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Single Cell Analysis inside Environmental Scanning Electron Microscope (ESEM)-Nanomanipulator System

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1. Introduction

The conventional approach to characterize cellular biology is called biochemistry. This developed science is used for studying physiological aspects, mainly genetics, by characterizing protein and other biomaterials. Since single cells are difficult to study, a collection of cells are used for characterizing cellular physiology and in turn used to describe behavior of single cell (Brehm-Stecher & Johnson, 2004). However, in addition to this advance understanding of cellular genetics, information about mechanical properties of cells is also needed. The molecular structure of the cell-wall is only partially understood, and its mechanical properties are an area of “near-total darkness” (Harold, 2005). Moreover, the approximation of single cell behavior from a group used in conventional approach also requires further justification whether it can be applied to all cell types (Shapiro, 2000). The knowledge of the cell mechanics could be valuable in the future for biomedical applications, for example, variations in cell mechanics of healthy and unhealthy cells can be linked to a specific disease.

Available experimental techniques to probe single cells include micropipette aspiration, optical tweezers, magnetic tweezers (Bausch et al., 1999), atomic/molecular force probes (Gueta et al., 2006), nanoindenters, microplate manipulators, optical stretchers (Thoumine et al., 1999) and nanoneedle (Obataya et al., 2005).. The functionality of nanoneedles is not limited only to the stiffness measurements but it can also be used for single cell surgery (Leary et al., 2006) which can be further applied to a novel single cell drug delivery system (Bianco et al., 2005) or as a delivery tool for nanoparticles (Brigger et al., 2002). Conventional drug therapy suffers from the problems of inefficacy or nonspecific effects; hence, nanosystems are being developed for targeted drug delivery (Stylios et al., 2005). In order to successfully deliver materials; e.g. bioactive peptide, proteins, nucleic acids or drugs inside the cell, carriers must be able to penetrate the cell wall or cell membrane without causing death or create any mechanotransduction to the cell (Goodman et al., 2004), i.e. the process

of converting physical forces to biochemical signals and integrating them into cellular responses (Huang et al., 2004). Therefore, the knowledge of biomechanics of the cell is crucial in providing *prior-estimation* of required insertion force to deliver drug material inside the cell. Without having this information, the insertion process may be unsuccessful due to inadequate applied force or the cell may be seriously damaged due to the excessive applied force.

This chapter focuses on the following two needs, i.e. the needs for the understanding of the mechanical properties of single cells and the needs for the novel nanotools for the single cells probing. The first need was fed by highlighting our findings on the effects of three factors, i.e. cell sizes, environmental conditions and growth phases, on the strength of the single W303 yeast cells. The second need was served by showing our findings on the development of nanoneedles which can be used for single cell local stiffness characterizations and single cell surgery.

2. ESEM-Nanomanipulation System

We have developed an integrated environmental scanning electron microscope (ESEM)-nanomanipulator system. Unlike the conventional SEM, ESEM enables soft, moist, and/or electrically insulating materials to be imaged without prior specimen preparation. Fig. 1(a) shows the image of the ESEM instrument. A low pressure (up to around 1333 Pa) gas can be accommodated around the sample. When this gas is water, hydrated samples can be maintained in their native state. Sample temperature can be controlled (0–40 °C) by using the cooling stage assembled inside the ESEM chamber. By controlling the chamber pressure (10–2600 Pa) and sample temperature, single cell mechanical characterization and analysis can be conducted by using a nanomanipulator and image analysis.

There are two main advantages of the ESEM system compared to the AFM system. The first advantage is that AFM system is difficult to provide a real-time sample observation and the image is mainly constructed after the scanning of the AFM tip on the sample surface is finished. Therefore, it is difficult, if not impossible, to directly observe the response of the object during the sample manipulation. On the other hand, ESEM system provides real-time sample observation which can be obtained before, during and after the manipulation. This real-time observation capability of the ESEM system has many advantages such as in analyzing the dynamic response of the sample and sample selection/sorting for certain properties like cell size, become much easier. The mechanical characterization and analysis capabilities which can be done on the sample can also be enhanced.

The second advantage is related to the manipulation aspect. The AFM system can only provide two-dimensional (2D) manipulation, due to the difficulty of the real-time observation. Although 2D manipulation can measure the stiffness property of the sample, it lacks in the flexibility of manipulation thus limiting the area of sample which can be analyzed. Unlike AFM system, ESEM-nanomanipulation system can provide both 2D and 3D manipulations on the sample, thus increasing the flexibility of the measurement such as the stiffness characterization in different area of the sample like bottom surface can be realized.

The characterization of mechanical properties of the cell requires no interference from other external pressures or forces on the cell surface. These external forces such as the osmotic pressure may prevent investigation of the true mechanical properties of cell experimentally.

ESEM system can provide environmental conditions for the cells in their native state without any effect of the osmotic pressure. Cells can be sustained by releasing low pressure of H₂O molecule gas inside the ESEM chamber to provide high relative humidity (up to 100%). Therefore, the stiffness data obtained from this experimental setting shows better representation of the mechanical properties of the cell.

Nanomanipulation is an effective strategy for the characterization of basic properties of individual nano-scale objects and to construct nano-scale devices quickly and effectively. Previously, we have constructed a hybrid nanorobotic manipulation system integrated with a transmission electron microscope (TEM) - nanorobotic manipulator (TEM manipulator) and a scanning electron microscope (SEM) - nanorobotic manipulator (SEM manipulator) (Nakajima et al., 2006). This system allows effective sample preparation inside SEM with wide working area and many degrees of freedom (DOFs) of manipulation. It has high resolution measurement and evaluation of samples inside a TEM capability. The sample chambers of these electron microscopes are set under the high vacuum (HV) condition to reduce the disturbance of electron beam for observation. However, to observe the water-containing samples, e.g. bio-cells, drying treatment processes are additionally needed. Hence, direct observations of water-containing samples are normally quite difficult in these electron microscopes. This limitation was overcome by using ESEM.

In the present study, we used the nanorobotic manipulators inside ESEM. It has been constructed with 3 units and 7 degrees of freedom (DOFs) in total (Fig. 1(b)). The ESEM-nanomanipulator system can be easily incorporated with various kinds of nanoprobe for single cell analysis as shown in Fig. 1 (c).

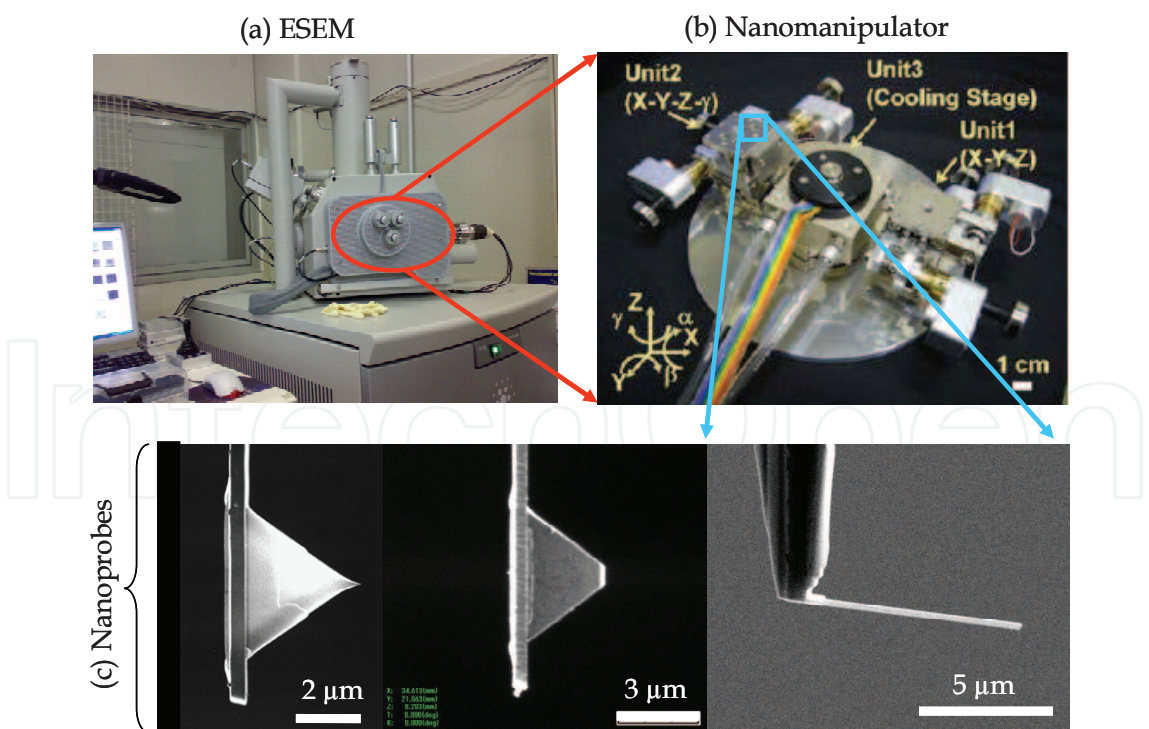


Fig. 1. Overview of the (a-b) ESEM-nanomanipulator system incorporated with (c) various kind of nanoprobe.

3. ESEM Observation of Single Cells

Wild type yeast cells (W303) were used for observation and measurements by ESEM nanomanipulation system. The W303 cells were cultured on YPD plates (1% yeast extract, 2% peptone, 2% glucose, 2% agar) in a 37 °C incubator for 24 hours and dispersed in pure water. Several micro liters of dispersed cells were placed on aluminum block on the cooling stage. The HV mode is operated at 16.7 °C and 3.03×10^{-3} Pa pressure. All yeast cells appeared concave and broken under HV mode. The ESEM mode is operated at 0.0 °C and ~ 600 Pa pressure and acceleration voltage is set at 15 kV. Under the ESEM mode, decreasing the pressure from ~ 700 Pa, water gradually evaporates and the samples can be seen underneath. Their images are shown with HV and ESEM modes as shown in Figs. 2(a)-(b). The remaining water can be seen at the intercellular spaces of yeast cells as black contrast (Fig. 2(b)). Almost all yeast cells appear sphere shaped by water-contained condition under the ESEM operation.

In order to reveal the influence of an electron beam observation under HV and ESEM modes, yeast cells were cultured once again after observation. Yeast cells were observed under the ESEM mode, and after the observation, we collected the cells from the observation stage (aluminum block) by a sterilized toothpick and inoculated onto the fresh YPD plate, and grew them overnight. To compare the cell viability with untreated cell, water-dispersed yeast cells were inoculated on the same plate for the control. The cultured plate is shown in Fig. 3. The plate was divided into three regions; cultured after water dispersion, SEM observation and ESEM observation. The numbers of yeast cells colony on the ESEM mode were higher than on the HV mode. From this experiment, the living cell rate on the ESEM mode is almost same order with initial condition of the water dispersion method.

