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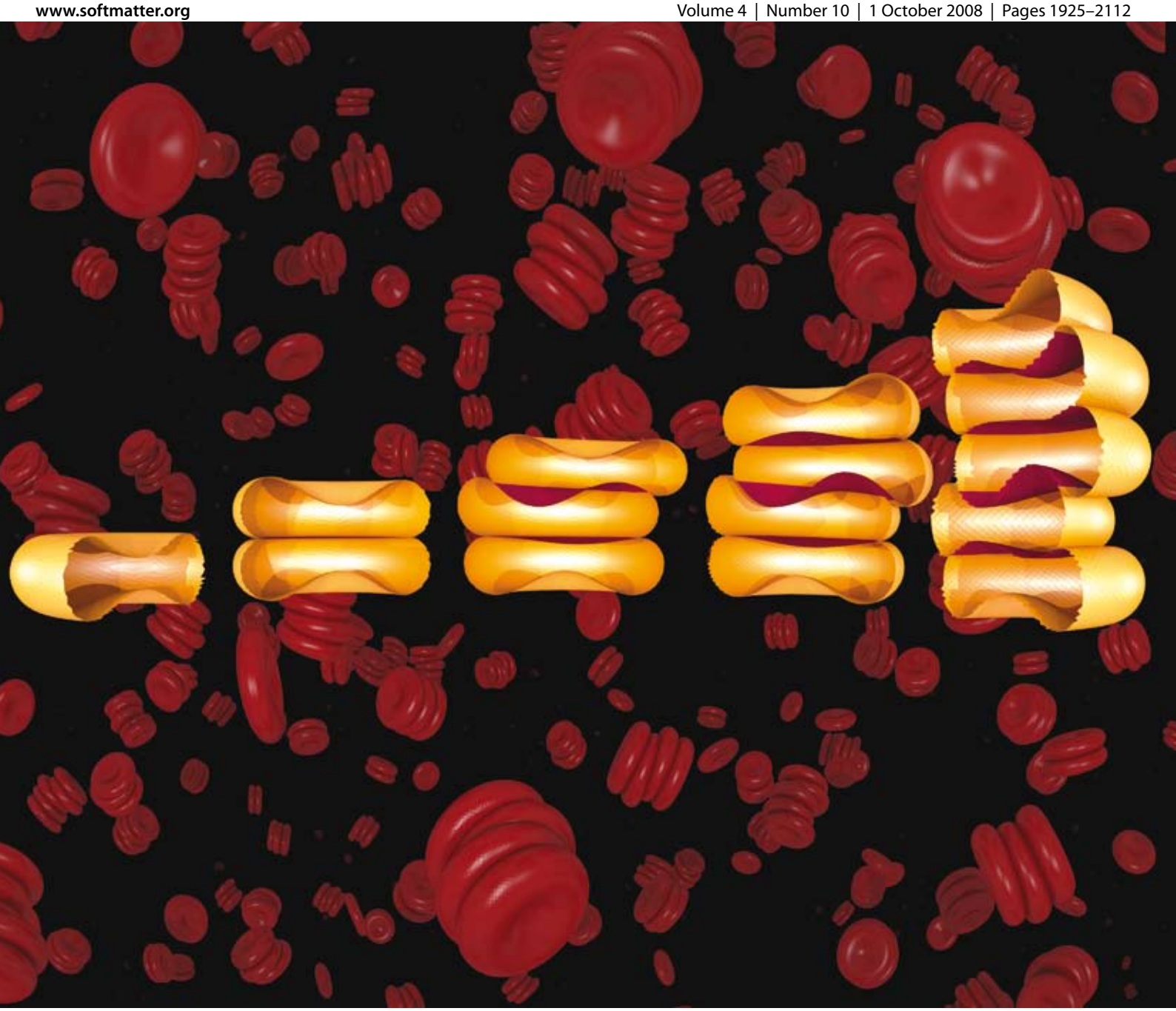

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Membrane elasticity molds aggregates of simple cells

P. Ziherl*[ab] and S. Svetina[bc]

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At first glance, quite a few tissues in simple animals or in the early development look similar to an assembly of soap bubbles. The undeniable appeal of this correspondence, the seemingly elementary surface-tension Hamiltonian, and the prospect of a mechanical model of biological structures have fueled research in the field for over a century. However, the correspondence is far from perfect and the Hamiltonian of soap-froth-like systems hard to solve. Yet the prospects of a physical interpretation of the form of some cell aggregates are good.

