CONTACT DEFORMATION OF LIPOSOME IN THE PRESENCE OF OSMOSIS

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ABSTRACT

The role of osmotic pressure on the geometry of adherent liposome remains an intricate question in membrane biomechanics. In this study, confocal reflection interference contrast microscopy (C-RICM) microscopy in combination with cross-polarized microscopy was applied to probe the adhesion contact mechanics and vesicle-substrate separation profile of deformed liposome on fused silica substrate. In parallel, a theoretical model, which describes the large deformation of lipid bilayer membrane under both out-plane bending and in-plane shear forces for simulating the global deformation geometry and is rigorously compared with our experimental data. Our experimental measurement showed that the adhesion contact area increases in dimension as the liposome volume deflated by osmotic pressure while the vesicle height decreases due to its global deformation

INTRODUCTION

Cell-substrate interaction plays an important role in many physiological functions including cell adhesion, tissue regeneration and inflammatory response [1]. A better understanding of the complex mechanisms involved in cell-substrate adhesion is crucial for the designs of biomaterial scaffold and engineered tissue equivalent. Several physiochemical parameters such as osmosis, temperature and ionic strength, are shown to modulate the contact mechanism and adhesion energy of adherent cell and model liposome. Therefore it is meaningful to quantitatively correlate the contact mechanics of adherent vesicle with the physiochemical driving forces involved in biological adhesion. Confocal reflection interference contrast microscopy (C-RICM) was shown to improve the upper limit of the experimental membrane-substrate separation (up to 4.5 mm) and increase the dynamic range of the probed contact zone for adhering capsules on a glass substrate [2]. In this study, we induce a reduction of osmotic pressure to adherent vesicles by evaporating water from the surrounding aqueous medium with high power illumination. C-RICM and cross-polarized light microscopy was then applied to elucidate the interfacial adhesion contact and vesicle-substrate profile of DSPC unilamellar vesicle against the change of osmotic pressure. At the same time, theory developed by [3] was introduced to calculate the global cell deformation profile of a spherical elastic vesicle adhering on flat substrates under a change of osmotic pressure.

EXPERIMENTAL

Our system for measuring the contact area and membrane-substrate profile was based on a laser scanning confocal microscope (Carl Zeiss, Germany) as described elsewhere. In brief, an Argon ion laser with a maximum power of 1 mM and excitation wavelength of 543 nm was used for illumination. A 63X oil immersion objective (Neofluar, N.A.: 1.25, Germany) was used. The optical train of the imaging system carried the excitation light to the membrane-substrate interfaces through the microscope objective. The constructive or destructive interference of reflected light from the two opposing reflective surfaces resulted in the formation of fringes propagating from the interfacial region of a strong contact zone to the peripheral region of the adhering liposome. Dilution of the original liposome suspension in 1X PBS buffer was incubated on a fused silica coverslip for an hour inside a humidity chamber of the microscope stage. A high-intensity halogen lamp was used for increasing the osmotic stress as a result of water evaporation. ZSM5 image analysis software (Carl Zeiss, Germany) was used for all image acquisitions and analysis. The membrane-substrate contact profile is directly determined from the inverse cosine transformation of the light intensity profile.

THEORY

When the liposome is brought into close proximity to the substrate and the membrane is fully compliant with the substrate surface to form a contact zone. Within the planar contact circle, there is assume to be stress-free, while without the contact region, both the out-plane bending and the in-plane shear stresses are assumed to govern the membrane deformation. When osmotic pressure is consequently applied, the outflow of the liquid from liposome's interior causes a volume decrease and contact area increase. The measured contact diameter is used as the input for our simulation of

the global contact deformation of the liposome. In all the contact stages, the liposome deformation is assumed axisymmetric (see Figure 1) and a is the radius of undeformed vesicle. The details of liposome mechanics and the definitions of most important parameters can be referred to previous works [3,4] Briefly, the constitutive equation was based on large deformation theory as mentioned above. The nondimensional parameter $C=a^2H/B$, which expresses the relative strengths of two elastic effects: in-plane shear modulus H (N/m) and out-of-plane bending modulus B (Nm), importantly govern the deformation shape of the vesicles in contact with the substrate. . As shown in Figure 1, (r, z) is used as the coordinate system for the original non-deformed sphere. (R, Z) is used as the coordinate system for the deformed vesicle, the meridional arc length is denoted as S, and ϕ is the angle measured from the local center of curvature to the deformed profile. The details of the governing equations and boundary conditions are reported elsewhere [4].

