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Research Paper

Mechanical behavior of cells in microinjection: A minimum potential energy study

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ARTICLE INFO

Article history:
Received 12 February 2013
Received in revised form
25 March 2013
Accepted 20 April 2013
Available online 7 May 2013

Keywords:
Mechanical behavior
Cell model
Microinjection
Potential energy

ABSTRACT

Microinjection is a widely used technique to deliver foreign materials into biological cells. We propose a mathematical model to study the mechanical behavior of a cell in microinjection. Firstly, a cell is modeled by a hyperelastic membrane and interior cytoplasm. Then, based on the fact that the equilibrium configuration of a cell would minimize the potential energy, the energy function during microinjection is analyzed. With Lagrange multiplier and Rayleigh–Ritz technique, we successfully minimize the potential energy and obtain the equilibrium configuration. Upon this model, the injection force, the injection distance, the radius of the microinjector and the membrane stress are studied. The analysis demonstrates that the microinjector radius has a significant influence on the cell mechanical behavior: (1) the larger radius generates larger injection force and larger interior pressure at the same injection distance; (2) the radius determines the place where the membrane is most likely to rupture by governing the membrane stress distribution. For a fine microinjector with radius less than 20% of the cell radius, the most likely rupture point located at the edge of the contact area between the microinjector and the membrane; however, it may move to the middle of the equilibrium configuration as the radius increases. To verify our model, some experiments were conducted on zebrafish egg cells. The results show that the computational analysis agrees with the experimental data, which supports the findings from the theoretical model.

1. Introduction

With the rapid development of biotechnology, more and more foreign materials (small molecules, DNAs, RNAs, peptides, and proteins) are required to pass through the cell membranes and have effects on cells (Green et al., 2007). Microinjection is one of the most effective methods to introduce foreign materials into living cells (Faccidomo et al., 2012; Fielder et al., 2011; Zhang and Yu, 2008). It is applied in many biomedical areas, such as gene injection (Matsuoka et al., 2007; O'Meara et al., 2010), in vitro fertilization (Kumagai et al., 2011; Umeyama et al., 2012), drug development (Park et al., 2010). Many researchers and companies have developed a variety of machine and robots to fulfill microinjection. Wang et al. (2008) reported a semi-automated

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1751-6161/$ - see front matter
http://dx.doi.org/10.1016/j.jmbbm.2013.04.017

micro-robotic system which could be applied to the flat adherent cell microinjection. An adaptive impedance force control approach was introduced into cell microinjection by Xie et al. (2010). Besides various kinds of equipment, several cell models are built to study the mechanical behavior of the cells in microinjection. Feng and Yang (1973) focused on the contact problem of an inflated nonlinear elastic spherical membrane compressed between two rigid plates. They described the membrane by the Mooney model and solved the three first-order ordinary differential equations to obtain a general stress–strain relation for the spherical membrane. Fan's group developed a phenomenological cell model to study the dynamic response of micropipettes during intracytoplasmic sperm injection. In their research, the Maxwell viscoelastic model was used to represent the cell. They successfully tracked the injecting pipette and provided the geometric characterization of the mouse oocytes during intracytoplasmic sperm injection (Diaz et al., 2010; Karzar-Jeddi et al., 2011). Chizari and Wang (2009) analyzed the material property of a living cell in microinjection by building a numerical finite element cell model. Tan et al. (2010) also proposed a numerical model based on quasi-static equilibrium equations and geometrical boundaries to characterize the mechanical response of a cell in microinjection.

However, most of these models presuppose the cell deformed configuration by experience. Essentially, it is the potential energy that governs the cell configuration in microinjection. The minimum potential energy principle determines that of all geometrically admissible deformed configurations, the equilibrium configuration of the cell should minimize the potential energy functional (Mason, 1980). Therefore, in this paper, a mechanical cell model is proposed based on the potential energy analysis, which reveals the natural deformed configuration of a cell in microinjection. Upon our model, we analyze the interior pressure, the membrane stress, as well as the influence of the microinjector radius in microinjection. This paper is organized as follows: in Section 2, some reasonable assumptions are listed and a description of the cell model is provided; in the solution section, Lagrange multiplier and Rayleigh–Ritz technique are used to solve the complicate model. Section 4 shows the results and discussion, including the cell configuration and some other analysis. The microinjection experiments conducted on zebrafish egg cells are presented in Section 6 so as to verify our theoretical model. Finally, 'Conclusions' are given.

2. Model

2.1. *Assumptions*

In order to describe the mechanical property of a cell, the complex organism is usually simplified to a model consisting of a cell membrane and homogeneous interior cytoplasm, which is widely discussed in the literatures (Alcaraz et al., 2003; Fabry et al., 2001; Lenormand et al., 2004). In these literatures, it is assumed that the membrane is a thin nonlinear layer and the interior cytoplasm generates hydrostatic pressure on the membrane. We take advantage of this structure and apply it to our theoretical model in microinjection. Throughout modeling, some other reasonable assumptions are introduced:

(1) The bio-membrane is isotropic, incompressible and with constant thickness before deformation. This assumption has been used in most of the continuum models of cell membranes (Cheng, 1987; Wang et al., 2004; Zhang et al., 1992).
(2) The cell is spherical, the cytoplasm is incompressible, and the injection time is short enough to ignore the osmosis process in microinjection. Because the injection time is at the scale of second, it is quite acceptable that osmosis can be ignored. Thus, the volume of the cell should be constant before the membrane ruptures.
(3) The contribution of the bending rigidity is neglected, because the bending rigidity is related to the cubic power of the membrane thickness while the extensional rigidity is proportional to the first power of the membrane thickness (Liu et al., 1996). When the membrane thickness is small, the contribution of the bending rigidity is negligible comparing to that of the extensional rigidity.

2.2. *Model description*

In experiments, the lower half of the target cell is usually fixed by a holding pipette to prevent the movement of the cell. Due to the small bending stiffness of the membrane, the experimental images (see Section 5) prove that the fixed half of the cell does not experience significant deformation during the tests, and for most of microinjection experiments, only the deformation of the injected half of the cell is concerned. Thus, a semi-sphere with fixed boundary is applied in our model to describe a cell in microinjection instead of simulating the entire sphere. The microinjector with radius r_{inj} is set to be vertical and axisymmetric, and points to the center of the cell, Fig. 1. According to the assumptions above, the deformed configuration should be axisymmetric due to the axisymmetric loads. A set of spherical coordinates (r,ϕ,θ) is introduced to define the configuration of the membrane before being deformed and the cylindrical coordinates (ρ,η,θ) to describe the configuration after injection, Fig. 1.

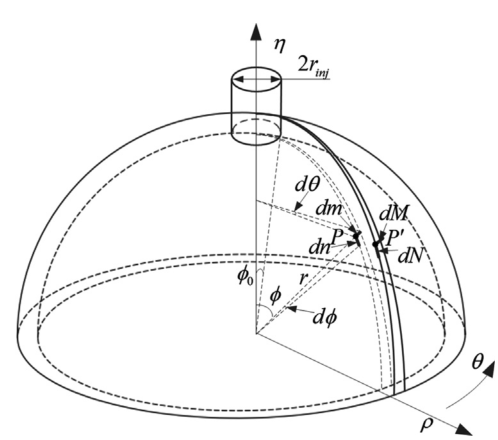

Fig. 1 – Geometrical description of the cell model.