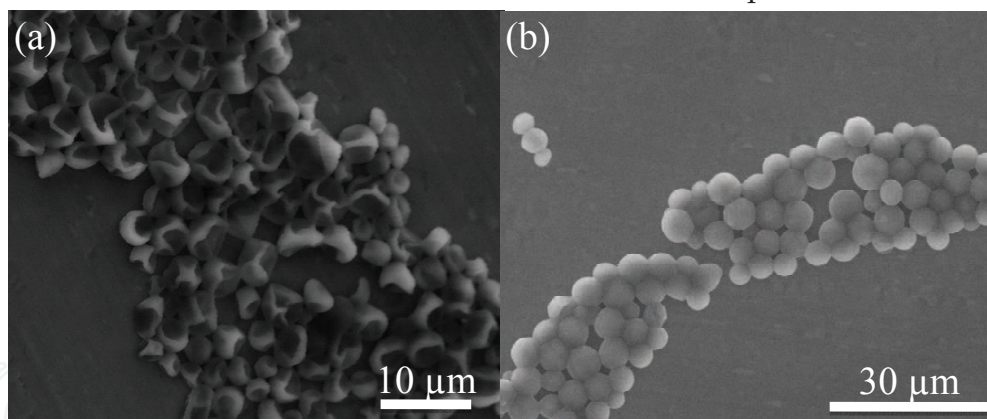


Fig. 2. Images of W303 yeast cells under (a) HV mode and (b) ESEM mode.

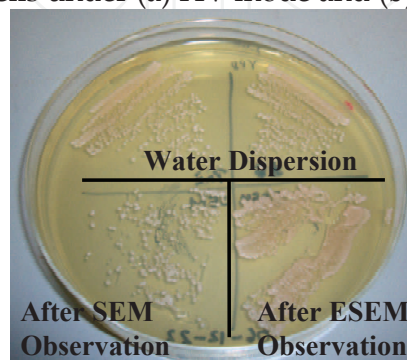


Fig. 3. Cell culture plate of W303 yeast cells after observations under SEM condition (bottom

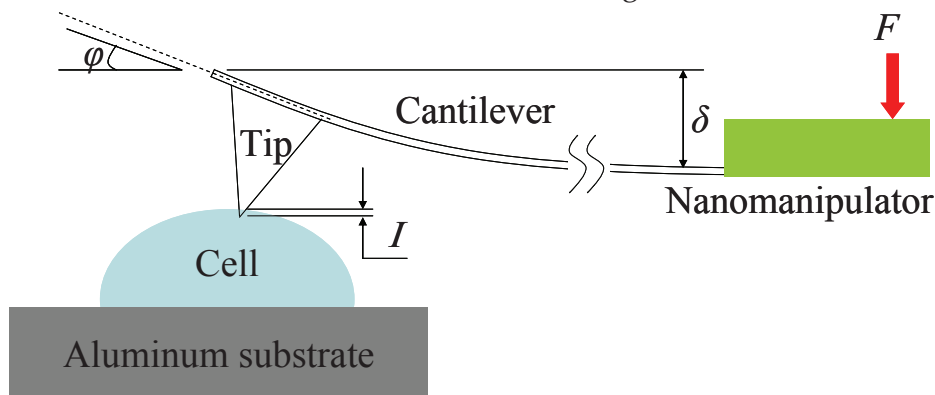
left of the plate area) and ESEM condition (bottom right of the plate area) as compared to the control cell culture by a standard water dispersion method (upper area of the plate).

4. The Effects of the Cells Sizes, Environmental Conditions and Growth Phases on the Strength of Individual *W303* Yeast Cells

4.1 Determination of the Penetration Force and the Cell Stiffness

The indentation experiments (Fig. 4(a)) were conducted by using the integrated ESEM-nanomanipulation system. The cells were fixed on the cooling stage in perpendicular direction with an AFM cantilever (0.02 or 0.7 N/m spring constant). The AFM cantilever is controlled by the nanomanipulator and used to apply the compression force on each cell. The penetration of a single cell by the AFM cantilever in this experiment was based on a direct observation of the cell when it was bursted by the AFM cantilever. Due to the shape and size of the indenters, i.e. pyramidal or tetrahedral tips (sharp or flat), the cell bursted phenomenon can not be avoided as soon as the cell wall is penetrated (Obataya et al., 2005a).

The ESEM can provide real-time observation, i.e. a cell being approached, touched, indented, and finally penetrated/bursted by the AFM cantilever can be seen clearly. Therefore, as far as this paper is concerned, the penetration of a single cell by the AFM cantilever was based on the occurrence of the cell bursting via real-time observation.



(a) Cell indentation experiment

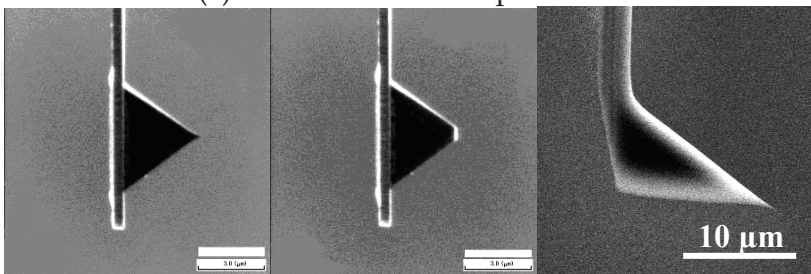


Fig. 4. Schematic diagram of (a) an indentation experiment by using (b) sharp and (c) flat AFM pyramidal cantilever tips under the ESEM mode. For the HV mode, (d) a tetrahedral cantilever tip was used.

Force (F)-cantilever indentation (I) curves were determined from the experiment. A typical F - I curve using a sharp cantilever tip shows a response of a cell before and after the penetration as shown in Fig. 5. The value of F was calculated by using (1), where k , φ and L are the spring constant, the displacement angle in radians and the length of the cantilever, respectively. Values of φ , L and I were determined from direct measurements of ESEM images using an image analysis software (ImageJ, developed at National Institute of Health), while k was obtained from the manufacturer (Olympus Corp.). Equation (1) was derived from the Hooke’s law, i.e. $F=k\delta$, where δ is the displacement of the cantilever which was obtained by using $\delta=\varphi(2/3)L$.

$$F = k\varphi(2 / 3)L \tag{1}$$

The Hertz-Sneddon models which based on the shape of the tips, i.e. conical, spherical, and cylindrical, were used to estimate the Young’s modulus of the cells as expressed in (2)-(4). Parameters of E , ν , α , R and a are the Young’s modulus, the Poisson’s ratio ($\nu = 0.5$ for soft biological materials (Lanero et al., 2006)) of the elastic half space (cell’s surface), the half opening angle of a conical tip, the radius of curvature of a spherical tip and the radius of a cylindrical tip, respectively. Values for α , R and a were obtained from ESEM images. Values for these parameters are summarized in Table 1.

$$F_{cone} = \frac{2}{\pi} \tan \alpha \frac{E}{(1 - \nu^2)} I^2 \tag{2}$$

$$F_{spherical} = \frac{4}{3} \frac{E}{(1 - \nu^2)} R^{1/2} I^{3/2} \tag{3}$$

$$F_{cylindrical} = 2 \frac{E}{(1 - \nu^2)} aI \tag{4}$$

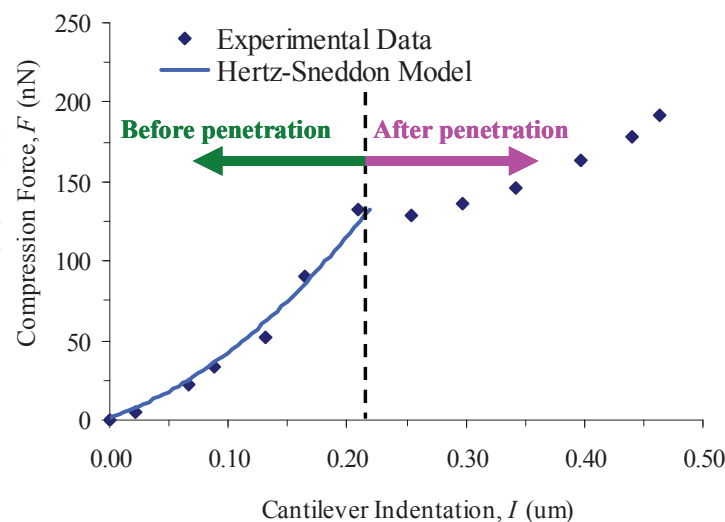


Fig. 5. Typical force – indentation (F - I) curve under ESEM mode using a sharp tip. The curve shows points before penetration (green arrow) and after penetration (pink arrow) of the cell.

The blue line shows the best fit of the Hertz-Sneddon model of the experimental data until the penetration point (dotted line).

Parameters	Values
ν	0.5
α	10°
R	20 nm
a	316 nm (ESEM mode)
	206 nm (HV mode)

Table 1. Values for parameters used in the estimation of the Young’s modulus of the single cells.

The models predict that the load depends on the indentation according to a power law related to the tip geometry (Lanero et al., 2006). In order to choose the correct tip geometry an equation of the form $F=ml^b$ was fitted to force versus indentation curves using commercial fitting software (DataFit), where the exponent b depends on the tip shape. For the sharp pyramidal tip under ESEM mode, we obtained a value of b close to $3/2$, characteristic of the spherical tip. The curves were then fitted by using the spherical model. Interestingly, under HV mode, the value of b close to 2 was obtained. Therefore, the canonical model was applied under HV mode. Finally, for the flat pyramidal tip, the b value close to 1 was obtained which is for the cylindrical model. The fitting model for the sharp tip using the spherical tip was reported by Lanero et al. (Lanero et al., 2006) while Touhami et al. (Touhami et al., 2003) used the conical tip as their fitting equation. Obataya et al. (Obataya et al., 2005a) used the cylindrical equation to model their flat nanoneedles. The Hertz-Sneddon models were derived from the classic Hertz’s mechanics model for linear elastic material (Sneddon, 1965). For flat cylindrical punch relating to an applied force, F , with an indentation depth, I , yields the following expression for the initial unloading (load is released after indentation) response, $dF/dI^{(1/2)(1-\nu^2)}/E$, where c is coefficient ($c=2/(\pi)^{1/2}$) and A is the contact area. The equation was based on the flat-ended punch, but holds true for any punch that can be described by a smooth solid revolution (spherical, conical, elliptical, etc.) (Pharr et al., 2002). Berkovich and Vickers indenters (3 and 4 sided pyramidal cones, respectively) commonly applied in instrumented indentation techniques cannot be described as bodies of revolution. However, it has been found experimentally and by means of finite element simulations that the deviation from the above equation of pyramidal and other geometrical shapes is negligible (Pharr et al., 2002). The constant $c=1.142$ for the pyramidal indenter and $c=1.167$ for tetrahedral indenter differ little from $c = 2/(\pi)^{(1/2)} = 1.1284$ from the above equation. In other words, the derived equation for the sharp conical tip can be used without large error, even the sharp indenter is not a true body of revolution.

The final equations of the Young’s modulus based on the Hertz-Sneddon models, i.e. (2)-(3), and the parameter’s values (Table 1) for three different tip shapes, i.e. conical (HV mode), spherical (ESEM mode) and cylindrical (ESEM mode and HV mode), are expressed in (5)-(7).

$$E_{cell} = (6.681)\frac{F_{cone}}{I^2}$$

(5)

$$E_{cell} = \left(3.977 \times 10^3\right) \frac{F_{spherical}}{I^{3/2}} \tag{6}$$

$$\begin{cases} E_{cell} = (1.187 \times 10^6) \frac{F_{cylindrical}}{I} & \text{(ESEM mode)} \\ E_{cell} = (1.825 \times 10^6) \frac{F_{cylindrical}}{I} & \text{(HV mode)} \end{cases} \tag{7}$$

Fig. 6 shows typical *F-I* curves using the spherical and cylindrical models under ESEM mode while the inset shows a curve using the conical model under HV mode as obtained in our experiments. The curves are shown until the bursting point of the cells which is the maximum force which the cell can sustain before the cell is bursted. After the bursting point, the force will drop for a while due to the reversed force and will increase again when the tip traverses inside the cell or touch the bottom of the cell wall (Fig. 5). Starting from Fig. 6, data after the bursting points are not shown in the following figures. The reason for this exclusion is that the data were no longer representing the cell stiffness property since the cells were seriously damaged.

