2012 International Conference on Biological and Life Sciences
IPCBEE vol.40 (2012) © (2012) IACSIT Press, Singapore

Analysis of Local Stiffness in ECM Embedded with Bioconjugated Magnetic Beads under the Influence of an External Magnetic Field

Yue Du [1, 4], Sahan C. B. Herath [1, 4], Qing-Guo Wang [2], Harry Asada [3,4] and Peter C. Y. Chen [1, 4 +]

[1] Department of Mechanical Engineering, National University of Singapore, Singapore
[2] Department of Electrical and Computer Engineering, National University of Singapore, Singapore
[3] Department of Mechanical Engineering, Massachusetts Institute of Technology, USA
[4] Biosystem and Micromechanics Interdisciplinary Research Group, Singapore-MIT Alliance for Research and Technology, Singapore

Abstract. In this paper, we present a finite-element model for determining the local stiffness of an ECM sample with bioconjugated magnetic beads under the influence of an external magnetic field. The viscoelastic deformation of ECM samples embedded with magnetic beads (that are bioconjugated with the collagen fibres in the ECM) in the presence and absence of an external magnetic field was analysed using the finite element method. Based on information about viscoelastic deformation and the external forces, the stiffness of the ECM can be computed for the cases when the magnetic field is absent and present. We conducted numerical simulation using our model on two typical scenarios and compared our simulation results with that obtained from COMSOL. We also applied our model on an actual ECM sample embedded with bioconjugated beads and compared our results with that obtained from stretching tests done on that sample. Our analytical results are in close agreement with that obtained from COMSOL and the stretching tests.

Keywords: Extracellular matrix, Stiffness, Pre-tension in ECM, Finite element analysis, Viscoelasticity.

1. Introduction

Cell morphology and cytoskeletal structure are known to be influenced by the stiffness of their surrounding microenvironment [1-3]. Various methods for modifying ECM stiffness have been used to explore the effect of ECM collagen gel rigidity on cell behaviour. These methods include changing the concentration [4, 5] or pH of the collagen [6, 7], or varying the boundary condition (e.g., floating gel or mechanically constrained gel) [2] and the polymerization condition of the ECM [8].

We have proposed an approach for manipulating the local stiffness of ECM by embedding magnetic beads that are bioconjugated with the ECM fibres, then applying an external magnetic field to alter the fibres' resistance to deformation [9]. In this approach, the local stiffness of an ECM sample can be manipulated in real-time by proper control of the external magnetic field. This provides convenience and flexibility in generating stiffness gradients within a single ECM sample for the exploration of cell behaviours.

In this paper, we present a finite-element model for determining the local stiffness of an ECM sample with bioconjugated beads under the influence of an external magnetic field. Section 2 describes our finite-element method for analysing viscoelastic deformation of ECM gel due to the forces exerted by the beads under an external magnetic field. In Section 3, we apply our finite-element model to study the effect of (i) a single bead, and (ii) two columns of aligned beads, on the stiffness of an ECM sample under pre-tension caused by limited point loads representing the forces exerted by the beads, and compare the result to that obtained using COMSOL. We also apply our finite-element model to an ECM sample with randomly

[+] Corresponding author. Tel.: (+65) 6516 8837
E-mail address: mpechenp@nus.edu.sg

distributed beads, and compare the analytical results with experimental results obtained from a stretch test done on an ECM sample. We discuss possible improvement for our proposed model and present the conclusion in Section 4.

2. Finite Element Analysis

Our method for analysing viscoelastic deformation of beads-embedded ECM is based on the finite element method for the elastic materials [10, 11]. The viscoelasticity of the ECM is represented by The Standard Linear Solid Model, consisting of two branches in parallel: one having a spring and a dashpot in series and the other having only a spring. When subjected to a constant stress, the stress tensor of each element, including elastic and viscous parts, can be expressed as

$$\sigma_{e_{int}} = E_1 \varepsilon_e + \eta \frac{E_1+E_2}{E_2} \dot{\varepsilon}_e \tag{1}$$

where $\sigma_{e_{int}}$ and ε_e are the stress and the strain for each element, respectively, E_1 is the stable elastic modulus after relaxation, E_2 is the instantaneous elastic deformation when a constant stress is applied, and η is the viscosity of the dashpot. The internal restoring force is