As an example, let us look at a recent, well-received experimental study of the building blocks of the eye of a fruit fly, the so-called *ommatidia*.[1] Through the microscope, the en-face configuration of the cone cells of an ommatidium is strikingly reminiscent of clusters of soap bubbles. But a closer inspection reveals irreconcilable discrepancies between the observations and the theoretical structures governed by surface tension alone.[2] To recover the topology and the exact geometry of clusters of cone and pigment cells, the soap-froth-type Hamiltonian must be modified, and a quantitative agreement can be achieved by introducing a term associated with the stretching of the cell cortex.[2]

From a methodological perspective, it is reassuring to see that both coarse and fine features of the ommatidium depend on the cell mechanics and that they are rather sensitive to the details of the model. Thus it is conceivable that by analyzing the structure of cell aggregates one could identify the dominant physical forces involved.

Cells consist of many morphogenic structures—the lipid bilayer membrane, the membrane skeleton, the cytoskeleton, the organelles… In an aggregate, cell shape results from the competition of the elasticity of these structures and the cell–cell interaction. The latter is based on the intermembrane forces which comprise the hydration force, van der Waals attraction, electrostatic repulsion, ligand–receptor attraction, undulation forces, *etc.*[3–7] The combined effect of these forces and cell elasticity is complex and multifaceted. For example, even in simple anucleate cells such as erythrocytes the cell–cell or cell–substrate contact zone is often patterned rather than featureless,[8,9] and it consists of a strongly bound domain interspersed by weakly bound and fluctuating patches of the membrane. These fluctuations may lead to a cell surface roughness which weakens adhesion.[10] In the case of specific interactions due to mobile ligands and receptors, the effective adhesion strength as a macroscopic quantity is determined by the thermodynamic equilibrium of these molecules on the membrane.[11–13]

All of this affects the shape of cells in an aggregate. But as a complete account of these phenomena is obviously very complicated, it seems reasonable to first concentrate on a stripped-down theoretical framework defined on length scales comparable to the size of the cell. Here we focus on the most studied model of this kind which consists of the bending elasticity of the lipid bilayer membrane (assumed to be unstretchable and

[a]*Faculty of Mathematics and Physics, University of Ljubljana, Jadranska 19, SI-1000 Ljubljana, Slovenia*
[b]*Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia*
[c]*Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Lipičeva 2, SI-1000 Ljubljana, Slovenia*

Primož Ziherl

Primož Ziherl is assistant professor at the Faculty of Mathematics and Physics, University of Ljubljana, and a researcher at Jožef Stefan Institute. He works in theoretical soft matter physics; his current interests include condensed phases of colloids, assemblies of lipid vesicles, and several topics in liquid crystals.

Saša Svetina

Professor Saša Svetina is Head of the Institute of Biophysics at the Faculty of Medicine, University of Ljubljana, and a researcher at Jožef Stefan Institute. His lifelong work in biophysics revolves around red blood cells and lipid vesicles, especially the area-difference-elasticity theory. Presently, he focuses on morphology of vesicles as well as on their aggregates and self-replication.

impenetrable)[14] and the contact adhesion potential proportional to the contact area (A_c):

$$W_a = -\Gamma A_c, \qquad (1)$$

where Γ is an effective adhesion strength.