The curve leading up to the failure is expected to give a combined elastic property of indenting the whole yeast cell and the localized deformation of the surface ruled by the properties of the cell-wall surface. Therefore, the reported *E* values in this paper indicate the maximum strength of the cell before bursting from a local indentation point. The *E* values must be higher than the local elastic cell property as the strength of the cell is dominated by the whole cell stiffness (Smith et al., 2000). Nevertheless, examining the cell strength based on the Young’s modulus parameter per se is not adequate since its value may remains constant at different situations, e.g. cell growth phases (Smith et al., 2000). Therefore, the penetration force was used to determine the cell strength under several factors, i.e. cell sizes, environmental conditions and cellular growth phases.

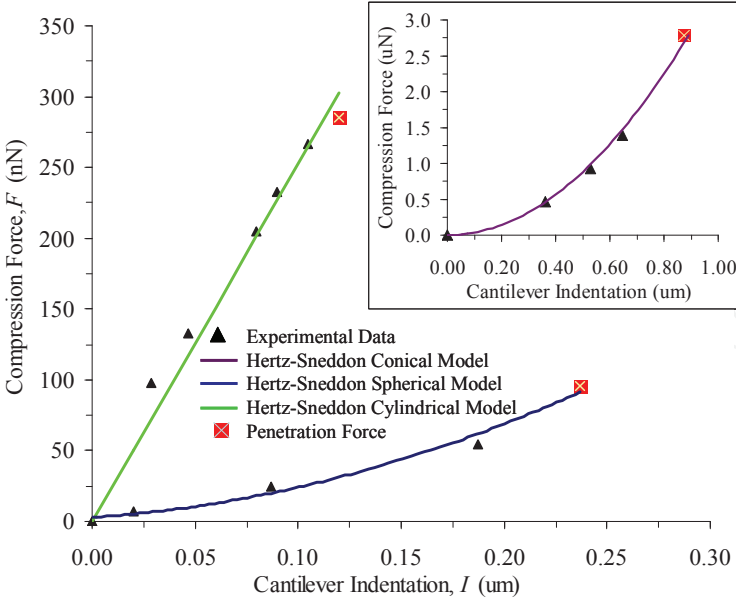


Fig. 6. Typical force – indentation (*F-I*) curves under ESEM mode using spherical and cylindrical models for sharp and flat tips, respectively. Inset shows the *F-I* curve under HV mode using conical model.

4.2 The Effect of the Cell Sizes on the Strength of the Single Cells

The penetrations of single yeast cells using sharp and flat cantilever tips (Figs. 4(b)-(c)) are shown in Figs. 7(a)-(b). The required force to penetrate a single yeast cell under the ESEM mode using a sharp tip for different cell sizes are shown in Fig. 8. The comparison between sharp and flat tips to penetrate each cell is shown in Fig. 9. The environmental settings for ESEM mode are set to 600 Pa and 0.0°C. These parameters are chosen to vaporize water so that the majority of the cell surface can be seen for mechanical manipulation.

Results clearly show a strong relationship between cell size and strength, the latter increases with an increase in cell size (Fig. 8). Yeast cell walls consists predominantly of glucan with (1,3)- β and (1,6)- β linkages, and mannan covalently linked to protein (mannoprotein) (Klis et al., 2006; Lesage & Bussey, 2006). Mechanical properties are a function of β -glucans, whereas mannoproteins control porosity (Lesage & Bussey, 2006). Thus it can be inferred that the β -glucans composition increases when the yeast cell size increases. This prediction is consistent with other studies stating that small and large yeast cells exhibit similar glucan and mannan contents, but further fractionation of the glucan showed five-times less alkali-insoluble glucan in the smaller sized cells (Srinorakutara, 1998). This may be another reason why the large yeast cells appear to be stronger than the smaller cells (Srinorakutara, 1998). The alkali-insoluble glucan plays a role in maintaining cell rigidity (Fleet & Manners, 1976). Nevertheless, the elastic properties of the cells at different sizes remain unchanged (Table 2). The small and large yeast cells contain different composition levels of alkali-insoluble glucan (Srinorakutara, 1998). The increase in this glucan in the large cells does not change the properties of the molecule itself but rather increase the number of molecules, without changing its elasticity properties. Consequently, the elasticity of the whole cell also remains unchanged and independent of cell size. On the other hand, since there are more glucan molecules in the larger cells, the thickness of the cell wall also increases (Smith et al., 2000). Hence, the amount of force needed to penetrate the cell also increases due to more molecule-barriers during the indentation which leads to penetration.

The average penetration forces for 3, 4, 5 and 6 μm cell diameter ranges are 96 ± 2 , 124 ± 10 , 163 ± 1 and 234 ± 14 nN, respectively (Fig. 8). This was expected since the yeast's cell wall is remarkably thick (100 - 200 nm) (Walker, 1998), whereas its plasma membrane is only about 7 nm thick (Walker, 1998).

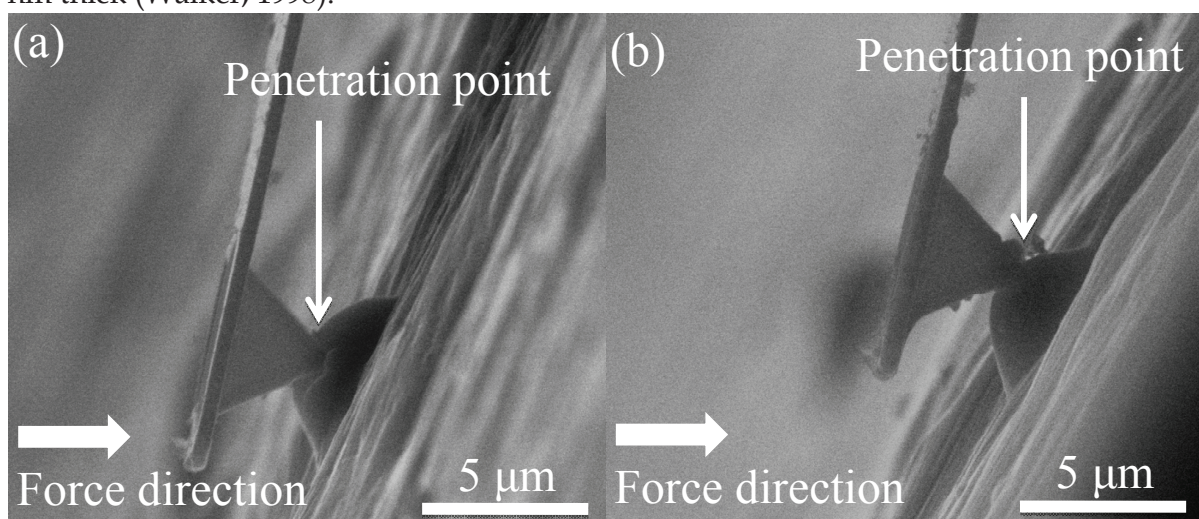


Fig. 7. Penetrations of yeast cells by different cantilever tips, i.e. (a) sharp cantilever tip and (b) flat cantilever tip.

These results are comparable to reported penetration force of 1 nN for a human epidermal melanocytes that is the only cell membrane without cell wall structure (Obataya, 2005). Since the mechanical strength of the cell is provided by the cell wall and not the cell membrane, therefore the melanocytes are easily penetrated compared to yeast cells. Additionally, the effect of local (small contact area between tip and cell surface and global (large contact area between tip and cell surface) penetrations are also an important factor in the amount of applied force. This can be seen from our results for the local penetration (96 to 234 nN) compared to reported average of $149 \pm 56 \mu\text{N}$ for global bursting of the yeast cell (Smith et al., 2000). The average Young’s modulus obtained from (6), i.e. $3.24 \pm 0.09 \text{ MPa}$ is reasonable compared to reported local cell stiffness values of 0.73 MPa (Pelling et al., 2004) and 1.12 MPa (Lanero, 2006), since the E values obtained in this paper representing not only the local elastic property of the cell but also the whole cell stiffness.

In addition to the cell size factor, we showed that the shape of the indenter also plays an important role in predicting the penetration force of a single cell. This can be confirmed from Fig. 9 where the penetration force can be reduced 40-50% by using a sharp cantilever tip compared to a flat tip. This provides a good indicator for future cell surgery where a minimal penetration force is required for preventing serious cell damage or undesirable mechanotransductions due to a large cell deformation (Huang et al., 2004). The Young’s modulus (E) of the cells estimated using the spherical model (sharp tip) and the cylindrical model (flat tip) obtained from (6) and (7), respectively, showed that the E values of the cell remained constant at about 3 MPa.

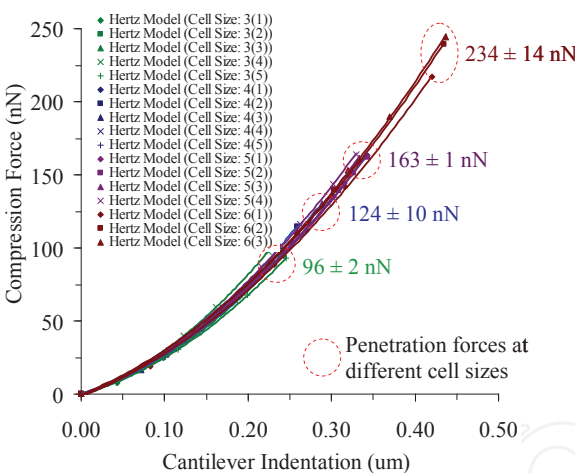


Fig. 8. Penetration force using a sharp tip at different cell sizes. Experimental data are fitted using the spherical model. The curves are shown until the penetration points. Inner bracket () indicates the cell number for each cell size. Cell size is in μm .

Cell Size (μm)	Young Modulus (MPa)
~ 3	3.27 ± 0.23
~ 4	3.28 ± 0.12
~ 5	3.31 ± 0.11
~ 6	3.29 ± 0.10

Table 2. Young’s modulus of the single cells at different cell sizes.

The link between the cell strength with the increase cell size as reported in this paper is somehow against the Laplace law. Laplace law governs the tension of an ideal thin-wall sphere, i.e. $Wall\ tension = Transmural\ pressure \times Radius\ of\ the\ curvature^{1/2}$ (Morris & Homann, 2000). From this equation, the more curved a membrane (small cell), the less tension experienced by the cell wall for a given transmural pressure (the pressure difference between inside and outside of the cell). The less tension the cell wall, the less possibility the cell wall to failure. This mean a smaller cell is less likely to be ruptured than a larger cell.

This notion could be true if the thickness of the cell remains constant independent of cell sizes. Since the cell wall plays a main role in cell stiffness than the membrane cell, any different in the cell wall thickness would give different stiffness property of the cell. Smith et al. (Smith et al., 2000) showed that the thickness of the cell wall increases as the cell growth. Kliss et al. (Klis et al., 2006) also revealed that cells increase their size during their lifetime before undergone a cell division. During this growing process, the composition of the β -glucan is increasing which inturn increases the thickness of the cell wall (Smith et al., 2000). The heterogeneity of the cell wall composition and cell wall thickness will give different stiffness property to the cell and therefore the Laplace law which governs the surface tension of the cell may play a minor role in predicting the strength of the cell.

