Rheology of living cell: modelling of stationary and motile behaviour

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Abstract

Cell motility observed in biological processes is modelled using continuum mechanics principles. A visco-elastic-active model is developed incorporating myosin retrograde flow, actin polymerization and substrate adhesion phenomenon observed in such cell. The model is solved using nodal FEM to predict non-motile and motile behaviours. Simulation of non-motile state is verified with the mathematical model. Motile behaviour is successfully observed by varying the parameters of the model.

Keywords: visco-elastic fluid, retrograde flow, actin flow, shape evolution

1 Introduction

Motility in cells is observed in a spectrum of biological processes like tissue regeneration, cancer propagation and immune system response. Morphogenesis at the cellular scale is an emerging field of research with recent advances in experimental methods that provide quantitative spacio-temporal information on the dynamical quantites such as traction and deformation and biological gene expressions [1] [2]. From the standpoint of physics, such advances give us impetus in obtaining necessary ingredients for modelling and also provide critical data to test the models, ultimately leading to better understanding and prediction capabilities [3].

The current paper aims to elucidate experimental observations on cell motility, incorporate relevant and tested observations to develop a rheological model based on continuum mechanics principles and conduct tests on the model. We model a single cell on a flat rigid substrate in both stationary and motile states and hope to gain a better insight into the cell scale morphological behaviour with this study venture.

The article begins with a brief explanation of the biological phenomema that influence the morphological changes in cells. We discuss the rhelogical model developed, solve analytically and numerically the resulting equations and put forth the predictions of the model for stationary and motile behaviour.

1.1 Morphology of a living cell

The main components of cell structure that dictate its shape are actin, myosin and adhesion complexes.

Actin is a globular protein scattered throughout the cytosol (inter-cellular 'fluid'). When actin monomers are assembled into a filament, the assembly acts as a cytoskeleton. The monomers assemble and disassemble at a rapid rate, thus rendering the cytosol a fluid-like property. Also actin polymers forming a network are linked to one another through actin cross-linkers that have a short time span of attachment (of the order of one second) [4].

Myosin is a complex protein and is usually found in association with actin, together forming actomyosin complexes. The charateristic feature of myosin in the ability to perform energy-consuming contractions. Myosin performs power strokes of contraction that help regulate cell structure and respond to changes in the stiffness of the external medium [5].

The actin network inside the cell membrane is attached to the molecules of the substrate or extracellular matrix by adhesion proteins. Hence flow of actin relative to the substrate is transmitted in the form of traction forces. However, adhesion complexes are not a rigid connection and involves slippage and relative motion between substrate and actin network. No clear mechanism effectively explains the coupling between adhesion molecules and actin network flow in force transmission to the substrate [2].

1.2 Dynamics of actin and myosin complexes

Myosin performs power strokes on the actin network. This involves binding to actin network, pulling and unbinding from it. This creates a contractile force on the actin resulting in the flow of actin. Mysoin is observed to be radially oriented, resulting in an inward flow of actin network [1]. This is reported for both stationary and motile cells and termed as retrograde flow [2].

Retrograde flow alone would eventually lead to lumping of cellular mass but we observe that cells maintain their shape. Actin polymerizes at the edges resulting in protrusion of cell boundary through the formation of cell body extensions called lamella. The tendency of polymerization is expansion of cell boundary. As actin is pulled inwards due to retrograde flow, actin monomers diffuse towards the cell boundary to polymerize. This is called actin treadmilling and this counterbalances retrograde flow enabling the cells to maintain constant cell shape.

2 Rheology

Existing mathematical models for migration of living cells explain the process with different parameters and structures [3]. We model cells as viscoelastic fluid with energetic properties. It is based on the mutual interaction of myosin contraction, actin polymerization and substrate adhesion mechanisms. [5]

2.1 Model - Constitutive relation and Governing equation

Consider a cell on a substrate. The actin network of the cell is interconnected with cross-links that unbind with a characteristic time τ_α. Any stress, such as resulting from myosin contraction on the network would lead to yielding in the network when the cross-linkers unbind. We assume that the cross-linkers un-bind from the old configuration of the network and re-bind elsewhere in the new configuration, so that the network elastic modulus remains the same.

