Fluid models and simulations of biological cell phenomena

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Abstract

One aspect of Biofluid research concerns the dynamics of coated droplets. Of specific interest is the manner in which the shape of a droplet, the motion within it as well as that of aggregates of droplets can be controlled by the modulation of surface properties and the extent to which such fluid phenomena are an intrinsic part of cellular processes. From the standpoint of biology, an objective is to elucidate some of the general dynamical features that affect the disposition of an entire cell, cell colonies and tissues. Conventionally averaged field variables of continuum mechanics are used to describe the overall "global" effects which result from the myriad of small scale molecular interactions. By this means, an attempt is made to establish cause and effect relationships from correct dynamical laws of motion rather than by what may have been unnecessary invocation of metabolic or life processes. The distinction between what must be a life process and what may really be the result of more ordinary inanimate mechanisms is an important and central question, the resolution of which will lead to a deeper understanding of biological activity as well as the origin of life.

Several topics are discussed where there are strong analogies between droplets and cells. These are: encapsulated droplets -- cell membranes; droplet shape -- cell shape; adhesion and spread of a droplet -- cell motility and adhesion; foams and multiphase flows -- cell aggregates and tissues. Evidence is presented to show that certain concepts of continuum theory such as surface tension, surface free energy, contact angle, bending moments, etc. are relevant and applicable to the study of cell biology.

1. Introduction

A primary objective of biologists is to understand the structure and function of basic molecular complexes that constitute life processes. At this fundamental level of inquiry -- the direct bio-chemical interactions between molecules -- fluid dynamics is not applicable (although mathematical analysis, presently eschewed, could be used more effectively). However, when the focus of study turns to the explanation of the comparatively large scale phenomena that result from a myriad of molecular reactions, continuum theory becomes a natural and often preferred means of investigation as it does when the corresponding transition in physics is made from single to many particle systems. This in fact is a pertinent analogy that well illustrates the anticipated role of continuum mechanics in biology. To say that the movements of or within a cell are explained by the contractile capability of certain protein molecules is about as satisfying, and as predictive, as the knowledge that all mechanics follows directly, in some way, from the application of Newton's laws of motion to each and every constituent particle of a medium. It is not generally recognized within biology that (a) the large scale dynamical consequences of the interaction of very many particles need not be discernible on the molecular scale or even predictable from that standpoint; (b) the net effect on the aggregate can be distinctive, new phenomena which transcend the behavior of the individual elements such as waves, instabilities, patterns and structure. As a result there is a paucity of cell physiological data that is relevant to continuum formulations and very few experiments attempt to measure the variables and parameters that characterize the rheology of a medium or its dynamic response. (Even less can be said about acceptance or the use of mathematics and mathematical models.) Unfortunately, biologists, like other scientists, can scarcely maintain control over the vast flow of information within their own specialties. However, the present situation does exemplify the opportunities and challenges inherent in the renewed and growing contact between two venerable disciplines, and these will be stressed here.

Continuum mechanics offers to cell biology, the means to study tissue morphogenesis, growth and differentiation, cell processes that involve dynamic change, motion and flow, as well as the conditions that maintain equilibrium. The description of patterns, flows and structures in terms of continuum state variables and rheological parameters will provide understanding of the dynamical principles that govern global phenomena. The use of dimensional analysis will enable data to be interpreted and correlated; experiments will be suggested and experimental techniques adapted; theoretical models and analogies will help formulate and test hypotheses and provide for detailed analysis of special events.

In return biology gives the continuum mechanician a miraculous "fluid" medium, an awe

inspiring chemical, metabolic system where almost anything conceivable can occur and seems actually to occur somewhere. With such wondrous material, what must be a life process and what can be the result of more ordinary inanimate dynamics become central questions to be resolved. The polymerization of contractile fibers is a favored omnibus explanation of much cellular activity but although this is indeed a most important mechanism, non-metabolic dynamic processes often give a simpler interpretation of events. This does not mean that when an inanimate mechanism is adequate, a more complicated life process is unnecessary. But it does require a rationalization of why in evolutionary developments a complex process has substituted for a simpler one. Questions of this type must lead to deeper understanding of biological processes.

Biological fluid dynamics, that is the fluid dynamics of cells and tissues, concerns that most complex material rheology yet studied. Although in most circumstances flows are slow, cellular material is chemically active in the extreme, most definitely a non-Newtonian colloid, emulsion or mixture subject to temporal and spatial changes of state from sol to gel, all within an ensemble or organelles of various compositions and functions -- minute deformable domains enclosed by a lipid/protein membranes of extraordinary properties. Moreover, even the simplest hydrodynamic analogies and simulations of cells and tissues lead to unsolved fluid problems of current interest in chemical engineering, microhydrodynamics, polymeric and interface sciences.

