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REVIEW ARTICLE

3-D cell modeling and investigating its movement on non-rigid substrates

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ABSTRACT

Introduction: Attachment of blood and tissue cells to the extracellular matrix (ECM) is done with the help of specific joints, which are made between cells' receptors and matrixes' ligands. This interaction is vital for many biological activities such as cell migration,cancer development, and wound healing. Objectives: The purpose of this study is to model a 3-dimensional cell by ABAQUS commercial software package and investigate effects of factors, including mechanical properties of focal adhesion, substrate stiffness and cell active stress on cell motility over a smooth non-rigid substrate.

Methods: In our model, the cell is assumed to be a continuum and continuum mechanics equations are used for the cell, also due to symmetry in the geometry of the problem, symmetric approach has been applied in order to conserve time.

Results: Simulation results of this modeling demonstrate that mechanical properties of focal adhesion between cell and substrate and also substrate stiffness have considerable effect on cell motility.

Conclusion: In fact, increase in substrate stiffness and also increase in focal adhesion strength cause an increase in magnitude of cell-substrate traction stress and on the other hand, increase in these two parameters causes cell motility velocity to continue its decreasing trend. Furthermore, magnitude of traction stress and cell movement speed highly depends on the active stress in the cell which perceptible increase can be observed in these two values when active stress increases. These results agree with experimental data reported by other authors.

Key words: Cell motility; 3-D Finite element model; Non-rigid substrate; Traction; Focal adhesion

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Introduction:

In mammals, migration of blood and tissue cells over an adhesive substrate apparently occurs through series of reversible reactions between protein receptors on cell membrane and ligands on the substrate [1]. Without adhesion of cells to each other and to the extracellular matrix, many vital activities of cells are not possible [2]. Adhesive proteins hold the tissue components close to each other and consolidate the tissue. Cell adhesion also becomes important in activities of migrant cells like leukocytes [3].

Formation and evolution of fetus from primary cells also depend on this phenomenon [4]. Some patients suffer from congenital diseases, which are caused due to poor performance of cell adhesions. Also, other patients have genes that cause malfunction in adhesive proteins performance. Cancer and tumorigenesis are among these diseases [5, 6].

Furthermore, adhesive proteins in viruses and bacteria are among factors regarding their penetration and displacement into the victim's body [7]. Other examples include relative connection of neural synapses. Memory formation and occurrence of Alzheimer's disease are resulted from activity of adhesion at synapses [8].

Purpose of this study is to present a model for simulation of cell movement by considering cell rheological properties and using ABAQUS finite element analysis software. Characteristics of cell motility are function of intracellular active stress, substrate's elastic properties, cytoplasm's viscous properties and also strength of cell-substrate connection. The presented model in this study is suitable for simulating 3-dimensional cell motility and investigating its unidirectional movement.

In this article, properties of fibroblast cell is used in which cell and substrate are considered as viscoelastic and elastic materials, respectively and adhesion between cell and substrate is also supposed to be a specific form of viscous friction..

Methods:

Initial configuration of the cell is assumed to be in ellipsoid form (on both sides) with semi-major and semi-minor axes equal to 25 and 12.5 micro-meters, and the cell is considered to be fixed on a rectangular substrate with length and width equal to 300 and 100 micro-meters. Figure 1 shows a schematic diagram regarding the cell and its dimensions. In this study, the cell and the substrate are considered to be a standard linear solid viscoelastic material and elastic material with elasticity modulus within the range of 0.5 to 100 kPa to investigate its effect on cell movement, respectively.

In the model presented in this article, which is very close to the actual case of cell motility, first the cell is assumed to be fixed on its location over the substrate, then by creating force (active stress) in the front part of the cell, which is created with the help of actins polymerization, the front part of the cell starts to stretch, while in the software the back-end is fixed to the substrate.

After 40 seconds [9, 10], the force in the front of the cell disappears and the frontal section of the cell becomes fixed and meanwhile the cell back-end is set free. Since tension and force is formed in the cell, it starts to move forward. Again after 40 seconds [10], the first stage (step) is repeated and this continues until cell motility can be modeled for 50 minutes timeframe which was defined as automatic time-steps in the software. It should be noted that this process was performed by explicit dynamics modeling, also due to symmetry in the geometry of the problem, symmetric approach has been applied in order to conserve time.