$$F_{e_{int}} = A_e E_1 \varepsilon_e + A_e \eta \frac{E_1+E_2}{E_2} \dot{\varepsilon}_e \tag{2}$$

where A_e is the cross-sectional area of each element. We use the linear shape function

$$B_e = \frac{1}{l_e} [-1 \quad 1] \tag{3}$$

where l_e is the length of each element. Thus, the strain of the element is

$$\varepsilon_e = B_e \begin{bmatrix} u_{e1} \\ u_{e2} \end{bmatrix} \tag{4}$$

where u_{e1} and u_{e2} are the displacement of the first and second node in the element. Equation (2) can be rewritten as,

$$F_{e_{int}} = A_e E_1 B_e \begin{bmatrix} u_{e1} \\ u_{e2} \end{bmatrix} + A_e \eta \frac{E_1+E_2}{E_2} B_e \begin{bmatrix} \dot{u}_{e1} \\ \dot{u}_{e2} \end{bmatrix} \tag{5}$$

$$K_{e_{elastic}} = A_e E_1 B_e \tag{6}$$

$$K_{e_{viscous}} = A_e \eta \frac{E_1+E_2}{E_2} B_e \tag{7}$$

The expressions for $K_{e_{elastic}}$ and $K_{e_{viscous}}$ are then assembled into $K_{elastic}$ and $K_{viscous}$, respectively, based on the principle that each node connected two elements. (Details of the assembly are omitted here due to space limitation.) Using the force balance of the internal and external force,

$$F_{int} = F_{ext} \tag{8}$$

where F_{int} is the sum of all the internal forces of the elements. Without the forces exerted by the beads in the presence of a magnetic field, F_{ext} may include traction forces due to cell migration and boundary forces in the ECM. Then, based on Equations (5)-(7), the assembled external force at time step *t* is equal to

$$F_{ext}^t = K_{elastic} u^{t-1} - K_{viscous} \frac{(u^t - u^{t-1})}{\Delta t} \tag{9}$$

where u^t is the displacement of the nodes, i.e.,

$$u^t = [u_1^t \quad u_2^t \quad \dots \quad u_n^t]^T \tag{10}$$

Since the displacements of nodes at the boundaries are zero, the corresponding rows in the stiffness matrix will be removed during calculation. Based on Equation (9), the displacement of each node at time step *t* is determined recursively from its displacement at time step $(t-1)$, i.e.,

$$u^t = \frac{\Delta t}{K_{viscous}^{-1}} \left(K_{elastic} u^{t-1} + \frac{K_{viscous}}{\Delta t} u^{t-1} - F_{ext}^t\right) \tag{11}$$

Taking into account the force F_{mag}^t exerted by the bead under the influence of a magnetic field, u^t is reduced to

$$u^t = \frac{\Delta t}{K_{viscous}^{-1}} \left(K_{elastic} u^{t-1} + \frac{K_{viscous}}{\Delta t} u^{t-1} - (F_{ext}^t - F_{mag}^t)\right) \tag{12}$$

3. Numerical Simulation and Validation of Results

3.1. Numerical Simulation of Pre-tension in ECM Due to Point Loads Representing Forces Exerted by Bead

The pre-tension caused by the forces due to one bead (i.e., one point) and two columns of beads in opposite directions are simulated using both the derived finite-element model and COMSOL. The viscoelastic parameters used are $E_1 = 1719$ Pa, $E_2 = 500$ Pa, $\eta = 137$ Pa $\cdot$ min. For the case of a single bead, the bead is set at 0.003 m away from one edge of the ECM. The opposite edge is fixed. The force exerted by the bead due to an external magnetic field is set at 1.2×10^{-7} N. In this scenario, the pre-tension in the area near the bead in the direction of the bead-exerted force is analysed, as is illustrated in Fig. 2a). For the beads-in-two-column scenario, each column is set at a distance of 0.003 m away from an edge of an ECM strip with the magnetic forces acting in opposite directions, as is illustrated in Fig. 2b). The effect of the pre-tension due to a single bead is sensitive to the mesh fineness around the bead. Hence, for comparison with COMSOL, in the calculation using our model the size of a mesh element is set to be similar to that used in the COMSOL simulation. This issue is not significant for the beads-in-two-column scenario, in which the pre-tension of the ECM in the direction perpendicular to the direction of the bead-exerted force is analysed.