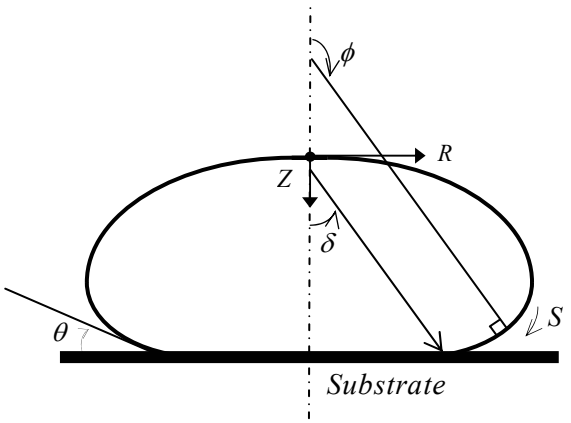

Figure 1. Schematics of computational configuration of the deformed liposome adherent on a planar substrate

RESULT AND DISCUSSIONS

Figure 2 shows the deformation profile of the liposomes in isotonic and hypertonic (high osmotic pressure) solutions, respectively. The profile of membrane-substrate separation y* is directly determined from the inverse cosine transformation of the light intensity profile from the interference fringe pattern. When the contact zone occupies larger fraction of the overall deformation profile (δ>0.2), the fitting of the experimental data is dependent on the magnitude of C. Our result indicates that the experimental profile is best fitted by theoretical profile at the bending dominant regime (C≈10). Current results agree well with the theoretical concept that the shape of a biomimetic vesicle under a larger contact deformation is mainly governed by the bending elasticity of the bilayer or membrane [5]. For the hypertonic case, we simulated the vesicle deformation profiles with different normalized osmotic pressure P; it is found that P=–5 is the best fit for the experimental data under the osmotic pressure. It is also noticeable that the total normalized height of liposome is decreased by 7.34% from y*/a=1.966 to y*/a=1.822, while the normalized osmotic pressure reduces from isotonic P=0 to P=–5. Thus, under osmotic pressure the calculated degree of vesicle deformation for P=0 and P=–5 increases from 1.02 to 1.118. Such increase in deformation is stemmed from the vesicle deflation in response to water outflow from vesicle interior. Our result indicates that our theoretical model correctly predicts the effect of osmotic pressure on the geometry of adherent profile. More importantly, from the definition of P, once the real osmotic pressure P* is determined, the bending modulus B will be obtained. The relative bending modulus for present study is approximately B=-3.9×10^{-16}P* (Nm), of which $B=a^3P^*/P$, where: a=12.5 μm, $P=-5$, and P^* is a negative value. The bending modulus found here is comparable with previously reported value from the literature where B=2×10^{-19} (Nm), the bending modulus of red blood cell that measured using micropipette aspiration [6].

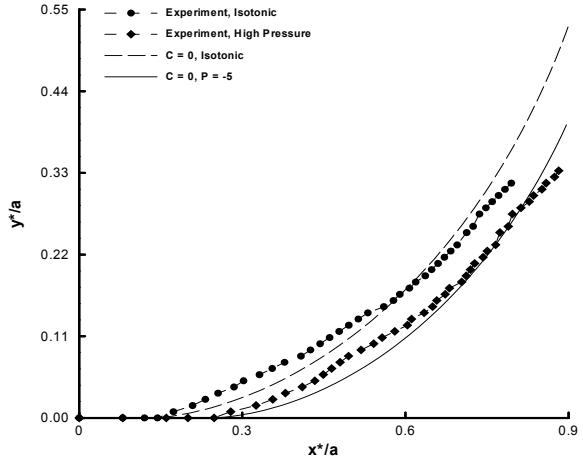

Figure 2. Comparison of calculated distributions of liposome deformation profiles with C-RICM results isotonic and hypertonic (osmotic pressure effect) solutions.

CONCLUSION

We have demonstrated that C-RICM in conjunction with our extended theory of vesicle contact deformation is an effective way for exploring the interfacial geometry of adherent DSPC liposome, in response to the change of osmotic pressure. The experimental results clearly show the liposome-substrate contact area and the degree of deformation increased with the reduction of osmotic pressure to hypertonic state. The good agreement between the experimental and theoretical results provides new insights into the biomechanical driving forces of liposome adhesions.

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