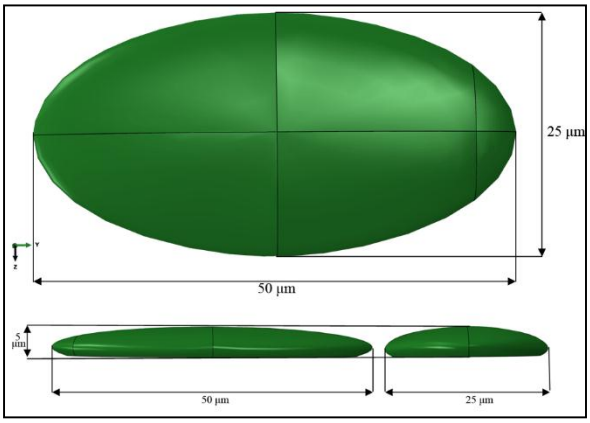

Figure 1: Schematic of cell and its dimensions in this study

Using the above-mentioned modeling, effects of different parameters such as substrate stiffness and adhesion strength on cell movement velocity, maximum magnitude traction stress exerted to the substrate and shear stress acting on the cell during the movement can be investigated for each model. Values of parameters used in this study are presented in Table 1.

Table 1: Parameters used in the simulations

Parameters	Description	Values	References
E_{sub}	Substrate elastic modulus	100,10,0.5 kPa	[2, 11, 12]
P_0	Maximum (anterior) intracellular active stress	100,200,400 Pa	[13]
k_s	Adhesion strength	10-35 Pa	Assumed in this study
η_s	Damper	100 Pa.s	Assumed in this study
R_{cell}	Cell semi-major axis	25 micro-meter	[14, 15]
r_{cell}	Cell semi-minor axis	12.5 micro-meter	[14, 15]
E_1, E_2, η	Cell material properties (standard linear solid)	296.65,450 Pa 4335 Pa.s	[16]

Cell material model

In next step, 3-D viscoelastic equations for the cell must be extracted so as to define the material properties in the software. Considering the presented model and since the applied force (active stress) on the cell is unidirectional, the 3-D equations regarding the standard linear solid are extracted as follows [17]:

$$\frac{\varepsilon_{11}(t)}{\sigma_0} = J(t) = \frac{1}{3}\left[\frac{1}{E_1} - \frac{E_2}{E_1(E_1+E_2)}e^{-t/\tau_\sigma} + \frac{1}{3K}\right] \quad (1)$$

$$\tau_\sigma = \frac{\eta(E_1+E_2)}{E_1 \cdot E_2} \quad (2)$$

Where E_1, E_2 are spring constants for the standard linear solid, K is elastic bulk modulus and η, J(t), τ_σ, ε and σ are damper concerning the model, creep compliance, creep time constant, strain and stress, respectively.

Therefore, magnitude of creep compliance in one direction for a 3-D standard linear solid with unidirectional applied stress is according to equation 1. For the sake of simplicity, this magnitude is considered to be constant and the same in all the three directions. Considering equation 1, by determining time range and using creep test in the software, cell viscoelastic properties are defined.

Mesh type and elements

Since the substrate is considered to be in shape of rectangular prism, hexahedral elements with fairly big meshes compared to cell elements (0.05 micro-meters) are used. This causes a reduction in computation and run-time of the analysis. Finally, number of elements and nodes are 600 and 1342 respectively.

But, since shape of the cell is rather complex, and also due to importance of stress analysis on the cell, tetrahedral elements C3D4 with small (0.02 micro-meters) meshes are used for the cell. For meshes smaller than 0.02 micro-meters, the solutions converged and for bigger meshes the solutions fluctuated and diverged. Therefore, the mesh size for the cell is considered to be 0.02 micro-meters. Finally the number of elements and nodes for the cell are 2648 and 776 respectively.

It should be noted that number of elements and nodes for the cell and the substrate are given in symmetric case, which can be seen in Figure 2:

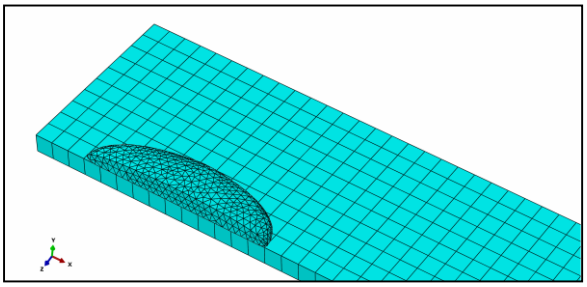

Figure 2: Schematic of the 3-D model of the cell on top of the substrate

Boundary condition

After the modeling process and defining the properties regarding the cell and the substrate, boundary conditions must be defined. To do that, according to the presented helical motion model and previous studies, magnitude of the propulsive active stress for a single cell is evaluated to be between 100-1000 Pa [13]. This force acts on a surface area equal to 50 square micro-meters [14, 15] which acts on the front part of the cell, in this study three different values of fibroblast active stress are used, which are: 200, 100 and 400 Pa.