Fig. 1a) and b) show the results obtained from COMSOL for the stress tensor and deformation at t = 26s for the two scenarios. Fig. 1c) and d) show the exact pre-tension generated in ECM. The (red) solid curve shows the result from our model and the (blue) dashed curve shows the results from COMSOL. Fig. 1c) shows the pre-tension in the area near the bead in the direction of the bead-exerted force. The maximal pre-tension at t = 26s calculated from our model is -1.4×10^{-6} m, while that from COMSOL is -1.2×10^{-6}m. A sharp pre-tension is generated in a very small area near the bead, and decays away from the bead. Fig. 1d) shows the pre-tension of ECM in the direction perpendicular to the direction of bead-exerted force. The deformation calculated from our model is between 2.9×10^{-7} m and 3×10^{-7}m, while that from COMSOL is between 2.8×10^{-7}m and 2.9×10^{-7}.

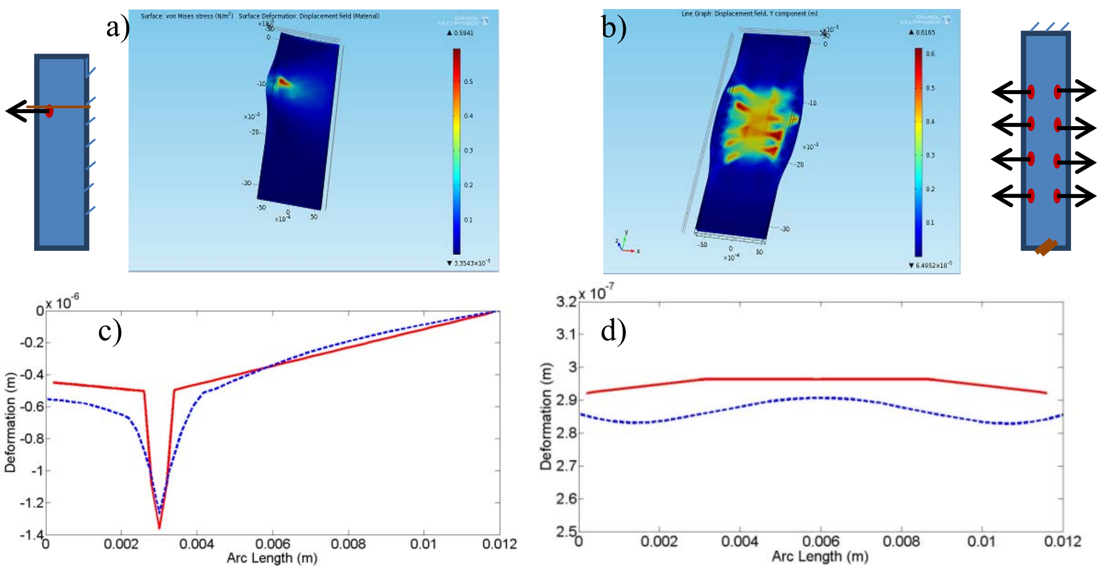

Fig. 1: Pre-tension created in ECM by point load

3.2. Numerical Simulation of Change in Stiffness of Actual ECM Sample with Bioconjugated Magnetic Beads under Magnetic Field

In this section we discuss the application of our model to predict the change in the measured stiffness of an ECM sample due to pre-tension generated by bead-exerted force, and compare our analytical prediction with the actual results obtained from a stretching test reported in [9]. The stretching test involved an ECM strip embedded (by bioconjugation with the collagen fibers) with beads. The strip was placed between two permanent magnets, with the bottom of the strip fixed and part of the strip in the middle directly exposed width-wise to the magnetic field. When the top of the strip is pulled, the beads in the mid-section of the strip exposed to the magnetic field experience a two forces, each can be resolved into a horizontal component pointing toward the magnets and a vertical component pointing downward. The net downward force on a bead provides resistance to the deformation of the strip. Since in this case the beads are randomly distributed throughout the ECM strip, it is not practical to locate the exact position of each bead in order to use COMSOL to study the resulting ECM stiffness. Our analytical model, however, can still be readily applied. The same parameter values used in the experiment (as summarized in Table 1) are also used in this simulation. The magnetic force is calculated according the formula derived in [12].