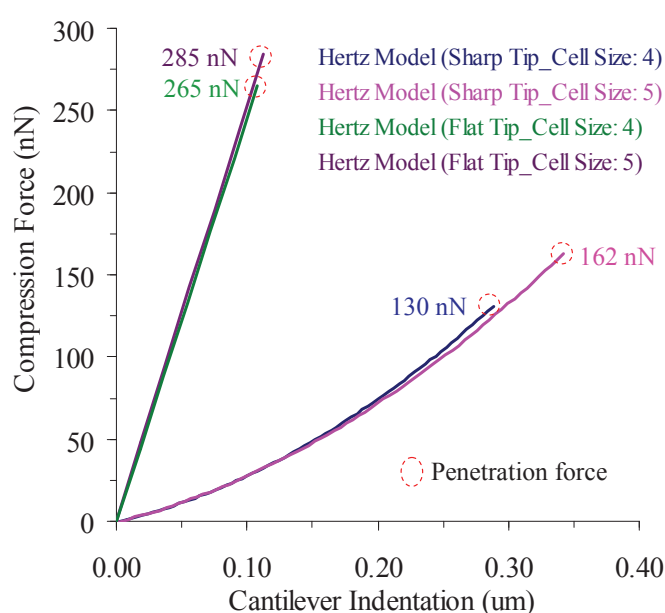


Fig. 9. Comparison of penetration force using sharp (using the spherical model) and flat tips (using the cylindrical model) for 4 and 5 μm cell sizes range. Young's modulus of the cells remains constant at about 3 MPa. The curves are shown until the penetration points.

4.3 The Effect of the Environmental Conditions on the Strength of the Single Cells

The required force to penetrate a single yeast cell under HV mode using sharp and flat tips for different cell sizes are shown in Figs. 10 and 11, respectively. Insets are images of penetration of a single yeast cell using sharp (Fig. 10) and flat (Fig. 11) cantilever tips. Higher spring constant of the cantilever, i.e. 0.7 N/m was used for the compression experiment as lower spring constant values failed to penetrate the cell. Fig. 4(d) shows the image of a tetrahedral cantilever tip used in this experiment. The penetration force to burst a

cell wall increases about twenty times from ESEM mode to high vacuum (HV) condition (cells which 5 μm diameter were used for comparison and analysis) (Figs. 8 and 10). Since this data under high vacuum condition is reported for the first time, it is difficult to compare with other reports. Nevertheless, the increase in penetration force from ESEM to HV modes which was obtained in our experiment can be logically understood.

Cell wall construction is tightly controlled since polysaccharide composition, structure and thickness of the cell wall vary considerably, depending on the environmental conditions (Klis et al., 2006). Therefore, we can infer that under extreme condition of high vacuum, yeast cell tries to survive by increasing its wall thickness as suggested by the increased compression force needed to penetrate its cell wall (Figs. 10 and 11). Other possibility is that under high vacuum condition, cells become too dry which eventually increases their cell hardness. These findings reveal that besides the shape of a cantilever tip, other external factors such as environmental conditions also influence the penetration force of a single cell. Like the ESEM mode, cell size remains the internal factor that influences the penetration force under high vacuum condition. By using a sharp tip, an average of 3 μN penetration force was required for a 5 μm diameter cell. As expected, this force increased (up to 14 μN) when a flat cantilever tip was applied, for cells with an average diameter of 4 to 6 μm .

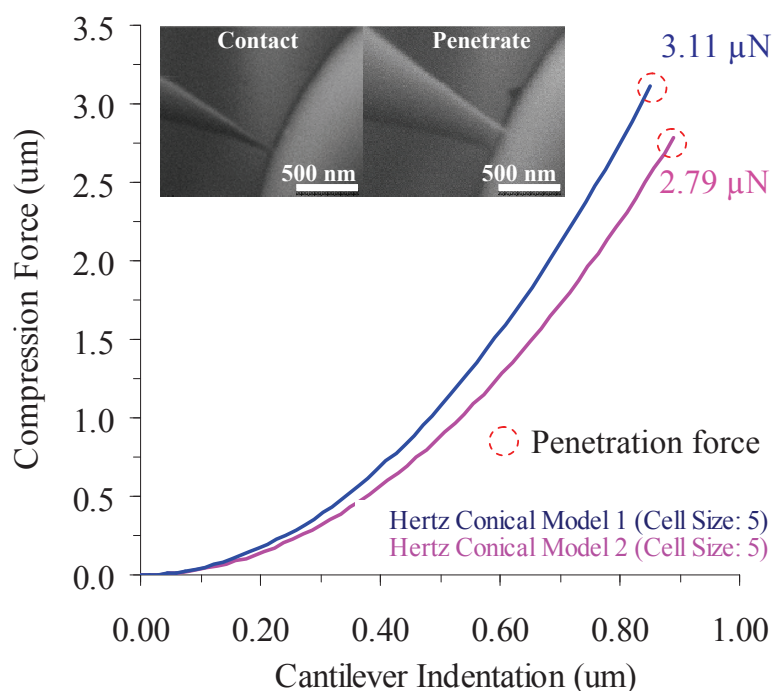


Fig. 10. Force-indentation curves until the penetration points using a sharp tip (conical model) for about 5 μm cell size under HV mode.

For a quantitative evaluation of the elasticity of the cell, the Hertz-Sneddon mechanics model for conical and cylindrical tips as expressed in (2) and (4) are used for sharp and flat tips, respectively. Average elastic properties of yeast cells under the influence of two environmental conditions are shown in Table 3, where the cell stiffness increases about ten times under HV mode. The cell surface becomes rigid when it is placed in the HV mode. It is believed that in the absence of water, cell surface becomes harder than in the presence of

water. This leads to a significant increase in the Young’s modulus (26.02 ± 3.66 MPa) obtained from (5) under HV mode as compared to the ESEM mode (3.31 ± 0.11 MPa) as expected.

4.4 The Effect of the Growth Phases on the Strength of the Single Cells

All cells have a unique growth phase curve during their life cycle which is normally divided into four phases; lag, log, saturation and death phases. In the present study we did not investigate the death phase. These phases can be easily identified from the optical density (OD) absorbance-time curve (Fig. 12). Lag phase located at the initial lower horizontal line where the cells adapt themselves to growth conditions and the cells mature and divide slowly. Log phase, can be seen from an exponential line where the cells have started to divide and grow exponentially. The actual growth rate depends upon the growth conditions. Saturation phase resembles a final upper horizontal line where the growth rate slows and ceases mainly due to lack of nutrients in the medium.

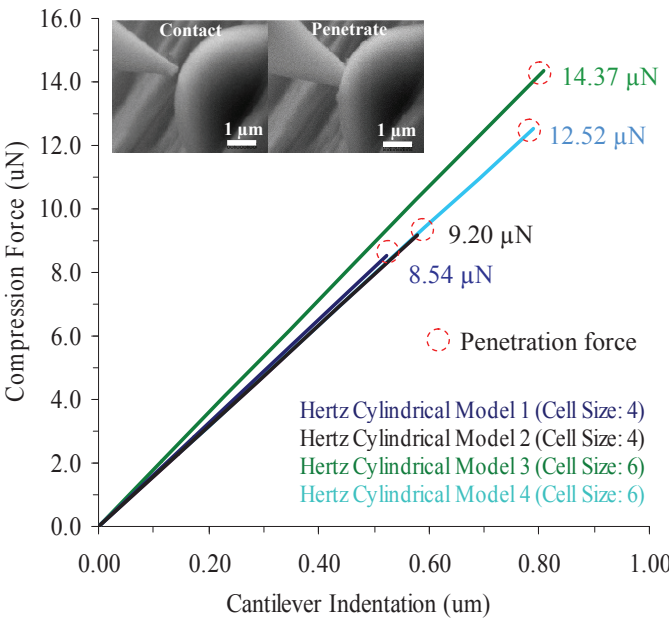


Fig. 11. Force-indentation curves until the penetration points using a flat tip (cylindrical model) for 4 to 6 μm cell sizes under HV mode.

Environmental Conditions (Pa)	Young Modulus (MPa)
ESEM mode (~ 600)	3.31 ± 0.11 (n=4)
HV mode (~ 0.00314)	26.02 ± 3.66 (n=6)

Table 3. Young’s modulus of the single cells at different environmental conditions.

During the death phase, all of nutrients are exhausted and the cells die (Tortora et al., 2003). It is believed that the mechanics of the normal cells have different properties at each of the phases. In this section, the mechanical properties of W303 yeast cells at four different growth phases (early, mid, late log and saturation) are discussed.

Since each cell has a unique growth phase, we determined the growth phase of *W303* yeast cells by measuring the absorbance at 660 nm using a Spectrophotometer (Hitachi: U-2000) at one hour intervals (Fig. 12). The growth rate (absorbance per hour) was about 0.23 as can be seen from the slope of the curve. Samples were stored at -80°C (Sanyo: MDF-291AT) to hold its growth before the compression experiment.

The compression experiments were done at 6 hour intervals starting at early log phase and ending at saturation phase. Penetration force was analyzed for each phase as shown in Figs. 13(a)-(d). As expected, the force needed to penetrate a single cell increased from early log to saturation phase (161 ± 25 , 216 ± 15 , 255 ± 21 and 408 ± 41 nN), whereas, the elastic properties of the cells seem constant for all the phases obtained from (6), i.e. 3.28 ± 0.17 , 3.34 ± 0.14 , 3.24 ± 0.11 and 3.38 ± 0.11 MPa (Table 4). These mechanical properties of the *W303* yeast cells at different growth phases are in an agreement with reported increment of average surface modulus of *S. cerevisiae* cell wall to be 11.1 ± 0.6 N/m (log phase) and 12.9 ± 0.7 N/m (saturation phase) with no significant increase in the elastic modulus of the cell, i.e. 112 ± 6 MPa (log phase) and 107 ± 6 MPa (saturation phase) (Smith et al., 2000). Their values for elastic modulus are quite high is also reasonable since they measured the whole elastic properties of the cell by compressing a single cell between two big flat indenters as compared to a local cell indentation in our case.

From this analysis, it can be inferred that the thickness of the *W303* cell wall is increased during the growth phase since the penetration force increases at each phase, and at the same time, maintaining their elastic properties during the growth phase as verified by the constant elastic modulus values shown in Table 4. The increase in the cell thickness does not affect the elastic properties of the cell but the penetration force will be increased due to more molecules need to be penetrated. We believe that the increase in the thickness of cell wall is mainly due to an increment of β -glucans molecules (Lesage & Bussey, 2006).