Also, according to the results obtained from [18], an approximate length equal to 8.8±0.2 micro-meters (which is related to a cell with length around 30 to 50 micro-meters) in first phase of the cell movement is fixed at the rear end of the cell and is attached to the substrate.

Except for the forces that act on the frontal section of the cell which are resulted from actins polymerization and are called active stress, another stress exists in the cell which is called passive stress. In fact, this stress shows tendency of the cell to contract and is neglected

in this study due to its negligible magnitude compared to magnitude of active stress.

In addition to active and passive stresses, another significant force act on the cell during its movement, which is called friction, which is existed due to consistent formation and disconnection of adhesion between the cell and the substrate. This friction force is modeled by a specific type of viscous forces, which is defined with the help of a spring and a damper.

To model such a friction, two methods can be used: in first method, an adhesive element can be defined that covers the whole surface area of the substrate and is located between the cell and the substrate with a negligible thickness and is considered to be a viscoelastic material with parallel spring and damper (Kelvin Model). In the second method, contact between the cell and the substrate can be set by defining a general contact in dynamics solution scope between the lower surface of the cell and the upper surface of the substrate and also by defining a viscous friction between the cell and the substrate in ABAQUS software.

In this modeling, both methods are used in order to ensure validity of the analysis. It should be noted that in the reality increase in adhesion strength between the cell and the substrate is equal to increase in concentration of receptors and ligands on the cell and substrate surfaces. Magnitude of damping is considered to be 100 Pa.s and the spring constant is also supposed to be within the range of 10-35 Pa. Figure 3 shows schematics of the cell-substrate connection:

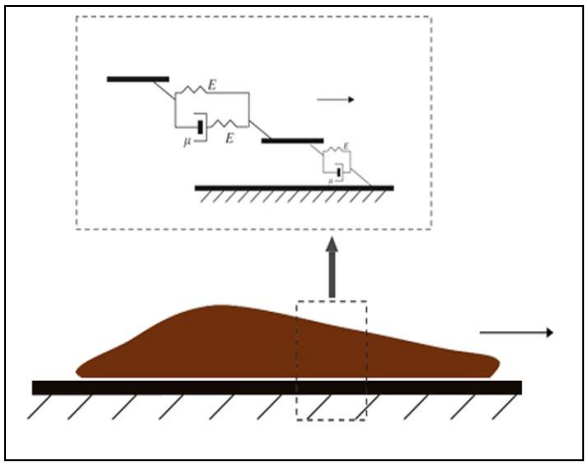

Figure 3: Schematics of cell-substrate connection and its material

Results:

In this article, maximum magnitude for traction stress and also average movement velocity of cell as a function of adhesion strength and over a substrate with stiffness equal to 0.5, 10 and 100 are plotted.

Figure 4 demonstrates cell average velocity for active stress equal to 100 Pa and for different values of substrate's stiffness and also adhesion strength of 20 Pa.

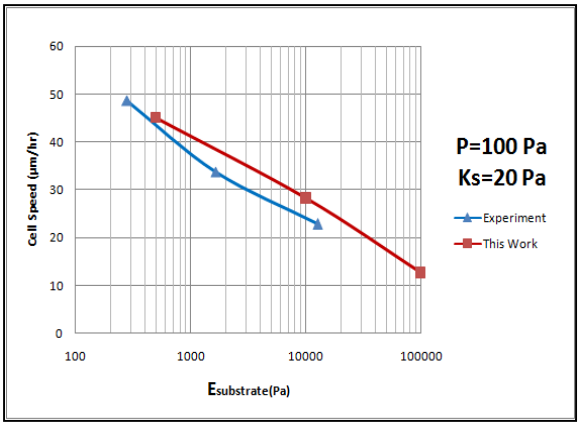

Figure 4: Variations of average cell velocity at different values of substrate's stiffness