Corresponding topics in biology and fluid dynamics are roughly the following: cell rheology -- viscoelasticity; cell membranes -- interfaces and monolayers; cell shape -- droplet dynamics; cell adhesion and motility -- liquid adhesion and spreading; cell aggregates -- foams, emulsions and multi-phase mixtures. Some specific problems will illustrate these connections and the feedback that continually generates new questions.

2. Survey

Some of the biological phenomena which my colleagues and I have studied using continuum theory are cell cleavage, cell adhesion and motility, cell membranes (as monolayer coatings on droplets), cell cultures and capillary growth.

There are essentially two classes of problems represented here. One concerns motion of and within a cell and the other deals with the dynamics of cell aggregates. In the former, the relevant length scale is the size of the cell, which though small, is still very much greater than molecular distances. In the latter the cell itself may be viewed as the basic element of the tissue fluid as long as the phenomenological scales are many cell lengths. (However, both scales are usually microscopic in the literal sense. Large and small distances are referred to then as microscopic or submicroscopic and ultrafine, respectively.)

A continuum theory based on surface tension, slow viscous flow and an approximate momentum equation similar to Darcy's law, was formulated[1] to describe the growth and movement of certain cell cultures in response to a distribution of nutrient. In this model, a new cell forms, expands to proper size, and pushes aside neighboring cells of the culture. The forces of displacement are transmitted with attenuation throughout the cel population. The total pressure developed in this way causes internal migration of cells and a drift of the entire colony as it builds in the direction of a richer supply of nutrient. The internal motion is assumed to be proportional to the negative gradient of the pressure and a surface tension force proportional to the mean curvature of the tumor surface maintains the colony as a compact and continuous mass. In essence then, the culture is similar to an incompressible fluid within a variable domain in which there are sources and sinks. This theory and elaborations of it[2] are found to yield good agreement with observations.

The competition between surface and interior forces that control the shape of the colony, motivated an examination of dynamic instability of such cultures[3]. This analysis established the conditions in which the slight surface distortions that arise lead to the possible division of or disintegration of the colony. This like other analyses[4] makes the important point to biologists that instabilities are a natural part of biological development.

This example of the control of shape by surface tension, and the form of the most prominent mode of instability suggested that cell cleavage could also be approached along similar lines. A theory of cytokinesis was presented[5] in which cytoplasmic streaming, furrow development, contractile ring formation and division are all direct and related effects of a dynamical instability that is caused by the modulation of tension at or near the membrane surface during anaphase. Based on earlier work[6], a completely fluid model of cleavage dynamics was constructed in which an effective surface tension simulates the sum of all forces exerted within the boundary structure of a cell. The dynamical process hypothesized was shown to be unstable and once triggered develops rapidly without further stimulation. The primary pattern of instability exhibits the typical shape of furrow formation at the equator; the time scale of this excitation is also consistent with

observations. However, this first model is much too simple to be an entirely realistic description of a cell, but it, with the accompanying experiments on the division of oil droplets and subsequent continuum analysis of the cell membrane as a thin elastic shell[7], well illustrate the capabilities of surface forces and the manner in which they may be implicated in the division process.

A common theme in all this work is the control of droplet shape by surface forces that arise from the distribution of a surfactant on a pure interface, or from a material which is in fact a separate surface fluid. Several droplet problems were examined in order to explore the range of phenomena associated with surface flows and to develop greater intuition regarding the possible relevance of such effects in cell biology. For example, it has been shown[8] that the deposition of mass to the surface layer is in itself sufficient to cause spiculation of a droplet if the layer material is nearly incompressible, i.e., the surface compressional modulus is large. At early times, the surface layer does indeed behave like a true incompressible fluid and crenations form. But these protuberances decrease in size and in number as the compressibility of the real coating takes effect, until finally the droplet again becomes spherical. Essentially too much surface area for the contained volume causes ripples. The manner in which excess area is actually accommodated by fully nonlinear changes of droplet shape -- whether by bumps, invagination, folds, or a break up into smaller droplets -- is a question under examination.

It is known that the deposition and desorption of surfactants also leads to interface instabilities[9], and in the combination of these effects (and a more complex model of the surface) lies a possible explanation of at least the onset of crenation, villation, and spiculation of real cells[10].

The collapse of surface monolayers is a closely related instability problem of interest which is being examined simultaneously. The mathematical formalism developed for droplets applies in this case as well.

A similar approach based on "thin shell" theory of solid mechanics was used to construct a model of capillary growth in tissue[11]. Here the surface area of the capillary "cylinder" which consists of endothelial cells, increases as the cells divide. This leads to sinuous and bulbous instabilities of the column. These distortions seem essentially the same as those of capillaries observed in situ[12] and in fact, the theory provides an explanation of and qualitative agreement with experimental data.