This model, which provides an accurate description of lipid vesicle assemblies, offers some insight into erythrocyte aggregates[15–17] although it neglects the membrane skeleton elasticity and the detailed structure of the contact zone. It also covers the shape of certain intracellular structures, *e.g.*, the Golgi apparatus. In the following, we argue that it is the competition of membrane bending and adhesion that is responsible for simple aggregate topologies not present in systems with vanishing bending modulus (*e.g.*, in soap froth[18] and its variants[19] or in cells equipped with a cortex[2]), and we explore the implications of this competition in contexts ranging from sedimentation of blood to embryonic development.

Bending as aligning force

It is instructive to begin thinking about the bending elasticity/contact adhesion model by comparing it with the surface tension Hamiltonian describing clusters of soap bubbles—primarily because their characteristic length scales are the same but also because surface tension constitutes the basic paradigm used to interpret the shape of simple cell aggregates.[20] A free soap bubble is spherical, and proving that this is the minimal surface at a given volume is a matter of elementary variational calculus. However, rationalizing the shape of bubble clusters is much more complicated "despite the simplicity of the governing principle of energy or area minimization", as noted in a recent lucid topical overview.[21] It is known that in equilibrium, the edges and vertices must obey the Plateau rules stating that bubble surfaces meet at an edge in threes forming angles of 120° and edges meet at a vertex in fours forming tetrahedral angles of 109.47°.[22] Yet the link between the local equilibrium and the global structure of a soap froth is very complex, and even the ostensibly plain questions of the structure of clusters of more than 2 bubbles[21] and the shape of a froth formed by bubbles of identical volumes—the Kelvin problem—are still open.[23,24]

Are vesicle aggregates more complex than clusters of soap bubbles? The shape of free vesicles is far less universal than that of free bubbles, and it depends to a large extent on the reduced volume defined as the ratio of the vesicle volume V and the volume of a sphere of area identical to the vesicle area A: $v = V/V_s$, where $V_s = A^{3/2}/6\pi^{1/2}$. Within the area-difference-elasticity theory, the stable shapes minimize the bending energy consisting of a local and a non-local term.[25] The local term is proportional to the integrated squared sum of the local curvatures

$$W_b = \frac{k_c}{2}\oint (C_1 + C_2)^2 dA, \qquad (2)$$

where k_c is the bending modulus. The non-local term describes the penalty for the deviation of the actual difference between the areas of the lipid monolayers from its equilibrium value,[26] and the possible shapes of an isolated vesicle include cup-like, disc-like, and cigar-like axisymmetric morphologies,[26] their derivatives[25] and non-axisymmetric hybrids.[27] A comprehensive account of the origin of the bending energy, the variants of the model, their solutions, and phase diagrams can be found in ref. 14.

The present understanding of the topology and geometry of vesicle aggregates is incomplete if not episodic. The experimental studies are far less extensive than Plateau's investigations of soap froths, and no analog of the Plateau rules exists. Still the situation is not dismal. One fortunate fact is that the vesicles are not very different from erythrocytes, which do aggregate. The ensuing speed-up of their sedimentation measures the presence of adhesion-inducing proteins in blood,[28] and due to its clinical importance this process is extremely well studied.[29] The available experimental data provide invaluable information which, if combined with the right theoretical concepts, should delineate the main structural features of vesicle aggregates.

Restricted topology

Upon adhesion, the shape of a vesicle is changed to make a part of the membrane morphologically compatible with its counterpart on the neighboring vesicle. Whatever the contour and the form of the contact zone, adhesion modifies the shape of the vesicle globally, and it generally renders the non-adhering parts of a vesicle in an aggregate less suitable for subsequent contact.

