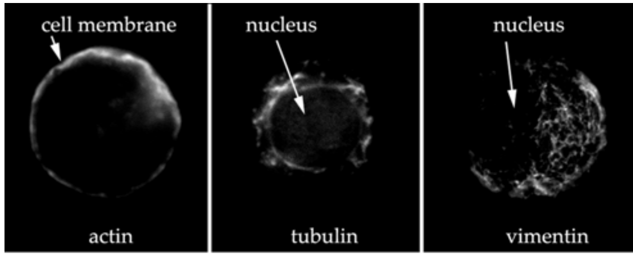

figure 1: Three of the main cytoskeleton types: actin filaments, microtubules and intermediate filaments (from left to right). They are visualized with fluorescent antibodies specific to a cyto skeleton component (here, actin, tubulin and vimentin).

2.2 Adhering spherical shells[3]

Consider an spherical shell adhering on a flat surface. When it undergoes a pulling force normal to the contact zone applied with a very small pipe (micropipette), the separation occurs at the force f_{sep}:

$$f_{sep}=\pi R W_a \qquad (1)$$

In the case of two adhering shells, equation 1 is still valid by taking R as the geometrical mean of the shells radii. If they are identical, it simplifies to :

$$f_{sep}=\frac{\pi R W_a}{2} \qquad (2)$$

2.3 Adhering elastic spheres[4,5]

In the case of a full sphere with a given elastic modulus, K, the mechanical equilibrium can be written by minimizing the sum of the different contributions to the energy: elastic energy, mechanical energy due to the displacement of the spheres and surface energy. When the sphere adheres on a substrate, the result is similar to that obtained with the spherical shells with a factor 3/2:

$$f_{sep}=\frac{3\pi R W_a}{2} \qquad (3)$$

As for the shells, equation 3 remains valid for two cells by taking R as the geometrical mean of the shells radii. If they are identical, equation 3 becomes:

$$f_{sep}=\frac{3\pi R W_a}{4} \qquad (4)$$

2.4 Experimental characterization of the mechanical properties of living cells.

In order to know whether a cell has indeed an elastic behavior, a possible approach is to apply a controlled adhesion and to compare f_{sep} and W_a. Equations 1 and 3 show that the following relation is expected:

$$\frac{f_{sep}}{R} \alpha \ W_a \qquad (5)$$

If this relation is experimentally verified, the prefactor will allow determining whether the studied cells behave more like spherical shells or like spheres.

Such a study can be conducted by forcing initially non-adhering cells to adhere through non-specific forces such as a depletion effect induced by long polymers in solution. The principle of this effect is well known. When the cells approach each other, the excluded volume for the polymer decreases generating an attractive entropic force between the cells. More quantitatively, the expression of the adhesion energy as a function of the volume fraction of the polymer ϕ is well-known[6]:

$$W_a=\frac{k_B T}{\xi^2}=\frac{k_B T}{a^2}\phi^{1.5} \qquad (6)$$

where a is the size of one monomer.

In the case of dextrane, equation 6 has been experimentally checked on lipid vesicles[7].It is therefore possible to test the mechanical behavior of cells by forcing them to adhere through depletion forces at different volume fractions ϕ. A suitable experimental technique consists of holding two cells in micropipettes with a controlled aspiration. The following experimental procedure can be used (cf. figure 2b): the aspiration is high in one of the pipettes (the right one on the figure), and lower in the other one. A mechanical traction (movement of one of the pipette along its axis) is applied to the cell doublet in order to separate them. Two cases can occur: If the adhesion is stronger than the lowest aspiration, the doublet remains intact and detaches from the pipette. If, on the contrary, the aspiration is predominant, the cell will separate. In the first case, the doublet is reacquired in the pipette and the aspiration is increase to try again to separate the cells. The cycle is repeated until the breakage of the adhesion during the nth cycle. The separation force is then well approximated by:

$$F_s= (\Delta P_{n-1}+\Delta P_n)\ \pi\ r_p^2/2 \qquad (7)$$

Where ΔP_{n-1} and ΔP_n are respectively the aspirations

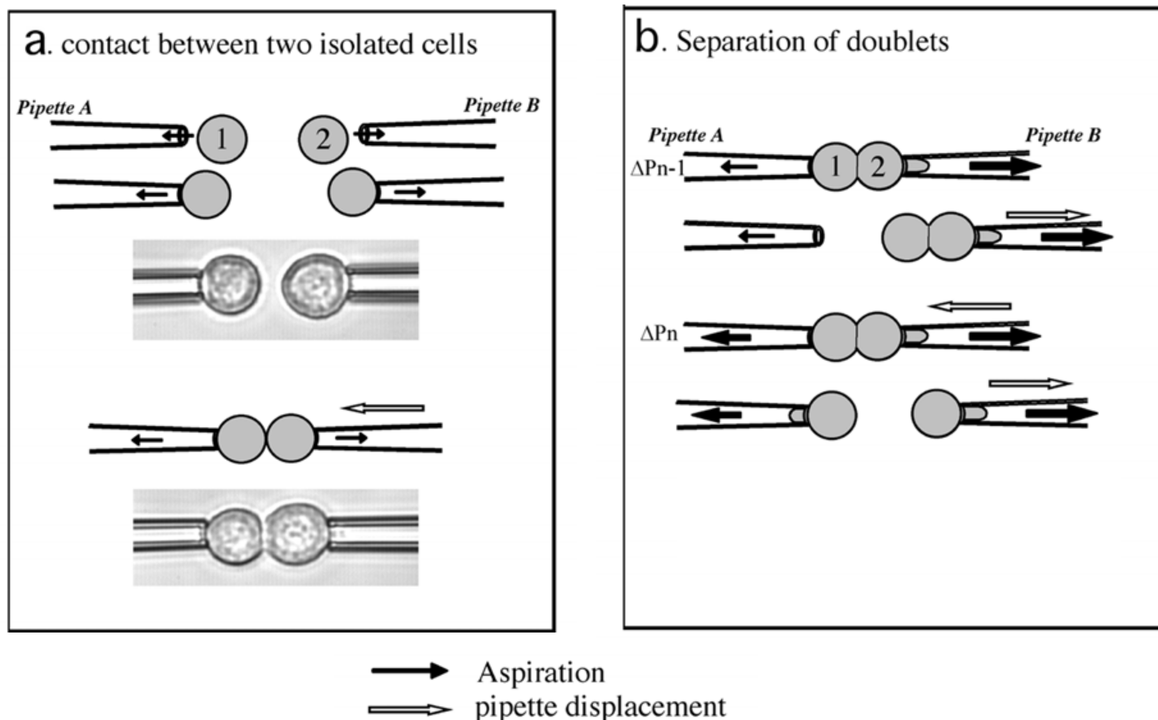

figure 2 : Experimental protocol: Two cells are grabbed by micropipettes, forced in contact (a) and mechanically separated (b) for increasing values of the aspiration in the left micropipette until the rupture of the adhesion.

for cycle n-1 and cycle n and rp the inner radius of the pipette (left pipette in figure 2).

The relation 7 was experimentally checked on murine sarcoma S180 cells with a microdynamometer (here, a microneedle).

The results obtained with these S180 cells show that they behave more like elastic sphere than like spherical shells. This conclusion was cross-checked by measuring the variation of the contact radius with the pulling force and deducing an elastic modulus through JKR theory. The obtained value is in good agreement with the ones from the literature. Also, these cells verified this theory.

The chemical disruption of the cytoskeleton lowered dramatically the measured separation force, showing that the mechanical behavior of the cell was indeed due to the cytoskeleton.

It turns out to be difficult to systematically predict what behavior a given cell will have. For instance, red blood cells that only have a cortical cytoskeleton behave more like a spherical shell.

Finally, it is important to mention that if the characteristic time of the experiment is longer than the time required for the cytoskeleton to reorganize, the cells would not have an elastic behavior (viscoelastic behavior) and the results would not be valid anymore.

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