Table.1: Parameters used in experiment

Parameter	Value	Units
The dimension of ECM	33×12×4	mm
Magnetic flux field	2	T
Volume of beads sample	0.325	ml
Deformation rate of ECM	10	mm/min
Diameter of magnet	12	mm
Thickness of magnet	4	mm
Magnetization	70	emu/g

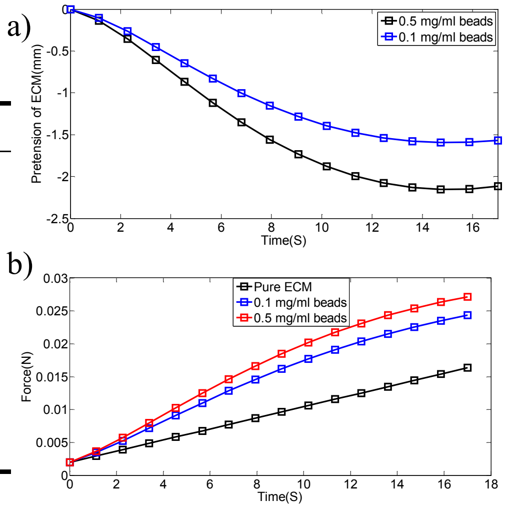

Fig. 2: Pre-tension in ECM and stretching force

Using the value for the viscoelasticity of pure ECM gel experimentally determined in [9], we obtain $E_1 = 1719$ Pa and η = 137 Pa · min for use in our model. Fig. 2a) shows the pre-tension (which opposes the stretching) created in the ECM gel. It can be seen that the pre-tension increases exponentially and finally approaches a stable value of −1.5 mm after 13 seconds because of the viscoelasticity of the ECM gel. Fig. 2b) shows the tensile forces needed to deform ECM gel at a constant rate of 10 mm/min in the absence (i.e., the dotted curve) and the presence (the solid curve) of the magnetic field. In the absence of the magnetic field, the force increases linearly with the deformation of the ECM gel. There is an offset 0.002N corresponding to the portion of force that is needed to deform the viscous part of ECM gel at constant rate. In the presence of the magnetic field, due to the pre-tension created by the beads that resists ECM deforming, a greater force is needed to deform ECM at the same rate.

Fig. 3 shows the stiffness calculated using our model and that determined experimentally as reported in [9] for two types of samples with different bead concentrations of 0.5mg/ml and 0.1mg/ml. The stiffness was estimated at t = 16s of the simulation, at which point the pre-tension was at the stable value. It can be seen that our analytical prediction of the stiffness of the ECM is close to the experimental results, especially for ECM with a higher concentration of beads. The stiffness of ECM with bead concentration of 0.5mg/ml calculated from our model is 0.0102 N/mm, whereas that obtained from experiment is 0.011 N/mm.

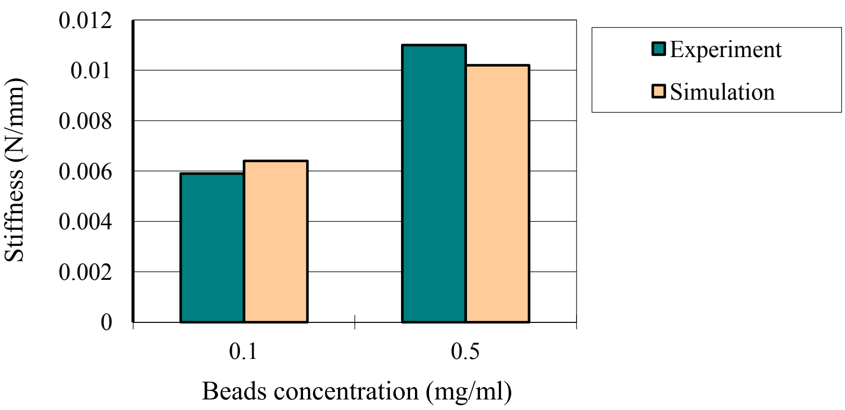

Fig. 3: Comparison of stiffness value from experiment and simulation.