For this scenario, the constitutive relations is given by 1.

$$\bar{\sigma} = \bar{\Sigma} + \eta\, d(\vec{u}) \qquad \text{, where } \Sigma = \frac{c_{myo} l_{myo}}{\tau_{myo}} \tau_\alpha E \tag{1}$$

where, $d(\bar{u})$ is the rate of deformation tensor with $\vec{u}$ being the velocity field and $\eta = \tau_\alpha E$ is the viscosity of the actin network. $\bar{\Sigma}$ is myosin strain rate which is directly proportional to the concentration of myosin c_{myo}, step length l_{myo} and inversely proportional to myosin characteristic time τ_{myo}.

Stresses in the network is balanced by the traction with the substrate which is assumed to be linear function of velocity for the non-motile and a non-linear function of velocity for motile case. For non-motile case and motile case, linear momentum balance respectively are

$$\nabla \cdot \bar{\sigma} = c_f\, \vec{u} \qquad \text{and} \qquad \nabla \cdot \bar{\sigma} = c_f(|\vec{u}|)\, \vec{u} \tag{2}$$

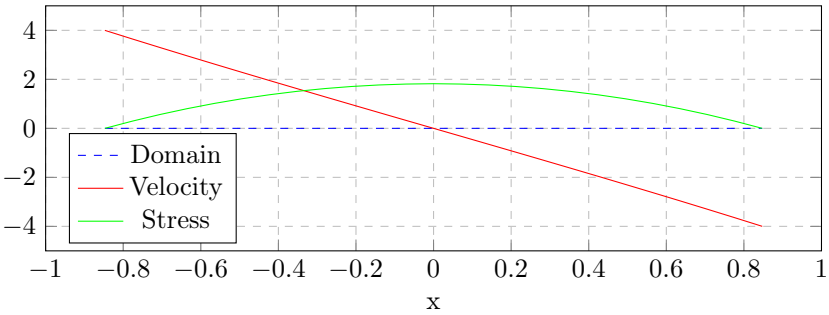
where the scalar c_f is the coefficient of friction of the cytoskeleton with the substrate.

3 Non-motile cell

A stationary cell on a flat substrate has an outward actin polymerization rate balancing the inward actin network flow due to myosin contractions. The substrate provides resistance to actin network flow through adhesion complexes.

3.1 Analytical solution

Figure 1: Equilibrium cell position = [-0.847,0.847], stress and velocity profile for an arbitrary value for parameters $\eta = 4$, $c_f = 1$, $\Sigma = 20$ and $v = 4$ (non-dimensional)



Consider a 1-D cell on a substrate. Let the cell at equilibrium have a length $2x_o$, extending between $[-x_o,\ x_o]$. The relevant equations 1 2 are reduced to: Solve for $\vec{u}$, $\bar{\sigma}$ and equilibrium position x_0 such that

$$\sigma = \eta \frac{du}{dx} + \Sigma \tag{3}$$

$$\frac{d\sigma}{dx} = c_f u \quad \text{in } \Omega \tag{4}$$

$$\sigma(x_0) = \sigma(-x_0) = 0 \quad \text{on } \Gamma \tag{5}$$

$$u(x_0) = -u(-x_0) = -v \tag{6}$$

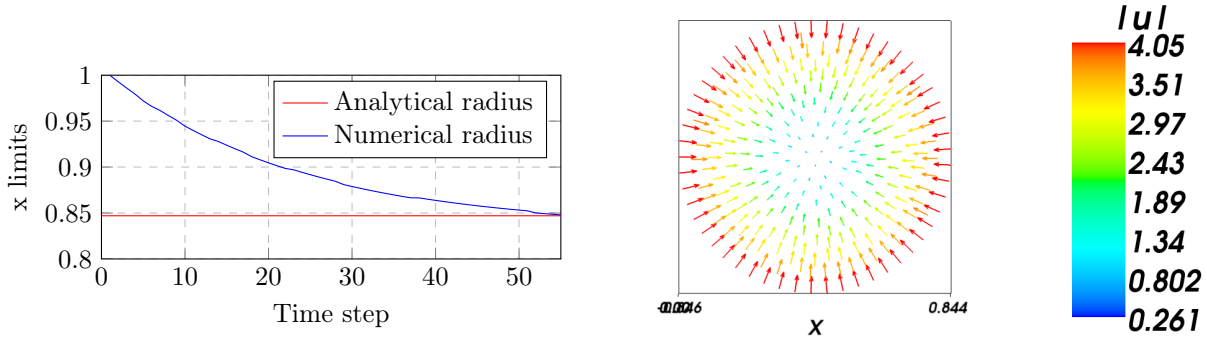
When the above ODE is solved, we obtain the solution for actin velocity u, and the stress in the cell body as shown in 1.