This notion can be well illustrated by comparison of the theoretical cross-sections of free vesicles and vesicle doublets with the reduced volume corresponding to that of a normal human erythrocyte (Fig. 1). At this v, free vesicles are axisymmetric and biconcave just like erythrocytes.[30] At small adhesion strengths they form flat-contact doublets with very pronounced concave non-contact parts of membrane, whereas at large adhesion strengths the doublets are nonaxisymmetric with a sigmoidal shape of the contact zone and a purely convex external appearance (Fig. 2).[17] This contact, reminiscent of the second lowest normal mode of a circular drum, is seen in electron micrographs of red blood cell doublets.[8,31]

Fig. 1 suggests that in order to maximize the contact zone area at minimal increase in bending energy, the vesicles stick to each other face to face whereas

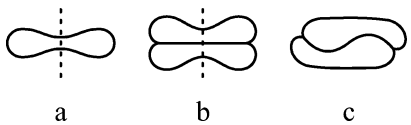

Fig. 1 Theoretical cross-sections of a free lipid vesicle (a), flat-contact doublet (b), and sigmoid-contact doublet (c) at reduced volume $v = 0.6$. In panels (a) and (b), dashed lines indicate the axes of rotational symmetry. The sigmoid-contact doublet is nonaxisymmetric: shown is the representative, mirror-plane cross-section. Adapted with permission of the National Academy of Sciences of the USA from ref. 17.

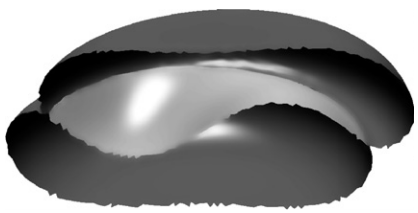

Fig. 2 Sigmoid contact zone in a doublet of vesicles of reduced volume of a normal human erythrocyte. The mirror-plane cutaway view clearly shows the complementary invagination and evagination of the contact zone. Reproduced with permission of the National Academy of Sciences of the USA from ref. 17.

rim-to-rim or rim-to-face contacts are disfavored. This effect is most pronounced in the moderate adhesion regime where the increase of the bending energy upon aggregation is just outweighed by the adhesion energy. As a result, bending elasticity is a powerful aligning force since it restricts the minimal-energy topologies considerably and makes a linear aggregate with chain-like connectivity preferred to other configurations (Fig. 3) even in conditions as harsh as rapid centrifugation.[32] In the moderate adhesion regime, vesicle aggregates differ most dramatically from aggregates of objects devoid of bending elasticity: the topology of such aggregates is very complex even in two dimensions.[21,18,19,33,34]

Three adhesion regimes

The different adhesion regimes are best quantified by comparing the bending and the adhesion energy. The former is independent of vesicle size[14] and is conventionally measured relative to the bending energy of a spherical vesicle which equals $8\pi k_c$. On the other hand, the adhesion energy does scale with vesicle size and is of the order of ΓA. Given than the vesicle area A can be expressed in terms of the radius of a sphere of identical area, $A = 4\pi R_s^2$, the reduced adhesion strength defined as the ratio of the characteristic magnitudes of the adhesion and bending energies is

$$\gamma = \frac{\Gamma R_s^2}{2k_c}. \qquad (3)$$

The scaling argument suggests that for $\gamma \ll 1$ adhesion is negligible and the vesicles are free, whereas $\gamma \gg 1$ defines the strong adhesion limit. The crossover moderate adhesion regime where linear aggregates are stable must be at $\gamma \approx 1$.

If adhesion is very strong, the stable aggregate maximizes the total contact area, and any effects of bending elasticity are subdominant.[35] Since the area of each vesicle is conserved, this is equivalent to minimizing the total non-contact area as if the aggregate itself were a soap bubble, and the stable aggregates were round clumps.

Structure of junctions

In the strong adhesion regime, the organization of aggregates is non-trivial, and this problem has not received much attention. It is instructive to think about it in view of the boundary conditions stemming from the balance of forces at the rim of the contact zone where an adhering membrane experiences a discontinuity of curvature.[36,37] In axisymmetric shapes, the jump of meridional curvature at the rim is[16]

$$\Delta C_m = \sqrt{2\gamma} \qquad (4)$$

Imagine now that at very large γ, vesicles in an aggregate adopt convex polyhedral shapes with flat faces, rounded edges, and rounded vertices. In this regime, only a small fraction of each membrane is not in contact with a neighbor, and it is reasonable to approximate the rounded edges by parts of a cylinder and the rounded vertices by parts of a spherical surface such that the radii of rounded patches are $1/\Delta C_m$.