The use of an effective surface tension to model the membrane and subcortical structure of the cell naturally gave rise to the question of whether adhesion and motility of cells[13] could also be simulated by droplets[14].

Interfacial tensions between membrane, substratum and ambient medium must be a part of the physical mechanism by which an almost spherical cell rapidly flattens after mitosis. Chemical changes in the cell surface at division probably increase the affinity of membrane molecules for those of the substratum or solid boundary providing thereby the main forces for the initial extension of a cell. The intrinsic process may be somewhat analogous to the spread of a liquid droplet on a solid. Although the cell attaches to the surface at only thousands of sites mainly along its periphery[15], while there are many billions of binding sites for a liquid droplet, this is still probably a sufficiently large sample for the same kind of statistical averaging to apply that underlies a continuum theory. It would then follow that such concepts as surface free energy, spreading and contact angle have loose analogs in cell adhesion and motility. (It should be noted that the spreading of a liquid droplet is in many respect an unsolved problem. The exact nature of the boundary conditions at the advancing liquid/solid boundary is unclear[16] and this reflects the uncertainty about the physics of fluid attachment of a surface which implies the condition of no-slip.)

We advanced a model[17] for the movement of a small droplet on a surface specifically adapted for the high viscosity of cells. This was based on the lubrication equations and used the concept of dynamic contact angle to describe the forces that act on the fluid at the contact line. The specific problems solved with this theory were the spreading and retraction of a circular droplet; the advance of a thin two-dimensional layer; the creeping of a droplet on a coated surface to a region of greater adhesion; the distortion of droplet shape owing to surface contamination. A more intensive analysis of the equations[18] showed that an advancing droplet becomes more pear-shaped in outline, with a broader advancing contact line and a narrower retreating edge. This has some of the features of motile cells; however, numerical methods will have to be employed to examine further the shape and stability of the edge contour.

The similarities between the spread of a cell and of a droplet that were noted by Greenspan and Folkman[19], have been exploited to a remarkable degree in the experiments of Folkman and Moscona[20]. They have shown that the shape of a cell can determine whether DNA

synthesis will be permitted; the technique used was to control the degree of spreading on coated the plastic slips, i.e., by modifying the interfacial tension in the known manner. The fact that cell shape can be proven to correlate with cell function substantiates some of the earlier hypotheses relating shape and function[21]. There is now considerable evidence[22] that surface tension and contact angle are meaningful and useful concepts in cell biology, but more work is required before the advantages of this viewpoint are accepted.

The study of the dynamics of encapsulated droplets has been extended by considering a surface coating which is an oriented, polar fluid of either a monolayer or a bilayer structure. The general theory of polar fluids[23] was adapted for such surface flows[24] and general constitutive laws were determined that relate surface stress, strain, bending moment and a non-Newtonian rheology[24]. The bending moment of the membrane arises from the preferred orientation of the molecules that form the surface layer. Its importance has been discussed in connection with the shape of the red blood cell[25] and with the growth of capillaries in tissues[26]. In both cases, the bending moment appears as a stabilizing factor because it acts to undo distortions of an equilibrium configuration and to cut off the growth of incipient wave instabilities below a certain wave length. However, this is not as yet a general conclusion valid in all circumstances. The interplay of moments, variable surface tension (pressure) and chemical exchange with the bulk fluid by the deposition and desorption of surfactants could easily produce a range of new, interesting and surprising effects.

We are currently examining the dynamics of liquid/liquid foams (i.e., coated droplets in an aqueous medium) with the focus on an accurate description of the motion that results from the addition of surfactants. The behavior of such systems is in many ways analogous to assemblages of cells in cultures and colonies. For example, spiral patterns of motion are observed in such biliquid foams[26]; likewise the development of "similar" spirals in cultured epidermal cells has also been described[27]. Whether the underlying mechanical and visco-elastic processes are also analogous remains to be seen[28]. One difficulty is that the fluid dynamics of foams is essentially an undeveloped area whose theoretical formulation is not completely understood. Accordingly, we have had to begin with certain fundamental, idealized problems that are several levels removed from biological applications, and this has indeed become a separate program of research.

The results of such a study should also be directly relevant to our continuing investigation of the growth and movement of certain cell cultures and solid tumors in response to a changing chemical environment. Development of a more elaborate compressible fluid model of tissues is necessary in order to substantiate or to generalize the empirical dynamical law that has been assumed. We would then be better positioned to inquire about the dynamic response of cultures to stimuli, and the effects of spatially varying rheological properites.

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