NANOMECHANICS AND NANOMANIPULATION OF SOFT BIOLOGICAL MATERIALS FOR TISSUE ENGINEERING APPLICATIONS

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Abstract: Nanomechanics and nanomanipulation of soft biological materials are of great importance for the development of new strategies for tissue engineering applications[1;2]. The techniques which allow to applying nanoscale force and displacemnt to the biological materials are only recently available due to the development of novel experimental instruments, such as confocal reflection interference contrast microscopy, optical tweezers, micro-manipulation method and nano-indentation technique. In parallel, various theoretical modelling and simulations, such as the Liposome Mechanics Theory and capsule-substrate adhesion model, have been developed for interpreting the experimental data and for facilitating the determination of the mechanical properties of biological materials at the cellular/molecular level. The applications of these new techniques for nanomechanical characterization and manipulation of biological cells and tissues for tissue engineering application will be addressed in this paper.

Introduction

Nanomechanics and nanomanipulation of soft biological materials, such as molecular, cell and tissue, are essential for the advancement of the tissue engineering. For example, applying nanoscale force and displacement as mechanical stimuli to engineered tissue construction, and for sorting and manipulating cells or molecules to nanofabricate de novo biomimetic systems, are in great need of both the better understanding of nanomechanics and the new techniques of nanomanipulations.

Recent advancements in bio-manipulation instruments, such as micromanipulation [3] and optical tweezers [4], have enabled the nanomechanical characterisation and manipulation of biological cells and soft tissue. Micromanipulation is capable of the micro-force measurement of a single biological cell and tissue membrane at large deformation, while optical tweezers allows to the force measurement as low as 100 pN. These measurements can be incorporated with mechanical modelling to facilitate the determination of the mechanical properties, such as the elasticity and rupture strength of cell and tissue membrane. In addition to the mechanical properties, interfacial characterization, e.g. cell-substrate adhesion, can be realized by our recently developed method which is based on Confocal Reflection Interference Contrast Microscopy (C-RICM)[5]. In parallel, various theoretical modelling and simulations, such as the Liposome Mechanics Theory and capsule-substrate adhesion model, have been developed for interpreting the experimental data and for facilitating the determination of the mechanical properties of biological materials at the cellular/molecular level. Various materials, such as biological/ biomimetic cell and tissue, have been examined by using these new techniques, and their results are presented in this paper.

Experimental Techniques

Confocal Reflectance Interference Contrast Microscope (C-RICM): This new technique has recently been developed by Sackmann and his co-worker for investigating the adhesive contact deformation of vesicle and biological cell on planar transparent substrate [6;7]. Based on the interference of the reflected light from the cell/vesicle membrane surface and the opposite substrate surface, their reported Reflectance Interference Contrast Microscope (RICM) which was built on a conventional epi-fluorescence microscopy, can achieve a dynamics range of 0.05-1 μm in the measuring membrane-substrate separation [6]. Even with the unique merits of RICM, it is not able to accurately measure the membrane-substrate distance beyond 1 μm and offers only limited spatial resolution in the contour profile of adhering vesicles [7]. The latest development of the technique based on laser-confocal microscope, i.e., Confocal Reflectance Interference Contrast Microscope (C-RICM), was shown to improve the upper limit of the dynamics range up to 4.5 μm [5].

Optical Tweezers: When a highly focused laser beam hits a particle with a higher refractive index than the surrounding medium, an optical trap is created and the photons will be refracted outward [8]. Since the number of photons near the centre of the laser beam being refracted outward is greater than the reverse condition, the net reaction force is always greatest in the direction pointing towards the centre of the beam. Consequently, the particle moves toward the beam centre, and, once there, remains, as the lateral forces acting on it will be in equilibrium. This type of force is often referred as a lateral gradient force. Any force applied to the particle is resisted by the lateral trapping

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force. Unless the other force is greater than the lateral trapping force, the particle experiences a reaction force that is directed towards the centre of the laser beam. As a result, the net force can be used to pull and confined micro-sized particles in the focal volume [9].