Within this model of aggregates of three-dimensional vesicles, the only possible geometry of a 3-way edge (Fig. 4a) is regular just like in a soap froth (Fig. 4b). However, edges where more than three sides meet are also possible, and their structure is more flexible than in 3-way edges. 4-way edges, for example, may assume a regular configuration with a 90° angle between the neighboring sides (Fig. 4c). But any other symmetric configuration such that the adjacent angles are supplementary and none is smaller than 60° is allowed as well (Fig. 4d). The limiting 60°–120°–60°–120° configuration consists of twin regular 3-way edges (Fig. 4e).

The reduced bending energy of a model polyhedral vesicle is dominated by the energy of edges which amounts to $(\sqrt{2}/48)\sqrt{\gamma}L/R_s$ where L/R_s is the reduced total length of edges per vesicle; the numerical prefactor corresponds to 3-way edges. In the strong adhesion limit $\gamma \gg 1$, the edge energy proportional to $\sqrt{\gamma}L/R_s$ is much larger than the total bending energy of the vertices per vesicle, which can be estimated by 1 (again in units of $8\pi k_c$, the bending energy of the reference spherical vesicle) by assuming that the radii of their caps are identical.

A larger total edge length also implies a smaller contact area per vesicle: both bending and adhesion terms favor structures which minimize the total edge length at a given reduced vesicle volume. To the best of our knowledge, the edge-minimizing structures have not been cataloged yet. Once they are, a detailed analysis of the statistical geometry of edges and vertices will be in order, and these data may be decisive for the recognition of aggregates shaped jointly by bending elasticity and adhesion.

Anisotropic aggregates

The most powerful manifestation of the bending elasticity as the factor that co-defines the aggregate topology is the rouleau (Fig. 3). Its one-dimensional connectivity and anisotropic shape are essential for several biological structures and processes, some of which are discussed below.

Golgi apparatus

This eukaryotic cell organelle consists of a stack of interconnected flattened lipid vesicles called cisternae, typically between 3 and 11. The Golgi apparatus processes

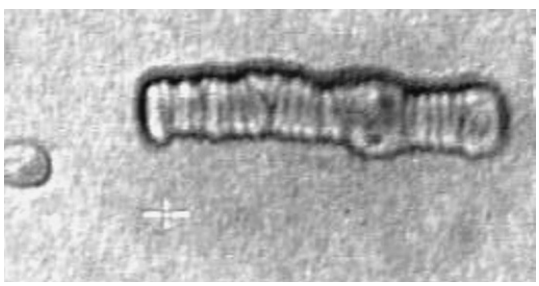

Fig. 3 Side view of a red blood cell rouleau with a clear linear structure resembling a stack of coins. Such aggregates are characteristic of a moderate adhesion regime, whereas at large adhesion strengths red blood cells form rounded clumps. Note that the rouleau shown is not perfectly regular. Reproduced with permission of the National Academy of Sciences of the USA from ref. 32.

