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Rupture of plasma membrane under tension

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ABSTRACT

We present a study on the rupture behavior of single NIH 3T3 mouse fibroblasts under tension using micropipette aspiration. Membrane rupture was characterized by breaking and formation of an enclosed membrane linked to a tether at the cell apex. Three different rupture modes, namely: single break, initial multiple breaks, and continuous multiple breaks, were observed under similar loading condition. The measured mean tensile strengths of plasma membrane were 3.83 ± 1.94 and 3.98 ± 1.54 mN/m for control cells and cells labeled with TubulinTracker™, respectively. The tensile strength data was described by Weibull distribution. For the control cells, the Weibull modulus and characteristic strength were 1.86 and 4.40 mN/m, respectively; for cells labeled with TubulinTracker™, the Weibull modulus and characteristic strength were 2.68 and 4.48 mN/m, respectively. Based on the experimental data, the estimated average transmembrane proteins–lipid cleavage strength was 2.64 ± 0.64 mN/m. From the random sampling of volume ratio of transmembrane proteins in cell membrane, we concluded that the Weibull characteristic of plasma membrane strength was likely to be originated from the variation in transmembrane proteins–lipid interactions.

1. Introduction

Understanding the mechanical behavior of cells has great implications in tissue engineering and regenerative medicine. Control of cell behavior and/or cell fate can be exercised if exquisite linkages between external stimuli and cell response can be established. To this end, quantitative description of cell behavior is necessary and it entails precise measurement of fundamental mechanical properties. Various experimental methods have been used in studying the mechanics of cells, and apparently, much attention has been focused on cell adhesion as well as elastic and viscoelastic behavior (Van Vliet et al., 2003), but less on the rupture behavior of living cells.

For many materials, synthetic or biological, their (mechanical) strength is unlikely a single number but more appropriately described by a statistical distribution. However, whether the strength of living cells or plasma membrane can be described statistically is a fundamental question that has not been properly addressed. Results of such studies will provide insights into the material structure and useful information on injury mechanics at the cellular level.

In this work, we have studied the rupture behavior of two groups of NIH 3T3 fibroblast cells under tension, using micropipette aspiration. The first group of cells was unstained while the second group was stained with TubulinTracker™ (TT) to observe the local deformation of microtubules (MTs) during aspiration. Results were analyzed by the Weibull model, an established statistical model used to describe the strength distribution of many engineering materials and some biological materials such as dentin (Staninec et al., 2002) and fibrillar collagens (Layton and Sastry, 2004), to test its suitability in representing cell membrane rupture data. In addition, we have quantitatively determined the cleavage strength of transmembrane protein–lipid through random sampling of transmembrane proteins (TMPs) volume fraction in cell membrane.

2. Materials and methods

2.1. NIH 3T3 mouse fibroblasts cell culture

NIH 3T3 mouse fibroblasts were purchased from ATCC (USA), and cultured using Dulbecco's Modified Eagle's Medium (DMEM) supplemented with L-glutamine, 10% fetal bovine serum, and 5% penicillin/streptomycin in a 35 mm Petri dish at 37 °C, 5% CO_2.

2.2. Microtubules fluorescence staining

After reaching 70–80% confluence, the fibroblasts were stained with 250 nM of TT Green reagent (Invitrogen) according to the manufacturer's protocol. The stained cells were harvested and transferred into a black, low-wall Petri Dish (FD3510B-100, World Precision Instruments, Inc.) with a coverslip (0.17 mm thick, 10 mm in diameter) coated with 0.1 mg/ml fibronectin (F4759, Sigma-Aldrich) and 200 μl of Hanks balanced salt solution (Invitrogen) for a 10-min incubation.

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