3.2 Numerical solution

The governing equations with boundary condition are given below, where η, c_f and Σ are constants

$$\bar{\sigma} = \eta\, d(\vec{u}) + \Sigma\, \bar{I} \tag{7}$$

$$\nabla \cdot \bar{\sigma} = c_f\, \vec{u} \quad \text{in} \quad \Omega \qquad \text{and} \qquad \bar{\sigma}\, \vec{n} = \vec{0} \quad \text{on } \Gamma \tag{8}$$



(a) Shape evolution of a non-motile cell for an arbitrary value for parameters $\eta = 4$, $c_f = 1$, $\Sigma = 20$ and $v = 4$ 1 (non-dimensional)

(b) Actin network velocity field at steady state. Observe that at the steady state, the actin network velocity at the boundary is the same as actin polymerization velocity

The weak form of the above equation obtained is solved using Rheolef, an open source efficient C++ library [6].

We now describe the key steps performed by the code given the values of actin polymerization velocity during each time step.

1. The actin velocity field in the cell body is calculated from the given values of parameters.
2. Actin polymerization velocity (along the normal to the cell boundary) is added to the value of actin velocity field at the boundary. This gives the value of velocity at the boundary with which the cell is advected.
3. For the advection of the whole cell, it is necessary that the advection field be defined in the whole domain. We interpolate the field from the value at the boundary to be of the form given below.

$$\nabla \cdot \vec{u_{adv}} = 0 \text{ in } \Omega \quad \text{and} \quad \vec{u_{adv}} = \text{advection velocity on } \Gamma \tag{9}$$

4. With the given value of time step, we advect the domain with the advection velocity field. There is a likelyhood of mesh crossover and overlap. We address this problem by remeshing the whole domain. This is done by creating a CAD file with only the nodes along the boundary. Then the program calls for bamg or gmsh to create a suitable mesh. This mesh is then used in the next time step following the steps 1,2 and so on. Thus we obtain the evolution of shape of the cell with time.

The 2D solution would be analogous to the 1D with circular symmetry. We run our program for the same values of parameters and we observe that the cell approached the equilibrium shape asymptotically (fig. 2a). The actin network velocity close to equilibrium is shown in fig. 2b and it can be seen that the radially inward velocity at the boundary matches the radially outward velocity of the actin polymerization.