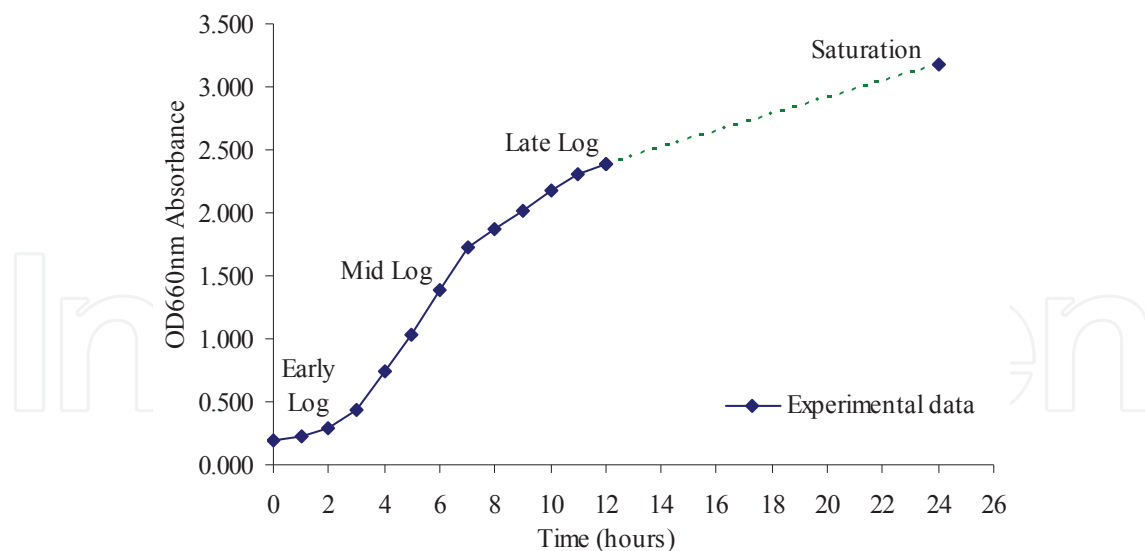


Fig. 12. The growth phase cycle for *W303* yeast cells based on the $\text{OD}_{660\text{nm}}$ absorbance measurement.

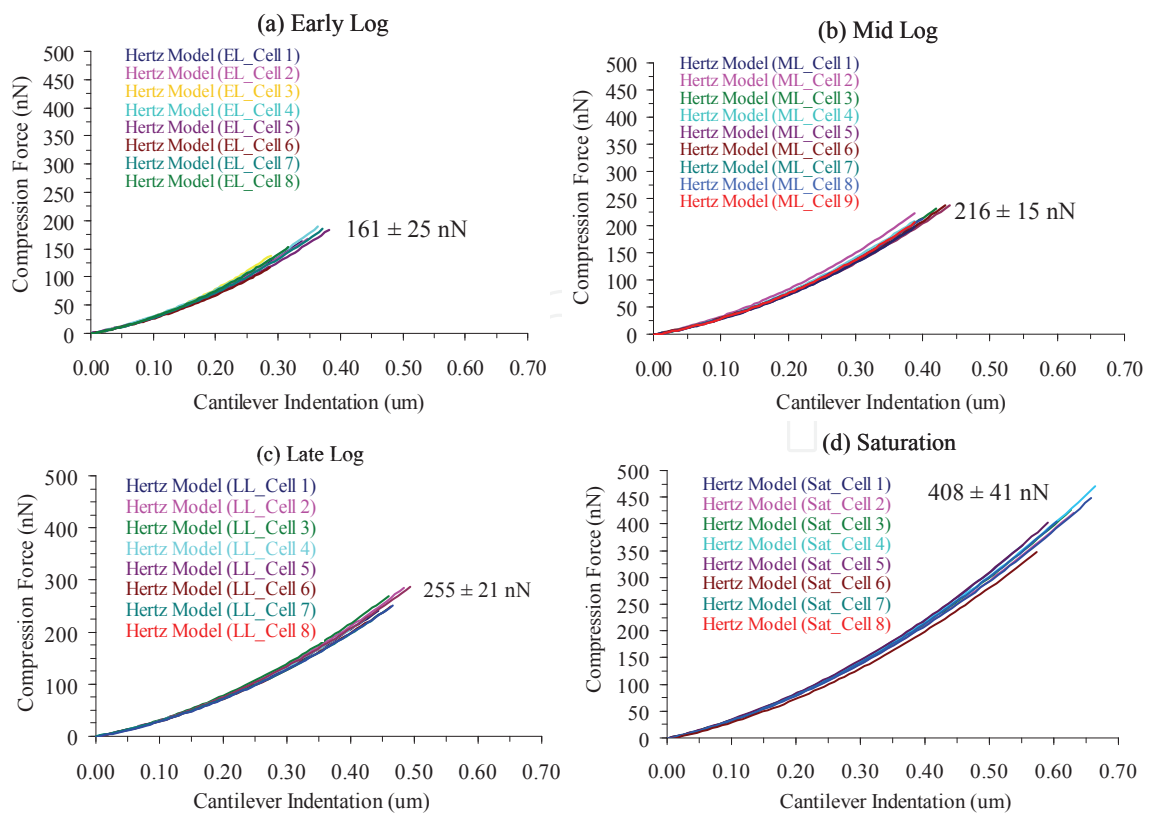


Fig. 13. The force-indentation curves (fitted using spherical model) for single cells at each growth phases (a) early log, (b) mid log, (c) late log and (d) saturation. The curves are shown until the penetration points.

Cell Growth Phase	Young Modulus (MPa)
Early Log	3.28 ± 0.17 (n=8)
Mid Log	3.34 ± 0.14 (n=9)
Late Log	3.24 ± 0.11 (n=8)
Saturation	3.38 ± 0.11 (n=8)

Table 4. Young’s modulus of the single cells at different cell growth phases.

5. Nanoneedles for Single Cells Mechanical Characterizations

5.1 Determination of Cell Stiffness and Cell Surgery Based on Nanomanipulation

We propose several approaches for achieving two biomechanic tasks, i.e. determining the stiffness of a single cell and performing single cell surgery as shown in Fig. 14. In the cell stiffness measurement, two approaches are designed. The first approach, to the best of our knowledge, is a novel technique in determining the stiffness of the cell. In this technique, the nanoneedle and the cell can be modeled as two springs in series. In order to model the nanoneedle as a spring, firstly, the nanoneedle should be able to buckle linearly and secondly, it should have lower or the same spring constant as the cell. This technique prevents the damage to the cell since the indentation is minimized from the buckling effect

of the nanoneedle which avoids excessive indentation being applied to the cell, the so-called “soft nanoneedle”. The schematic of this approach is shown in Fig. 14(a). The second approach for the measurement of cell stiffness is based on a “hard nanoneedle” which relies totally on the deformation of the cell in order to measure its stiffness. By knowing the applied compression force and the deformation of the cell, the stiffness of the cell can be measured (Fig. 14b). In order to fabricate the hard nanoneedle, strong material which is hard to bend is needed. We used Tungsten material to construct the hard nanoneedle. To prevent excessive indentation force on the cell by the hard nanoneedle, this process has to be performed by avoiding any sudden pressure which can interrupt chemical activities of the cell, e.g. mechanotransduction effect. The other preventive step is to use a lower cantilever spring constant. We used 0.09 N/m cantilever spring constants. The hard nanoneedle can also be used for single cell surgery. In theory, all hard nanoneedles must be able to penetrate the cell, however, in practice, for the yeast cells, only some nanoneedles (Si-Ti and W_2 nanoneedles) can penetrate the cells while others (e.g. $W_{0.09}$ nanoneedle) are only limited to cell stiffness measurement only. This is because to penetrate the cell, more compression force is needed. This excessive force may exceed the force limits of the nanoneedle causing failure. This so-called “cell surgery” operation is shown schematically in Fig. 14(c).

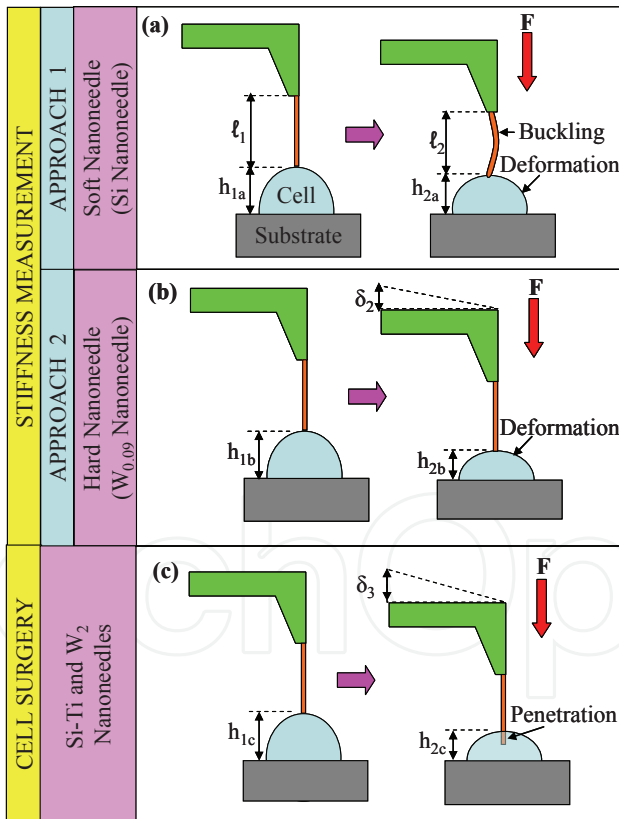


Fig. 14. The schematics diagrams for the nanoneedles indentation experiments indicate local single cell stiffness measurement using (a) soft Silicon (Si) nanoneedle and (b) hard nanoneedle which is based on Tungsten deposition on the cantilever (spring constant = 0.09 N/m) ($W_{0.09}$). Single cell surgery is shown in (c) by using hard Si-Ti and W_2 nanoneedles.

5.2 Fabrication of Soft and Hard Nanoneedles

Four kinds of nanoneedles were fabricated. Fig. 15 shows the general fabrication processes for each of the nanoneedles. The standard Platinum coated tetrahedral cantilever tips having a spring constant of 2 N/m (Olympus Corp.) were used in the fabrication of Si, Si-Ti and W₂ nanoneedles. Whereas for the W_{0.09} nanoneedle, the standard sharp pyramidal cantilever tip which has a 0.09 N/m spring constant (Olympus Corp.) was used.

The soft Silicon (Si) nanoneedle was fabricated by using only Focused Ion Beam (FIB) etching as shown in Fig. 15(a). The parameters for the etching process are 30 kV of acceleration voltage and 8.561×10^{18} of ion dose. The time required to complete the process is between 20 to 45 min. The diameter and the length of the Si nanoneedle are about 150-170 nm and 7 μ m, respectively.

The quantitative analysis of the detailed material of the soft nanoneedle was performed using an energy dispersion spectrometry (EDS) method (Ametex Inc.). In general, this type of spectroscopy identifies and counts the impinging X-rays based upon their characteristic energy levels. The X-ray spectrum, used in quantitative analysis, is generated by the nanoneedle in response to the spot from an electron beam on the center area of the nanoneedle. The intensity of the X-rays is measured by EDS as shown in Fig. 16. From the EDS analysis, the main element of the soft nanoneedle is Silicon (83.03 %) while the rest is Oxygen.

The first type of hard nanoneedle, i.e. Silicon-Titanium (Si-Ti) nanoneedle, was fabricated by coating the former Si nanoneedle with Ti material by sputtering as shown in Fig. 15(b). The time for the sputtering operation is about 3 min to obtain a coating of approximately 20 nm of Ti material. The coated area of the Ti material is only on the one side of the nanoneedle facing the Ti source during the sputtering operation. Nevertheless, this coating increases the strength of the basic Si nanoneedle from easily buckling to unbuckling. The typical diameter and length of the Si-Ti nanoneedles are about 185-200 nm and 4 μ m respectively.