Figure 5 shows the results of cell average velocity for active stress equal to 200 Pa over substrates with different values of elasticity modulus and different values of adhesion coefficient:

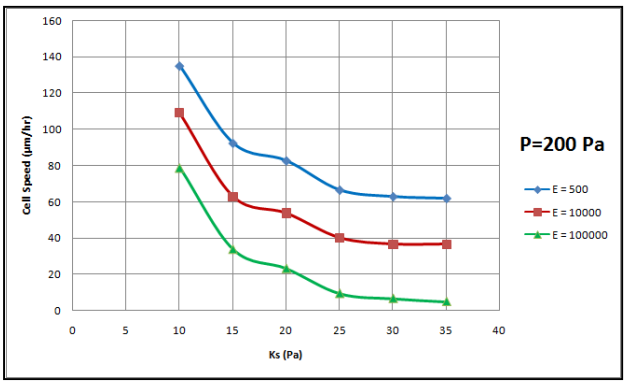

Figure 5: Variations of average cell velocity over different substrates with active stress equal to 200 Pa with respect to adhesion strength

Figure 7 demonstrates maximum magnitude of the traction stress for different values of substrate stiffness and adhesion and also for active stress equal to 100 Pa:

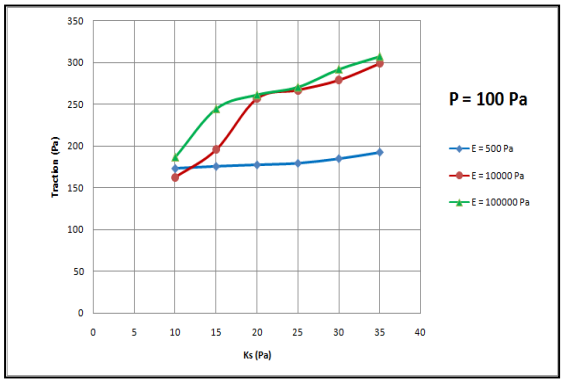

Figure 7: Variations of maximum magnitude of traction stress acting on the cell floor on different substrates when the active stress is equal to 100 Pa with respect to adhesion strength

Among other results obtained in this study is average shear stress acting on the cell floor which according to Figure 8 and 9, by increasing substrate stiffness, magnitude of shear stress on the cell floor increases:

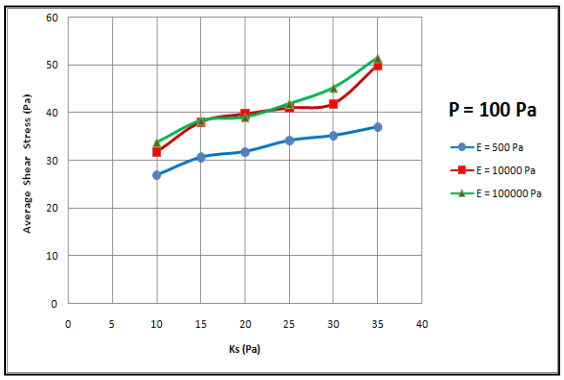

Figure 8: Variations of average shear stress on the cell floor over different substrates when the active stress is 100 Pa with respect to adhesion strength

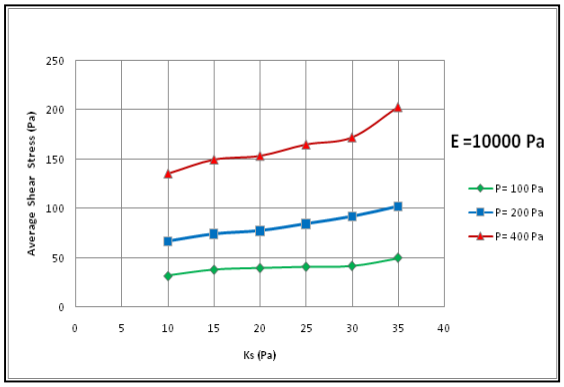

Figure 9: Variations of average shear stress on the cell floor over a substrate with stiffness equal to 10000 Pa when the active stress is 200, 100 and 400 Pa with respect to adhesion strength

Discussion

According to the obtained results in the figure 4, these values are highly acceptable when compared to experimental results of Ghosh et al. 2006 [12].