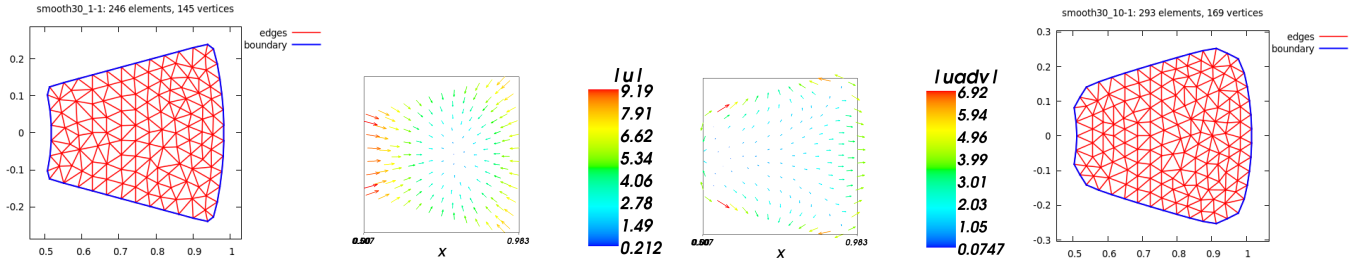
4 Motile cell

In a motile cell, experiments show that non-uniform bi-modal adhesion is observed in different regions of cell body with sticking adhesion at the leading edge and slipping adhesion at the rear and the sides, and thus force transmission to the substrate is via different mechanisms [7]. There is a negative feedback between actin network flow and adhesion strength. Close to regions of lower actin velocity, adhesion is high and there is force transmission through the 'gripping-like' action. Whereas in the regions of higher actin velocities, the strength of adhesion is lowered. Force transmission is through 'slipping-like' action. This observation is incorporated in the model by switching the value of coefficient of friction c_f based on the value of the actin velocity. We propose a switch, controlled by the norm of the actin velocity field and hence c_f is a nonlinear function of velocity field

$$c_f(|\vec{u}|) = c_{f0} + (c_{f1} - c_{f0})\frac{tanh(|\vec{u}| - uc)}{2} \tag{10}$$

Observation of cytoskeletal distribution of actomyosin filaments suggests that certain filaments have a prefered orientation during migration although isotropic distribution is observed generally [1]. This, we believe, is crucial in maintaining the velocity gradient in the cell required to initiate and sustain cell motility. We model this by additional terms for myosin and actin polymerization along the radial direction, which we choose as the prefered direction of motion for the cell. The orientation tensor $\bar{A}$ is defined as below, where e_r and e_θ are unit radial and orthoradial vectors

$$A_{ij} = (e_r)_i(e_r)_j + 0.5 * (e_\theta)_i(e_\theta)_j \tag{11}$$



(a) Initial domain of the motile cell with an anisotropic mesh (b) Actin network velocity field. (c) Advection velocity field (d) Cell shape after 10 time steps

Figure 4: Boundary evolution

The governing equations with the boundary condition is thus of the following form. The problem now reads: Monitor the shape evolution of the migrating cell and find $\vec{u}$ and $\bar{\sigma}$ such that

$$\bar{\sigma} = \eta\, d(\vec{u}) + \Sigma_0\, \bar{I} + \Sigma_1\, \bar{A} \tag{12}$$

$$\nabla \cdot \bar{\sigma} = c_f(|\vec{u}|)\, \vec{u} \quad \text{in } \Omega \tag{13}$$

$$\bar{\sigma}\, \vec{n} = \vec{0} \quad \text{on } \Gamma \tag{14}$$

With the boundary evolving with the velocity u_{adv} such that

$$\vec{u_{adv}} = \vec{u|_\Gamma} + v_{p1}\, \vec{n} + v_{p2}\, \bar{A}\, \vec{n} \tag{15}$$

The variational formulation and the procedure followed by the program to solve the problem is similar to the one detailed in the non-motile case with only the following changes:

1. Since c_f depends on the actin velocity field, the equation is nonlinear. Hence we use fixed point iteration to solve the equation for $\vec{u}$. We begin with an initial guess of the velocity field $\vec{u_0}$ and the equations are solved to obtain the new velocity field $\vec{u_1}$. The process is continued until the difference between norm of two successive values of the velocity field is less than a specified tolerance ϵ.

$$||(\vec{u_{k+1}} - \vec{u_k})|| \leq \epsilon \tag{16}$$

2. Also the motile case, myosin contractile stress and actin polymerization are no longer purely isotropic. The orientation tensor allows forf additional parameters to control the shape evolution of the migrating cell. Note that the coordinate system does not advect with the cell and remains fixed.