The second type of hard nanoneedle (W_{0.09}) was fabricated by first flattening the apex of the sharp pyramidal cantilever tip by using FIB etching. Then W material was deposited on the flat surface by using a small area of FIB deposition, i.e. 100 nm² as shown in Fig. 15(c). The ion dose parameter for deposition is 2.672×10^{20} . The time for the deposition is about 13 min. The typical diameter and the length of the W_{0.09} nanoneedles are about 250 nm and 3 μ m respectively.

Finally, the third type of hard nanoneedle (W₂), was fabricated by first flattening the apex of the sharp tetrahedral cantilever tip by using FIB etching, then followed by W deposition of large area of FIB deposition, i.e. 160 000 nm², and finally trimmed to produce nanoneedle structure by using FIB etching as shown in Fig. 15(d). The diameter and length of the W₂ nanoneedle are around 150-170 nm and 3 μ m. The actual images of each nanoneedle are shown in the Fig 17. All of the fabricated nanoneedles have flat ended tips.

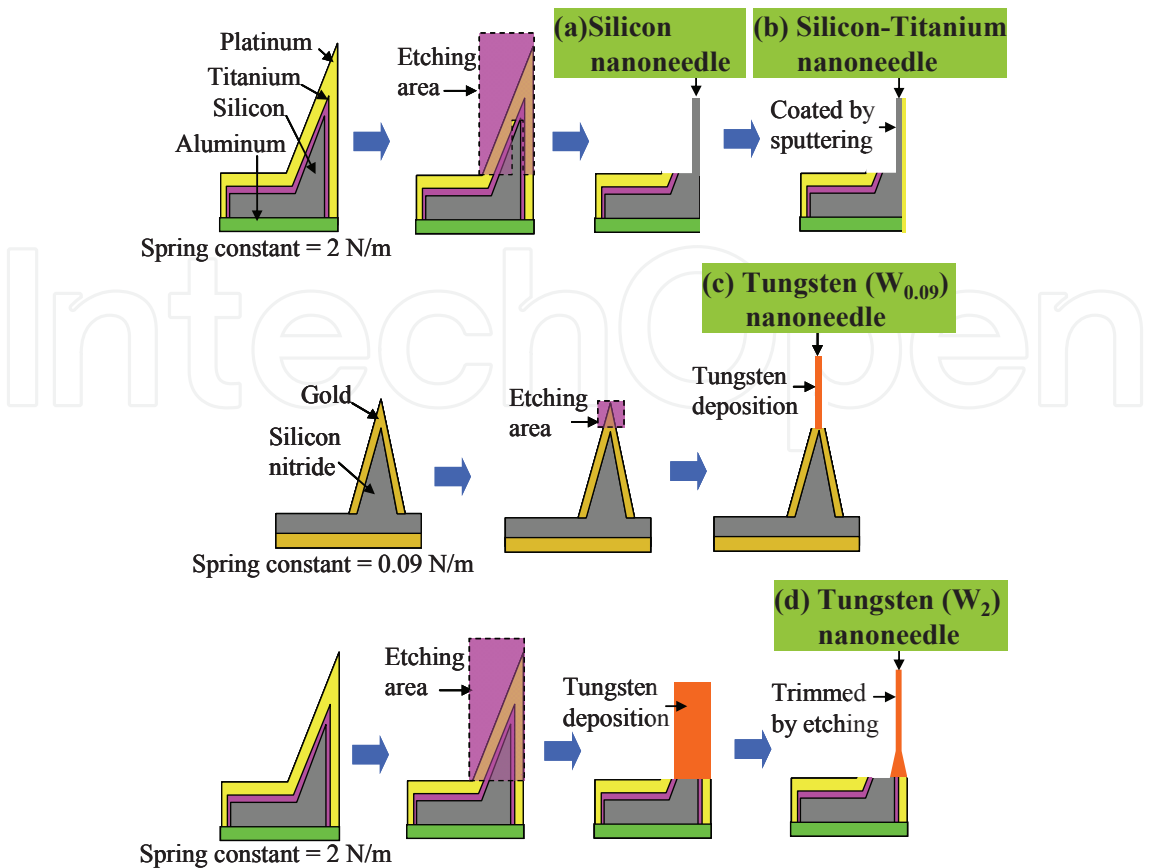


Fig. 15. Fabrication procedures of nanoneedle: (a) Silicon nanoneedle, (b) Silicon-Titanium nanoneedle, (c) Tungsten ($W_{0.09}$) nanoneedle and (d) Tungsten (W_2) nanoneedle, respectively.

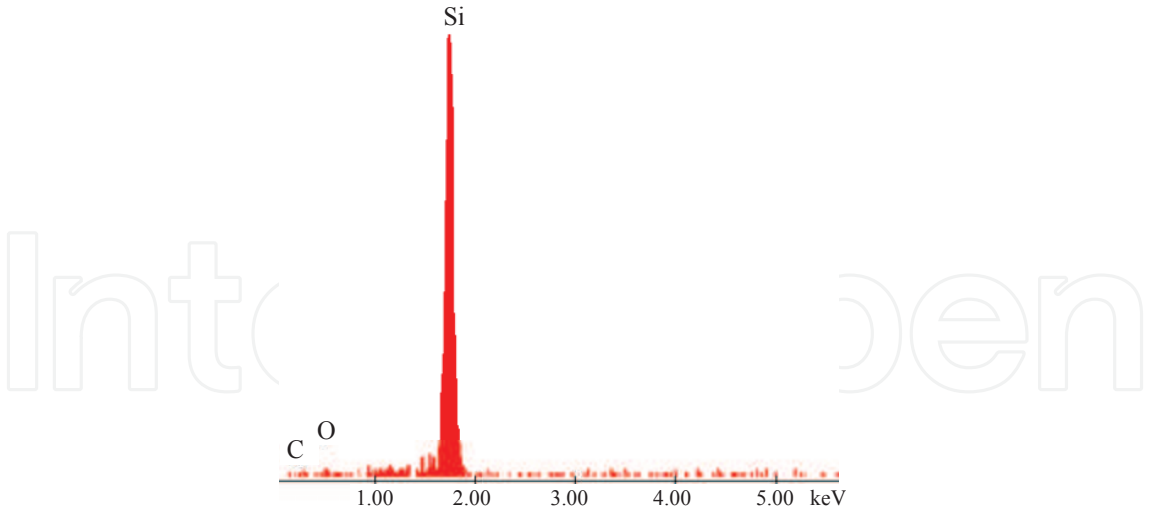


Fig. 16. The determination of the precise material of the soft nanoneedle was done by using energy dispersion spectrometry (EDS) method. From this data, the main component of the soft nanoneedle is Silicon as expected.

5.3 Application of Soft Nanoneedle: Single Cell Whole Stiffness Characterization

The whole stiffness response of a single cell, i.e. the deformation of the entire cell upon the applied load from a single point indentation, to the best of our knowledge, has not been previously reported. The difficulty to achieve such data is due to the stiff indenter which may penetrate or burst the cell. Our soft nanoneedle can be used for preventing the cell penetration by its ability to buckle during indentation. The mechanism of the cell deformation is stated as the following; upon indentation of the soft nanoneedle on the cell, local deformation occurred below the tip of the nanoneedle. Further indentation has induced the whole cell to deform in addition to the local cell deformation. The ability to produce a large local point indentation could give more information regarding the mechanical property of the organelles inside the cell.

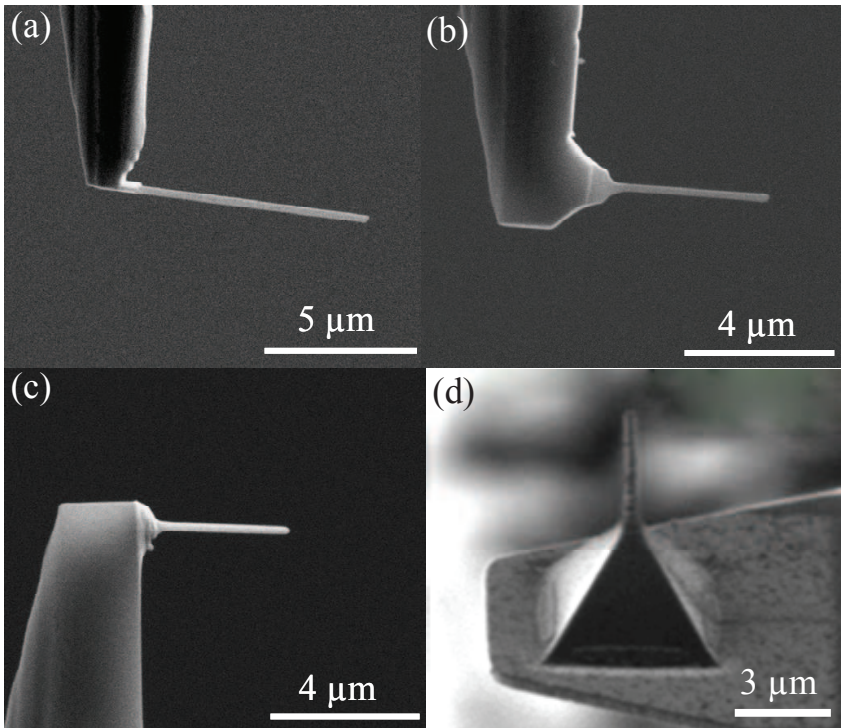


Fig. 17. Actual images of (a) Si, (b) Si-Ti, (c) W₂ and (d) W_{0.09} nanoneedles.

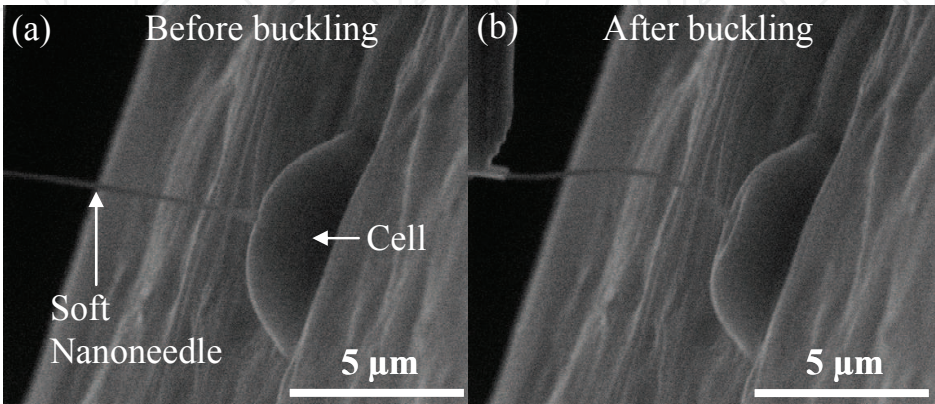


Fig. 18. Single cell global stiffness measurement from a single point using the Si nanoneedle. Two images show the Si nanoneedle at (a) straight condition and (b) buckling condition.