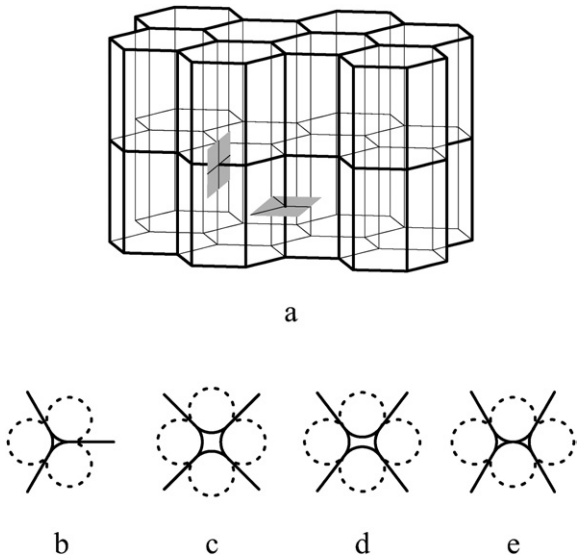

Fig. 4 Structure of edges in a vesicle aggregate at large adhesion strengths: schematic of a part of a bulk model regular aggregate of hexagonal upright prismatic vesicles (a) and blowups of the transverse cross-section of the 3-way edge (b) and the regular 4-way edge (c) indicated by the two shaded areas in panel (a). Also shown are an intermediate (d) and the degenerate 4-way edge (e). Edges of valence larger than 4 are also possible. In panels (b–e), straight solid lines represent adhering membranes, curved solid lines are non-contact parts of membranes. Dashed lines indicate cross-sections of cylinders of identical radii whose lengthwise segments form an edge.

and separates proteins which enter on the *cis* side facing the endoplasmic reticulum and exit on the opposite *trans* side, and it is also involved in transport of lipids. The cisternae are functionalized with enzymes and constitute a sequential reactor whose performance relies on its structure.

The Golgi apparatus is a dynamic structure with transport vesicles continuously being secreted by the apparatus and fusing with it. Yet it is possible to interpret the observed number of cisternae in terms of competition of adhesion energy of the disc-like cisternae and their bending energy concentrated at the rim.[38] By assuming that the apparatus is in a stationary state and that the total area and volume of the cisternae are fixed, the minimal-energy number of cisternae was found to agree well with the typical physiological values at realistic values of the material parameters. In addition, it has been shown that the number of cisternae is most robust if the incoming vesicles themselves are disc-like rather than spherical or tubular.[38]

Red blood cell rouleau

The physiological and diagnostic importance of red blood cell aggregation has driven theoretical studies of the formation of rouleaux for over three decades[15,39,40] The progress was hampered by technical limitations and initially, the theoretical results were restricted to axisymmetric rouleaux although it was known that such shapes are inadequate in the moderate adhesion regime.[15] Nonetheless, the predicted flat-contact axisymmetric rouleau at adhesion strengths well beyond the aggregation threshold and the intervening curved-contact rouleau are consistent with experimental observations.[16]

Recently, we have proposed a possible scenario of the rouleau formation[26] relying on numerical studies of vesicle doublets using Surface Evolver.[41] The scenario involves the sigmoid contact[17] as the contact zone morphology which allows the vesicles in a rouleau to retain a biconcave shape not very different from the shape of free red blood cells. A stack based on the sigmoid contact zone appears to be the best candidate rouleau morphology in the moderate adhesion regime,[35] and it may help to quantify the erythrocyte aggregation threshold as an important physiological parameter.

Convergent extension

The role of anisotropic structures in multicellular animal organisms can hardly be overstated. It all starts in the early embryonic development when the blastula, a shell formed by a single layer of cells, is transformed from a topological sphere into a topological torus called the gastrula. This process begins with blastula invagination followed by directional growth of the invaginated block of cells towards the opposite side until the tube-like invagination fuses with the blastula wall. Directional growth relies on convergent extension whereby cells rearrange by intercalation so that the cell aggregate becomes longer and narrower (Fig. 5).[42]

Convergent extension is also crucial for the establishment of the embryo's head-to-tail body axis,[42] *e.g.*, the ascidian notochord formation where the monolayer 40-cell plate transforms into