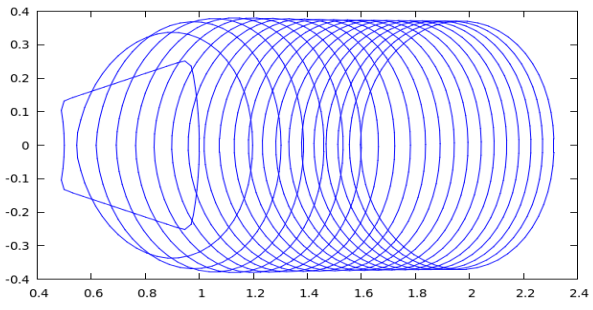


Figure 3: Cell shape evolution for every 100 time steps

The choice for the initial shape of the cell has two reasons, one being that since our cell has to have motility along the radial direction (through the definition of orientation tensor $\bar{A}$), we intended to have no effect of the orientation tensor on the lateral sides of the cell (vector $\bar{A}\,\vec{n}$ is zero) but only on the leading and and trailing edges. Secondly, it is similar to the fan-shaped structure exhibited by a motile cell [3]. We later found out that the condition for the lateral sides being perpendicular to vector $\bar{A}\,\vec{n}$ was found to be non-essential. Any shape that produces a difference in the actin velocity field at the leading and trailing edges was sufficient in initiating migration.

Shape evolution of the cell is shown in fig. 3 for parameter values of $\eta = 1$, $c_{f0} = 10$, $c_{f1} = 1$, $\Sigma_0 = 15$, $\Sigma_1 = 20$, $v_{p0} = 4$ and $v_{p1} = 6$.

Tracking the shape evolution after each time step involved solving the nonlinear equation to obtain the actin network velocity (fig. 4b), addition of velocity on the boundary and actin polymerization velocity to obtain the advection velocity at the boundary, obtaining a smooth variation of this advection field over the domain (fig. 4c), advection of the domain, remeshing of the domain with the boundary nodes to obtain the evolved and remeshed domain 4d.

As can be seen in fig. 3, the cell evolves into a more elliptical shape. This is expected since we have isotropic actomyosin activity and an additional radially oriented component. After a few time steps, we observe a steady height

for the cell, but the cell width is observed to grow very gradually. This corresponds to the action of relative strengths of actin and myosin, opposing each other.

It is interesting to note that as time progresses the cell slows down, and tends towards a more circular shape 5. This is also evident in the decrease in the difference between the mass centre and the mean distance between leading and trailing edges of the cell. Also the difference in the velocities at the leading and trailing edges decreases. We could think of this process as dissipation of potential energy of the shape of the cell to attain a lower energy circular shape. This suggests us that in order to have a steady motion of the cell, we require the shape to be more or less the same while being advected. But it is natural for a deformable cell to tend to a low energy state. Hence, there must be another factor that results in a steady motion of the cell. We could think of the present motion to be one where the unknown factor is 'switched off' resulting in a velocity decline towards a steady state.

5 Summary and future work

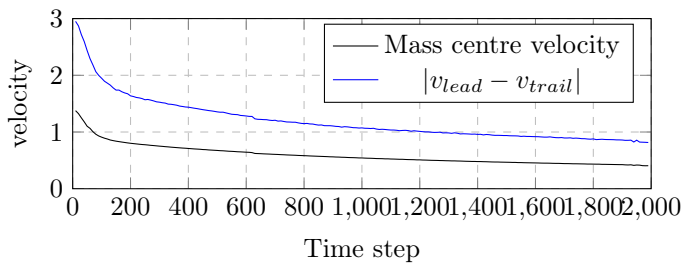


Figure 5: Shape evolution of a motile cell for value for parameters $\eta = 1$, $c_{f0} = 10$, $c_{f1} = 1$, $\Sigma_0 = 15$, $\Sigma_1 = 20$, $v_{p0} = 4$ and $v_{p1} = 6$ (non-dimensional)

In this paper we developed a rheological model based on the biological processes of actomyosin and adhesion occuring in a living cell with the help of observations recorded experimentally during non-motile and motile states. The cytoskeleton of the cell was modelled as a viso-elastic-active fluid. The resulting equations were solved analytically and numerically to determine the evolution of shape for non-motile state of the cell. We could successfully explain the influence of different parameters on the cell behaviour. This opened doors for more involved research into the complex process of cell migration, where we introduced new approximations for cell motility based on available experimental observations. We could successfully simulate cell migration and this lead to further insight into the factors affecting it and possible improvements for further investigation.

Work will be carried out towards obtaining steady state migration. This could be applied to study the migration patterns in live cell countours obtained from experiments. Also, this would serve as a foundation to a 3D study of cell motility.

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