4. Discussion and Conclusion

The work report in this paper contributes to the mathematical analysis of the manipulation of ECM stiffness by creating pre-tension in ECM using micro-size magnetic beads under the influence of an external magnetic field, providing the basis for fine control of the stiffness of ECM gel. The viscoelastic deformation of ECM samples embedded with bioconjugated magnetic beads in the presence and absence of an external magnetic field was analyzed by the finite element method. Based on information about viscoelastic deformation and external force, the stiffness of the ECM can be computed for the cases when the magnetic field is absent and present. We conducted numerical simulation using our model on two typical scenarios and compared our simulation results with that obtained from COMSOL. We also applied our model on an actual ECM sample embedded with beads and compared our results with that obtained from stretching tests on that sample. results are in close agreement with that obtained from COMSOL and the stretching tests.

Compared to other methods of manipulating ECM stiffness by changing the chemical composition of collagen gel, the method described in this paper is more versatile because it enables the control of the desired local stiffness at a particular region (with the presence and absence of magnetic beads) of an ECM sample and at a specific time (by switching on or off the external magnetic field). Such control will be useful in studying cell behaviour in a microenvironment with varying stiffness.

The analysis presented in this paper has not addressed the issue of how the mere existence of the beads in the ECM may influence the stiffness of the ECM in the absence of the magnetic field. This issue concerns the physical properties of the beads and the ECM, and the bio-conjugating reaction between beads and ECM. Taking this issue into account in the analysis will enable the resulting model to predict more accurately how the ECM will deform in the presence of an external magnetic field. Another issue that needs to be explored is the inclusion of cell traction forces in the model of ECM deformation behaviour, since such forces may interfere with the magnetic forces that are supposed to create the desired pre-tension in the ECM.

5. References

[1] S. Huang, C. S. Chen and D. E. Ingber. Control of cyclin D1, $p27^{Kip1}$, and cell cycle progression in human capillary endothelial cells by cell shape and cytoskeletal tension. *Mol. Biol. Cell*. 1998, **9** (11): 3179-3193.

[2] A. L. Sieminski, R. P. Hebbel, and K. J. Gooch. The relative magnitude of endothelial force generation and matrix stiffness modulate capillary morphogenesis. *Exp. Cell Res*. 2004, **297** (2): 574-584,

[3] S. Weng, and J. Fu. Synergistic regulation of cell function by matrix rigidity and adhesive pattern. *Biomaterials*. 2011, **32** (36): 9584-9593.

[4] S. Kanzawa, H. Endo and N. Shioya. Improved in vitro angiogenesis model by collagen density reduction and the use of type III collagen. *Ann. Plast. Surg.* vol. 1993, **30** (3): 244-251.

[5] A. Stéphanou, G. Meskaoui, B. Vailhé and P. Tracqui. The rigidity in fibrin gels as a contributing factor to the dynamics of in vitro vascular cord formation. *Microvasc. Res.* 2007, **73** (3): 182-190.

[6] N. Yamamura, R. Sudo, M. Ikeda and K. Tanishita. Effects of the mechanical properties of collagen gel on the *in vitro* formation of microvessel networks by endothelial cells. *Tissue Engin.* 2007, **13** (7): 1443-1453.

[7] V. Nehls and R. Herrmann. The configuration of fibrin clots determines capillary morphogenesis and endothelial cell migration. *Microvasc. Res.* 1996, **51** (3): 347-364.

[8] C. F. Deroanne, C. M. Lapiere and B. V. Nusgens. In vitro tubulogenesis of endothelial cells by relaxation of the coupling extracellular matrix-cytoskeleton. *Cardiovasc. Res.* 2001, **49** (3): 647-658.

[9] Peter C. Y. Chen, S. C. Herath, Dong-An Wang, K. Su, K. Liao and H. Asada. Active manipulation of uniaxial ECM stiffness by magnetic anchoring of bio-conjugated beads. *ASME Summer Bioengineering Conference*. Pennsylvania. 2011, pp. 1-14.

[10] T. R. Chandrupatla and A. D. Belegundu, *Introduction to Finite Elements in Engineering*. Prentice Hall, 1996.

[11] G. Dhatt and G. Touzot. *The Finite Element Method Displayed*. John Wiley and Sons, 1984.

[12] J. Lipfert, X. Hao and N. H. Dekker. Quantitative modeling and optimization of magnetic tweezers. *Biophys. J.* 2009, **96** (12): 5040-5049.