According to the obtained results of both studies, it can be observed that average cell velocity deceases with increase in substrate stiffness and this might be due to that since the back end of the cell is fixed to the substrate in odd time-steps, when the force is applied to the front part of the cell, the substrate also slightly moves forward with the cell, therefore the less the substrate elasticity, the more the forward movement of the substrate, and therefore the more progress of the middle section of the cell within 50 minutes and then the more average cell velocity.

Also, the error which appears in this simulation with respect to the experimental study is as follows:

$$\%Error = \frac{|our\ data - accepted\ value|}{accepted\ value} \times 100 \quad (3)$$

This value for cell movement velocity over the substrate with elasticity modulus equal to 500 and 10000 Pa would be:

$$\%Error\ (500\ Pa) = \frac{|45 - 45.47|}{45.47} \times 100 = 1.033\%$$

$$\%Error\ (10000\ Pa) = \frac{|28.24 - 25.73|}{25.73} \times 100 = 9.75\%$$

The result obtained in figure 5 shows that increase in adhesion strength between the cell and the substrate, which is equal to increase in concentration of the receptors and ligands on the cell and substrate surfaces in the reality, also causes the average cell velocity to decrease. This result was expected because when considering schematic diagram in figure 6, adhesion strength can be considered as springs, which are connected to receptors on the surface of the cell, therefore, an increase in sprig constant of these springs causes the magnitude of the required force for separation of these connections to increase.

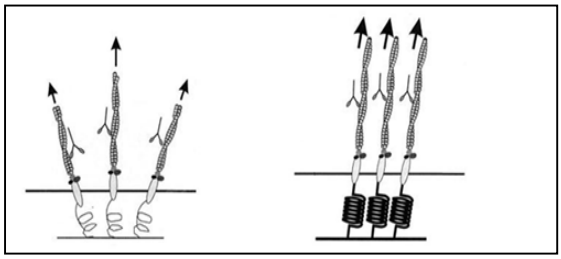

Figure 6: Schematic diagram showing the connection between cell receptors and substrate ligands which are shown in form of springs (derive from Lo et al., 2000 [15])

As was expected, cell movement velocity shows a decreasing trend by increasing substrate stiffness and also increasing adhesion strength coefficient, furthermore, it can be observed that decrease in substrate elasticity from 100000 to 10000 Pa causes average cell velocity to increase by 116.2 percent, and also decrease in substrate elasticity from 10000 to 500 Pa causes average cell velocity to increase by 47.6 percent. These percentage values for active stresses equal to 100 and 400 Ps are 114.85%, 50.98% and 119.22% and 41.66%, respectively.

Furthermore, in stress and force analysis section, magnitude of traction stress applied to the substrate can be calculated and then can be compared to the experimental results and the previous modeling;

Considering the present definition for the traction stress, the following equation can be used:

$$\vec{T} = \vec{n}[\sigma] \qquad (4)$$

Where $\vec{T}$, $\vec{n}$ and σ are 3-D surface traction vector, surface normal vector and material stress tensor, respectively.

Now, due to the figure 7, the results of this study can be compared to the results in Pan et al., 2009 [19] and Fournier et al., 2010 [20] and Maskarinec et al., 2009 [21]. According to the results of the aforementioned studies, maximum magnitude of traction stress acting on the substrate in experimental studies was within the range of 120 to 450 Pa which is in good agreement with the results obtained in this study.

Conclusion:

As was mentioned earlier, without adhesion of cells to each other and to the extracellular matrix, many vital activities cannot be performed by cells. Despite defects (incompleteness) of the presented models in regard to cell motility, by comparing the results of this research with other experimental results we understand that they are acceptable.

Based on the obtained results from the presented models for cell motility and also the results of experimental studies, it can be concluded that regardless of cell type, cell movement velocity over a substrate is a function of substrate stiffness, adhesion strength, receptors concentration and ligands concentration.

Regarding the modeling presented in this article, in spite of all the assumptions and simplifications that helped us solve the model in a better way, it must be noted that this model is able to show effects of different mechanical factors on cell movement velocity and formed stresses in the cell and can also be a valuable model in studies on cancer development and lesion healing.

Now, considering the obtained results in this study, to increase or decrease cell movement velocity over a substrate, some suggestions can be made. For an instance, in order to accelerate process of wound healing, conditions can be provided by changing the cell adhesiveness and substrate stiffness so that the cell movement velocity increases and consequently the healing process takes place faster. On the contrary, for the glands and cancerous tumor, by changing the above-mentioned factors, cell movement velocity can be reduced so that tumor metastasis or tumor growth can be minimized.

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