Recently, optical tweezers which uses the forces exerted by a focused laser beam entrap and move micron and sub-micron particles has emerged as a novel tool for manipulating cells and macromolecular structures and for performing sophisticated biomechanical/biophysical characterization. In our studies, we uniquely combined optical tweezers and advanced Computation Fluid Dynamics (CFD) to interrogate the shape transformation of human red blood cell (RBC) and unilamellar vesicle under thermal and flow induced deformation [4;9]. This technique allows to determining the force exerted on the cell membrane with accuracy as high as 1 Pico-Newton. In addition, the viscoelastic deformation behaviours of cell membrane can be characterized with a characteristic time constant, i.e. the ratio of membrane viscosity to elasticity. A number of interesting phenomenon, including straining-hardening process of RBC and thermal soften effect on phospholipid bilayer membrane, are shown in the experimental force-deformation relationship. Overall, our studies provided a new experimental approach in the characterization of cell membrane dynamics under physiological conditions [10;11].

Micro-manipulation Method: Micro-manipulation technique has been developed to measure the force-displacement of a single particle or vesicle that is compressed between two parallel plates. The method is capable to characterize not only the mechanical properties of the particle or vesicle but also the particle-/vesicle-substrate interfacial adhesion, and even achieve the both characterizations at the same experiments. In other word, it can measure both long-range and short range forces of colloidal particle simultaneously. This has been demonstrated in our earlier works which reported the application of the technique to capture the "jump-into-contact" and "pull-off" phenomena as well as the mechanical deformation of a single soft particle. It has been proved to perform a wide range of measurements, i.e., for both solid and hollow particles under either ambient or liquid-immersed environments, up to a large strain. It has also been applied for the nano-mechanical characterization of deformation behaviours of biological cells. The force resolution and displacement of the latest version of the instrument is 0.1 mm and 10 nm [12].

Nano-indentation Technique: Recently, we have developed a novel indentation technique, which has been successfully applied to measure the mechanical/viscoelastic properties of biological membrane [13-15]. Briefly, the method utilises a computerised long-focal CCD microscope system which allows the measurement of a side-view image of suspended circular membrane under the weight of a steel ball or under the indentation of a rigid indenter [14]. A theoretical model was constructed to correlate quantitatively the viscoelasticity to the time-dependent deformation profile of the membrane. The advantages of the measurement technique are compelling and can be briefly summarised: i) the gripping-related problems commonly existed in the extensometry test have been eliminated, ii) the testing apparatus is relatively simple, iii) the stress distribution in the deformed sample is bi-axial and axisymmetric, which is close to the natural status of biological membranes, especially for many biological membrane which have curved circular shapes in nature, iv) the set-up allows us simultaneously to stimulate the tissue construct and to characterise its mechanical properties due to the constant applied load, v) the sample can be fully immersed in liquid solution or in a chamber of well-controlled humidity and temperature, similar to a physiological environment, with no risk of damaging the instrument's electronics, vi) the technique can easily perform creep testing without a need for force feedback control, since fixed load is spontaneously ensured due to the constant weight of the ball used in the set-up , and vii) compared with classical micro/nanoindentation methods, the current method is free from the intractability of imperfect indenter shape, substrate effect, and defining initial contact, and has less influence from friction .

Theoretical Analyses:

Capsule-substrate adhesion model: We have lately developed a theoretical model to portray adhesion between biological vesicles and a flat substrate in the presence of osmosis. The theory leads to new prediction in cell-substrate adhesion and has an impact in tissue engineering and drug delivery.

A thin-wall capsule, modelled as an incompressible liquid droplet encapsulated by a spherical elastic membrane, was allowed to adhere onto a rigid or non-deformable substrate. The detailed contact mechanics, based on linear elasticity, has been quantified by a theoretically model which was recently developed to correlate the relationship between the ratio of the contact circle radius to capsule radius a/R, wall elastic modulus E and thickness h, and interfacial adhesion energy W [16]. Briefly, the equilibrium geometry of the liquid-filled capsule adhering on the substrate is modelled as a truncated sphere with a mid-plane radius R and $a/R = a$ where a is the contact zone radius. The capsule wall is under a uniform equi-biaxial stress, $\sigma = C\varepsilon$ where C is equivalent to Eh / (1–υ) in a linear system under small strain with E and υ, the elastic modulus and the Poisson's ratio, respectively. Only stretching energy is considered in the model, while bending is ignored. The average biaxial strain is given by

$$\varepsilon = \frac{1}{2}\left[\frac{2 + 2(1-\alpha^2)^{1/2}}{4/R^2 - \alpha^2} - 1\right] \qquad (1)$$