The global stiffness measurement of single cells from single point indentation was performed using a Si nanoneedle as shown in Fig. 18. The measurement was performed using a standard indentation procedure. The Si nanoneedle started to buckle after the cell deformed for about 0.5 μm . From this point, the buckling rate of the Si nanoneedle increased with the increase of the indentation depth. Fig. 19 shows the force-cell deformation response of a single cell using the Si nanoneedles. By using (5) (based on Hertz-Sneddon conical tip model) as described earlier, the spring constant and Young modulus of single *W303* yeast cells were estimated as presented in Table 5. The values of k_{cell} for about the same physical parameters of two yeast cells, i.e. $0.92 \pm 0.12 \text{ N/m}$ and $0.95 \pm 0.36 \text{ N/m}$ show strong mechanical property similarity. The values which represent the whole cell spring constants are reasonable as compared to the reported local spring constant of the *Saccharomyces cerevisiae* yeast cell ($0.06 \pm 0.025 \text{ N/m}$) (Pelling et al., 2004). The values of whole E_{cell} , i.e. 3.64 MPa and 3.92 MPa are also rational since they represent the whole cell stiffness property as compared to the reported local Young modulus of the yeast cell, i.e. $0.72 \pm 0.06 \text{ MPa}$ (Pelling et al., 2004).

Our method shows improved data sensitivity as compared to the other global stiffness measurement method that relies totally in a compression of a single cell between one big indenter and a substrate. Data from this method may not represent the actual global cell stiffness as extra dissipation force may be included in the measurement as reported by (Smith et al., 2000) for the global cell spring constant and Young modulus of yeast cell as 11.1 N/m and 112 MPa.

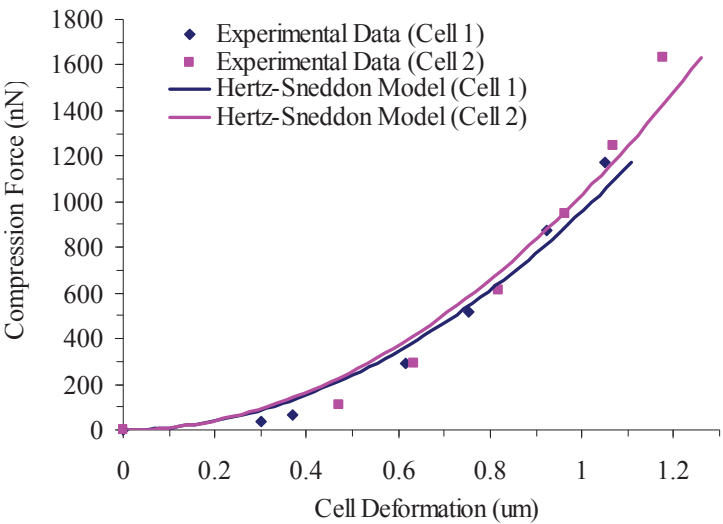


Fig. 19. Force-deformation curves of single cells using the Si nanoneedle. Experimental data were fitted with the Hertz-Sneddon conical tip model.

	Cell Physical Parameters		Cell Stiffness Characteristics	
	Height (μm)	Diameter (μm)	Spring Constant (N/m)	Young Modulus (MPa)
Cell 1	2.824	6.524	0.92	3.64
Cell 2	3.062	6.417	0.95	3.92

Table 5. The global characteristics of two single cells from local single point indentation using buckling Si nanoneedle.

5.4 Application of Hard Nanoneedle: Single Cell Local Stiffness Characterization

The local stiffness property, i.e. the deformation of the surface of the cell upon the applied load, on several points on mother cell and daughter cell, to the best of our knowledge, has never been reported. In order to achieve multi-point indentations on a single cell, we have used the standard indentation technique together with the image analysis to measure the cell deformation and compression force. The compression force was calculated using (1) and the value of φ was obtained from the bending of the cantilever. These values (cell deformation and compression force) were then fitted in (4) (Hertz-Sneddon cylindrical tip model) to obtain the local stiffness of the cell. $W_{0.09}$ nanoneedle which has small value of cantilever's spring constant (0.09 N/m) was used in this experiment as shown in Fig. 20. Three random indentation points on each mother and daughter cell were performed as shown in Figs. 20(a) and (b) respectively. The cells can be recognized as mother and daughter cells from a direct image observation or from the cell volume comparison. The shape of the cells was assumed to be a prolate spheroid, and the volumes were calculated by using the formula $V=(\pi/6)lw^2$, where V is the volume, l is the height, and w is the width at maximum circumference (diameter) (Johnston et al., 1977). Thomas et al. (Thomas et al., 1980) reported about 38% difference in cell volume between fully separated mother and daughter cells of *Candida utilis* type of yeast cell. In our experiment, the volume difference of the mother and daughter cells is about 45% and this is reasonable since the daughter was not fully grown as it was still intact with its mother at the time of measurement. The results from the indentations are shown in Fig. 21 and data were summarized in Table 6. From the data, all the three points on the mother and daughter cells show very good agreement to each other in local stiffness profile, i.e. for mother cell (1.47 MPa, 1.43 MPa and 1.47 MPa) and for daughter cell (1.11 MPa, 1.09 MPa and 1.09 MPa). On the other hand, the data revealed the heterogeneous local stiffness property between mother and daughter cell. This can be comprehended since the daughter cell was still in its early growth phase and the insertion of new cell wall macromolecules, as required for its strength, into its existing polymer network (Klis et al., 2006) was still carried out at the time of measurement and therefore its Young's modulus exhibited slightly lower value than the mother cell.

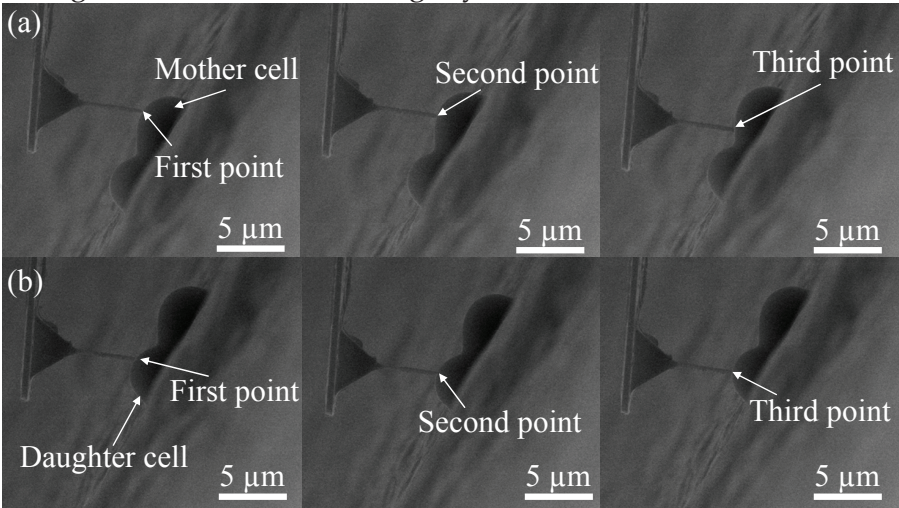


Fig. 20. Local stiffness measurement using the $W_{0.09}$ nanoneedle on three points of the (a) mother cell and (b) daughter cell.

The local stiffness characteristic of the mother cell as reported here falls in the same stiffness range as the data obtained from the literature. Peeling et al. (Pelling et al., 2004) reported 0.72 ± 0.06 MPa of the local stiffness property of the yeast cell. Lanero et al. (Lanero et al., 2006) reported 1.79 ± 0.08 MPa, 1.12 ± 0.02 and 2.0 ± 0.2 MPa for coated cell, uncoated cell and coated cell-bud scar of the *Saccharomyces cerevisiae* respectively. As compared to the *Saccharomyces cerevisiae* strain, baker-type yeast cells which has been widely used for the cell stiffness analysis, we have shown, for the first time, the mechanical property of the W303 strain, wild-type yeast cell.

5.5 Application of Hard Nanoneedle: Single Cell Surgery

The large stiffness and the small diameter of the Si-Ti and W₂ nanoneedles have bring additional functionality to those nanoneedles. Fig. 14(c) shows the schematic of single cell surgery. In this experiment, we are not interested in the measurement of the elastic property of the cells but rather the ability of the nanoneedles to penetrate the cell without creating any cell bursting. As compared to our previous work (Ahmad et al., 2007), the penetration activities were followed by the cell bursting which killed the cell. This is unwanted scenario if we want to perform the future drug delivery and the single cell surgery. From the experimental real-time observation under ESEM, we can see clearly that a single cell was punctured by a nanoneedle without any cell bursting as can be seen from Fig. 22. Fig. 23 shows the force-cell deformation response of a single cell using Si-Ti nanoneedle and W₂ nanoneedle at three stages of indentation, i.e. early indentation (blue line), after penetration (pink line), and late indentation, i.e. the nanoneedle was touching the substrate (red line).

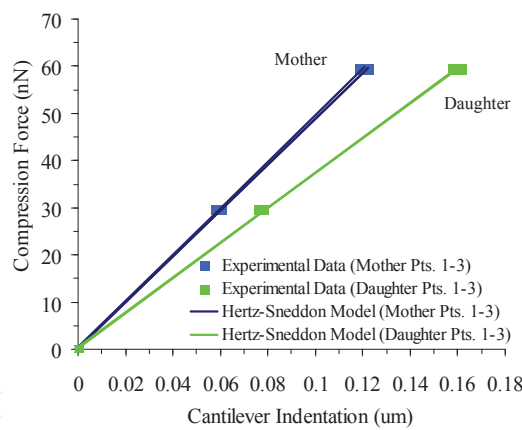


Fig. 21. Force-indentation curves of mother and daughter cells using the W_{0.09} nanoneedle. Experimental data were fitted with the Hertz-Sneddon cylindrical tip model.

		Cell Height (μm)	Cell Diameter (μm)	Young Modulus (MPa)
Mother cell	Point 1	2.981	5.565	1.47
	Point 2			1.43
	Point 3			1.47
Daughter cell	Point 1	2.488	4.467	1.11
	Point 2			1.09
	Point 3			1.09

Table 6. The local stiffness characteristics of mother cell and daughter cell using w_{0.09} nanoneedle.

At the first stage, the nanoneedle was indenting the cell without penetration of the cell as can be seen from the first inset of Fig. 23(a). The second stage begins when the nanoneedle started to penetrate the cell. The values of force and cell deformation at the point of penetration by using Si-Ti and W_2 nanoneedles were about 550 nN ($0.42\text{ }\mu\text{m}$) and 778 nN ($0.56\text{ }\mu\text{m}$). The increase in the penetration force by using the W_2 nanoneedle was due to the small bending of the nanoneedle prior penetration. This stage can be recognized from the drop in force value as can be seen from the second inset of Fig. 23(b). Since the penetration force before the penetration was quite high, the drop force was large due to the high momentum of the reversed force. Further indentation in the second stage had brought the nanoneedle in contact with the other cell wall that was supported by the substrate as can be seen by the sudden rise of force value as shown in the third inset of Fig. 23(c). In order to confirm the success of the injection of the nanoneedle without cell bursting, we intentionally hit the nanoneedle on the other side of the cell wall to break it as shown in Fig. 24. The injection without cell bursting is very important in single cell surgery and drug delivery systems. Nevertheless, the work on single cell surgery is still under investigation and more researchers are needed in this area to develop better devices for single cell surgery. One of the works in single cell surgery was done by (Obataya et al., 2005b). They measured force between 1 to 2 nN to penetrate a human epidermal melanocyte cell. The value of the force is small compared to the penetration force obtained in this paper because unlike the yeast cell, the human epidermal melanocyte cell does not have the cell wall. The cell wall which exists in the yeast cell is the main component which governs the stiffness properties of the cell (Klis et al., 2006).