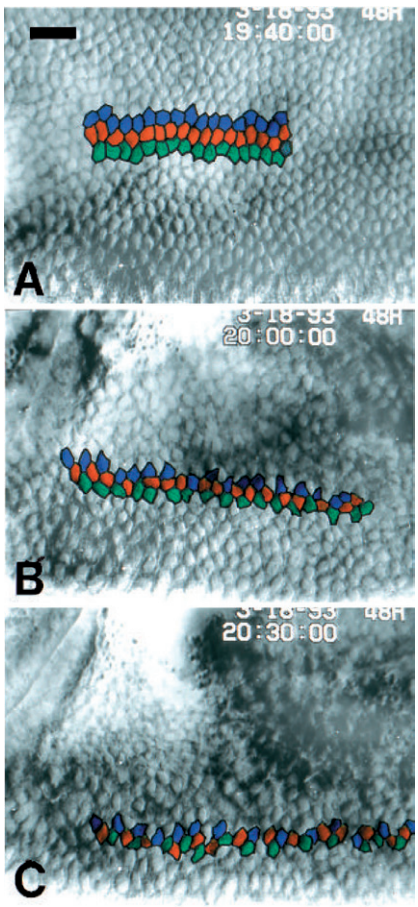

Fig. 5 Subsequent stages of convergent extension: cells gradually intercalate to rearrange into a narrower, longer structure. Micrographs of the process in *Drosophila* embryo germband, partly colorized for easier cell tracing; reproduced with permission of the Company of Biologists from ref. 44.

a single-file stack of cells.[43] Many aspects of the plate-to-rod transformation are reproduced by model aggregates of two-dimensional vesicles, which are linear at moderate adhesion strengths and sheet-like in the strong adhesion regime.[45] In the rouleau-like linear aggregate each vesicle has two neighbors, whereas the sheet-like aggregate is essentially a polygonal tiling of the plane except that the vertices of the polygons are rounded consistent with eqn (4). The simplest way of inducing the transformation of a sheet-like aggregate of 2D-connected vesicles into a stack of 1D-connected vesicles, which may be regarded as convergent extension, is by decreasing the adhesion strength.[45]

Bending elasticity and differential adhesion hypothesis

This interpretation supports an earlier theory of convergent extension[46,47] based on the differential adhesion hypothesis.[48,49] In this two-dimensional theory, cells are modeled by rectangles with 2 long and 2 short sides, and the three types of contacts (long–long, long–short, and short–short) are each assigned a different adhesion strength. For a suitable choice of parameters, this gives orientationally ordered aggregates which are elongated in the direction perpendicular to the long cell axis.[46,47]

The postulated differential adhesion used in ref. 46,47 can be considered as a coarse-grained description of the preferential bonding of two-dimensional vesicles which adhere to each other exclusively at their long sides unless the adhesion strength is large enough.[45] As this is a result of the competition of membrane bending elasticity and adhesion, so is the differential adhesion. This link is far-reaching: using differential adhesion as an effective model of cell–cell interaction, one can reproduce a range of complex biological phenomena such as cell sorting, cell dispersal, and engulfment.[50–52]

Outlook

Although the predicting power of physical models is increasingly more valued by biologists,[20,53] a common objection to the simple mechanical views presented here is that a real cell is far more complicated. This is, of course, true. Yet it is likely that not all of the cell structures are equally relevant for the morphology of aggregates. For example, the red blood cell membrane skeleton is essential for the spiculated form of echinocytes[54] but not for the large-scale shape changes upon rouleau formation. For some cell aggregates, the theory discussed here may be quite adequate, and it is amazing that it can elucidate certain questions in topics as diverse as embryogenesis, structure of cell organelles, and clinical diagnostics.

This framework also covers an emerging class of objects referred to as synthetic life.[55] As a compartment, an artificial cell is best materialized by a lipid vesicle, and long-lived vesicles operating as protein-expressing bioreactors have been constructed.[56] In addition, vesicles may also be capable of autonomous self-replication provided that the supply of lipids, water, and other ingredients is appropriate. If the growth rate of the membrane and its permeability to water and solutes are properly synchronized, a spherical vesicle will evolve into a symmetric budded shape so that after fission, the process can be repeated indefinitely.[57,58] Should it take place in an aggregate held together by adhesion-inducing agents, self-replication will be co-regulated by intermembrane attraction. In this context vesicle–vesicle adhesion will assume a whole new role.

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