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In the absence of external influence, the capsule spontaneously adjusts its distance of approach towards the substrate until equilibrium is achieved. The adhesion energy is shown as

$$W = (1 - \cos\theta)C\varepsilon + C\varepsilon^2 \qquad (2)$$

based on the experimental measurements of the mid-plane diameter R (by using Phase Contrast Microscopy) and the radius of contact zone, a (by using the RICM), W can be found by the equations (1) & (2), once the value of Eh is determined by an experimental characterization.

Liposome mechanics 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 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. 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. The details of the governing equations and boundary conditions are reported elsewhere [5].

Results and Discussion

Figure 1 shows the deformation profile of the liposomes in isotonic and hypertonic (high osmotic pressure) solutions, respectively. The profile of membrane-substrate separation 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 ($C\approx10$). 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].

Figure 2 shows a series of phase contrast images of a DPPC-ULV that is hold by the optical trap against various fluid flow velocities of 16.0 to 46.0 μm/sec at room temperature (22 °C). It is noted that due to the viscous drag effect the vesicle deforms, elongates, and finally produces a stream-wise geometry when it is acted upon by fluid shear stress at gel phase. For consistency, the same vesicle was probed against the increase of fluid flow velocity at elevated temperatures. Figure 2 shows a series of phase contrast images of the same vesicle at 48.6 °C under flow velocities of 16.82 to 56.27 μm/sec. The results indicate that the progression of gel to liquid crystalline transition has made the trapped vesicle more deformable under various fluid velocities. This phenomenon is clearly shown by the more significant elongation of the trapped vesicle along the direction of flow at 48.6 °C in comparison with that at 23 °C (Fig. 2).

Conclusions

We have demonstrated several new techniques incorporated with novel theoretical analyses which can be applied to nano-mechanically characterize and manipulate biological cells and tissues. These systems should provide powerful tools to perform the nanomechanical stimulation, manipulation and test of biological cells for developing the next generation of tissue engineering equivalents.

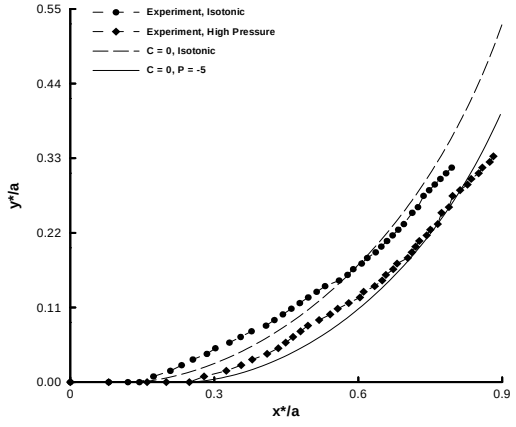

Figure 1: Comparison of calculated distributions of liposome deformation profiles with C-RICM results isotonic and hypertonic (osmotic pressure effect) solutions, from [5] with permission.

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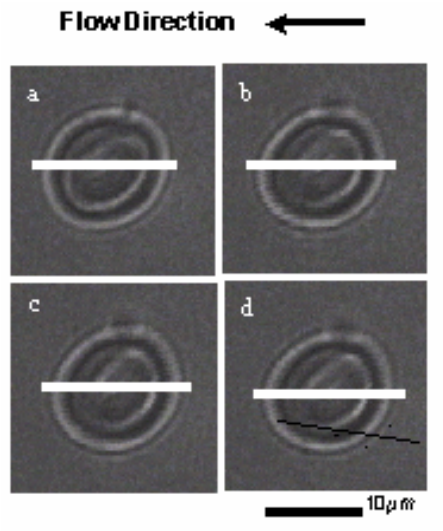

Figure 2: Deformations of DPPC vesicle at room temperature (23.0 ^{o}C) under different flow velocities; scale bar represents 10μm: (a) static, (b) 16.0 μm/s, (c) 31.0 μm/s, (d) 46.0 μm/s.

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