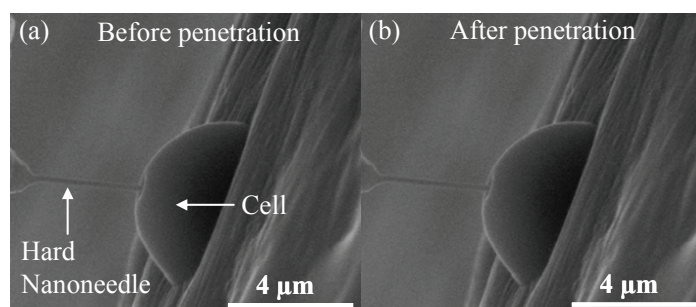


Fig. 22. Single cell surgery without cell bursting using Si-Ti nanoneedle.

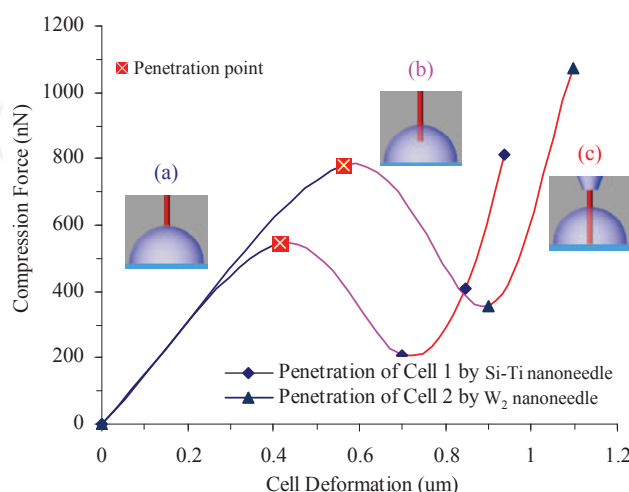


Fig. 23. Force-cell deformation curve using Ti-Si and W_2 nanoneedles at three different stages, i.e. (a) before penetration, (b) after penetration and (c) touching the substrate.

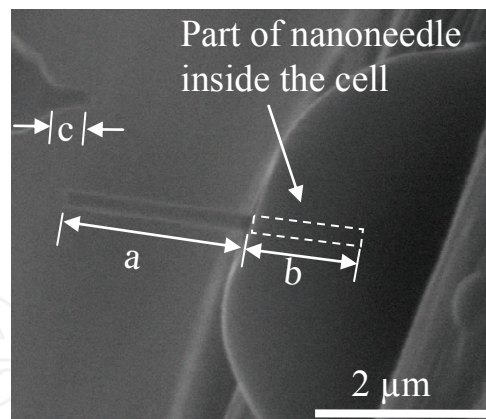


Fig. 24. Single cell surgery without cell bursting using Si-Ti nanoneedle. The dotted line represents the inserted nanoneedle inside the cell. The values for a , b and c are $2.16\ \mu\text{m}$, $1.19\ \mu\text{m}$ and $0.30\ \mu\text{m}$ respectively.

6. Conclusion

In this chapter, two important needs for the single cells analysis, i.e. the understanding of the mechanical properties of single cells and the implementation of the nanodevices in single cells mechanical property characterizations, have been addressed. The advantages of the integrated ESEM-nanomanipulation system rely on its capability to perform *in-situ* local direct observation and manipulation of a biological sample and the ability to control the environmental conditions. The ESEM-nanomanipulation system has a great capability for conducting single cells analysis. To the best of our knowledge, we, for the first time, have demonstrated the effect of the internal influences (cell size and growth phases) and the external influence (environmental conditions) on the cell strength. The penetration force is proportional to the increase in cell size, during the cell growth and under high vacuum condition. The elastic modulus of the cell increases dramatically under high vacuum condition but remain unaffected during the cell growth. Furthermore, we have highlighted the mechanical properties characterization of individual yeast cells from W303 strain using four types of nanoneedles, i.e. Si, Si-Ti, $W_{0.09}$ and W_2 nanoneedles. We have demonstrated via experimental verification that all four types of nanoneedles described are very effective for yeast cell local stiffness characterization. This capability has numerous future applications especially in human disease detection. In addition to the mechanical characterization, Si-Ti and W_2 nanoneedles can also be applied in single cell surgery due to their strength. Penetrations of single cells have been successfully performed using either of these nanoneedles. This single cell surgery can be further applied in the future single cell drug delivery applications.

7. References

- Brehm-Stecher, B.F., & Johson, E.A. (2004). Single-cell microbiology: Tools, technologies, and applications. *Microbiology and Molecular Biology Reviews* 68, 538-559.
- Harold, F.M. (2005). Molecules into Cells: Specifying Spatial Architecture. *Microbiology and Molecular Biology* 69, 544-564.
- Shapiro, H.M. (2000). Microbial analysis at the single-cell level: tasks and techniques. *Journal of Microbiological Methods* 42, 3-16.

- Bausch, A.R., Moller, W., & Sachmann, E. (1999). Measurement of local viscoelasticity and forces in living cells by magnetic tweezers. *Biophysical Journal* 76, 573-579.
- Gueta, R., Barlam, D., Shneck, R.Z., & Rouso, I. (2006). Measurement of the mechanical properties of isolated tectorial membrane using atomic force microscopy. *Proceedings of the National Academy of Sciences* 103, 14790-14795.
- Thoumine, O., Ott, A., Cardoso, O., & Meister, J.J. (1999). Microplates: a new tool for manipulation and mechanical perturbation of individual cells. *J. Biochem Biophys Meth* 39, 47-62.
- Obataya, I., Nakamura, C., Han, S.W., Nakamura, N., & Miyake, J. (2005a). Nanoscale Operation of a Living Cell Using an Atomic Force Microscope with a Nanoneedle. *Nano Letters* 5, 27-30.
- Leary, S.P., Liu, C.Y., & Apuzzo, M.L.I. (2006). Toward the emergence of nanoneurosurgery: Part III - Nanomedicine: Targeted nanotherapy, nanosurgery, and progress toward the realization of nanoneurosurgery. *Neurosurgery* 58, 1009-1025.
- Bianco, A., Kostarelos, K., & Prato, M. (2005). Applications of carbon nanotubes in drug delivery. *Current Opinion in Chemical Biology* 9, 674-679.
- Brigger, I., Dubernet, C., & Couvreur, P. (2002). Nanoparticles in cancer therapy and diagnosis. *Advance Drug Delivery Reviews* 54, 631-651.
- Stylios, G.K., Giannoudis, P.V., & Wan, T. (2005). Applications of nanotechnologies in medical practice. *Injury, Int. J. Care Injured* 36, 6-13.
- Goodman, M.B., Lumpkin, E.A., Ricci, A., Tracey, W.D., Kernan, M., & Nicolson, T. (2004). Molecules and Mechanisms of Mechanotransduction. *The Journal of Neuroscience* 24, 9220-9222.
- Huang, H., Kamm, R.D., & Lee, R.T. (2004). Cell mechanics and mechanotransduction: pathway, probes, and physiology. *Am J Physiol Cell Physiol* 287, C1-C11.
- Nakajima, M., Arai, F., & Fukuda, T. (2006). In situ Measurement of Young's Modulus of Carbon Nanotube inside TEM through Hybrid Nanorobotic Manipulation System. *IEEE Transactions on Nanotechnology* 5, 243-248.
- Lanero, T.S., Cavalleri, O., Krol, S., Rolandi, R., & Gliozzi, A. (2006). Mechanical properties of single living cells encapsulated in polyelectrolyte matrixes. *Journal of Biotechnology* 124, 723-731.
- Touhami, A., Nysten, B., & Dufrene, Y.F. (2003). Nanoscale Mapping of the Elasticity of Microbial Cells by Atomic Force Microscopy. *Langmuir* 19, 4539-4543.
- Sneddon, I.N. (1965). The Relation Between Load and Penetration in the Axisymmetric Boussinesq Problem for a Punch of Arbitrary Profile. *Int. J. Engng Sci.* 3, 47-57.
- Pharr, G.M., Oliver, W.C., & Brotzen, F.R. (2002). On the generality of the relationship among contact stiffness, contact area, and elastic modulus during indentation. *J. Mat. Res.* 7, 619-627.
- Smith, A.E., Zhang, Z., Thomas, C.R., Moxham, K.E., & Middelberg, A.P.J. (2000). The mechanical properties of *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences* 97, 9871-9874.
- Klis, F.M., Boorsma, A., & Groot, P.W.J.D. (2006). Cell wall construction in *Saccharomyces cerevisiae*. *Yeast* 23, 185-202.
- Lesage, G., & Bussey, H. (2006). Cell wall assembly in *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews* 70, 317-343.

- Srinorakutara, T. (1998). Study on the effect of diluent osmotic pressure on yeast cell strength and cell size using a novel micromanipulation technique. *World Journal of Microbiology & Biotechnology* 14, 719-725.
- Fleet, G.H., & Manners, D.J. (1976). Isolation and composition of an alkali-soluble glucan from the cell walls of *Saccharomyces cerevisiae*. *Journal of General Microbiology* 94, 180-192.
- Walker, G.M. (1998). *Yeast Physiology and Biotechnology*. Chichester: John Wiley & Sons.
- Obataya, I. (2005). Nanoscale Operation of a Living Cell Using an Atomic Force Microscope with a Nanoneedle. *Nano Letters* 5, 27-30.
- Pelling, A.E., Sehati, S., Gralla, E.B., Valentine, J.S., & Gimzewski, J.K. (2004). Local Nanomechanical Motion of the Cell Wall of *Saccharomyces cerevisiae*. *Science* 305, 1147-1150.
- Lanero, T.S. (2006). Mechanical properties of single living cells encapsulated in polyelectrolyte matrixes. *Journal of Biotechnology* 124, 723-731.
- Morris, C.E., & Homann, U. (2000). Cell Surface Area Regulation and Membrane Tension. *Journal of Membrane Biology* 179, 79-102.
- Tortora, G.J., Funke, B.R., & Case, C.L. (2003). *Microbiology: An Introduction*: Benjamin Cummings.
- Johnston, G.C., Pringle, J.R., & Hartwell, L.H. (1977). Coordination of growth with the cell division in the yeast *Saccharomyces cerevisiae*. *Experimental Cell Research* 105, 79-98.
- Thomas, K.C., dawson, P.S.S., & Gamborg, B.L. (1980). Differential Growth Rates of *Candida utilis* Mother and Daughter Cells Under Phase Cultivation. *Journal of Bacteriology* 141, 1-9.
- Ahmad, M.R., Nakajima, M., Kojima, S., Homma, M., & Fukuda, T. (2007). In-situ Single Cell Mechanical Characterization of W303 Yeast Cells inside Environmental-SEM. *Proc. of the 7th IEEE International Conference on Nanotechnology*, vol. 1. Hong Kong, pp. 1022-1027.
- Obataya, F., Nakamura, C., & Han, S.W. (2005b). Mechanical sensing of the penetration of various nanoneedles into a living cell using atomic force microscopy. *Journal of Biosensors & Bioelectronics* 20, 1652-1655.

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The main purpose of this book is to describe important issues in various types of devices ranging from conventional transistors (opening chapters of the book) to molecular electronic devices whose fabrication and operation is discussed in the last few chapters of the book. As such, this book can serve as a guide for identifications of important areas of research in micro, nano and molecular electronics. We deeply acknowledge valuable contributions that each of the authors made in writing these excellent chapters.

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