Nano Mechanical Behavior of Bio Composites

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Abstract:

One of the most important questions regarding the emergent mechanical strength of plant cell walls is: How do the mechanical properties of the individual components within the cell wall matrix compare to one another and evolve during elongation. In this research, the unique capability of the AFM was used to measure the mechanical properties of onion and celery epidermis on the nanometer scale. The Peak Force Quantitative Nano-mechanical Mapping (Peak Force QNM) technique was utilized to obtain images of the onion microfibrils where quantitative values of the mechanical properties provide the contrast for the image. For the first time, a series of high resolution images of intact onion microfibrils were taken to study the stress relaxation of individual fibrils after applying strain to the epidermis. This study was repeated to investigate the individual fibrils nanoscale movement, or possible reorientation, after mechanical elongation of the onion epidermis. The next step involved taking a sequence of images which were used to investigate the effect of pectin on microfibrils. The literature showed that pectin could inhibit the unfolding of Xyloglucan chains [1]. This was studied by adding Pectate lyase to both onion and celery epidermis wall to cut the polygalacturonic acid chains

Introduction:

Extensive research has shown that the bulk properties of plants are comprised from a complex interdependency between the properties of the individual components (i.e., matrix polymers, polysaccharides, and cellulose microfibrils) and the comprised architecture (i.e., the structure and orientation) within a cell wall [2-7]. In order to develop a predictive mechanical model for cell walls, accurate measurements of the mechanical properties of the individual components of a living plant cell wall is required. However, the published measurements of the Young's modulus of plant cell walls have mostly relied on interpreting averaged measurements or have obtained the results based on unrealistic conditions. Basically, uniaxial tension tests (~ mm) were used to measure the elastic properties of the materials. For measuring the elastic properties of an individual cellulose microfiber in a synthetic composition of pure bacterial cellulose [7, 8], this value must be extrapolated. Similarly, Raman spectroscopy has been used to identify crystalline changes in bulk samples of isolated bacterial cellulose. In the Raman spectroscopy method, an estimate

for the Young's modulus of an individual cellulose microfiber is calculated through monitoring the amount of changes in the C-O stretching bond on the cellulose polymer backbone [9]. One of the disadvantages of this technique is the assumption of complete crystallinity of the cellulose, which is not independently confirmed. Another disadvantage is the unknown effect of the uniformity of orientation of the microfibers under measurement, which would result in bias in the magnitude of the crystallographic changes.

Recently, many research studies have attempted to find a three point bending test utilizing an AFM cantilever to measure force and deflection on a single cellulose microfiber spanning a nano-manufactured gap [10-12]. Aside from the common questions regarding the uniformity and crystallinity of the cellulose microfibril, this research raises a set of issues more specific to the scale of the experiment. For instance, this study used nanometer scale electrostatic forces as a dominant force in mechanical experiments. Such interactions challenge the normal set of assumptions and interpretations of such experiments.

Studying nanoscale phenomena to achieve a quantitative measurement using an AFM, concluded more direct and higher resolution method of measuring the elastic modulus. This method used Force-Distance (F-D) curves of an AFM in tapping mode and advanced theories that correlate the F-D curve to a relative measure of elastic modulus [13], and further relate to an absolute measure of elastic modulus as demonstrated in hydrated states [4]. The advantage of this technique is that it extends beyond measuring the elastic properties of individual components of the cell wall matrix. These quantitative measurements are continuously made as the AFM probe scans the sample, resulting in enough values for mechanical strength to comprise the contrast data for the individual pixels of the high resolution image. Consequently, a geometrically (topographically) uniform cellulose microfibril, with variations in crystallinity (amorphous regions) for example, would become evident in a mechanical modulus contrast derived image.

In this research, the quantitative mechanical measurement capability of the AFM was used to measure the relative mechanical properties of both onion and celery epidermis on the nanometer scale. By using the Peak Force QNM AFM technique, quantitative images of the onion and celery epidermis microfibrils were obtained with the mechanical properties providing the contrast for the image. A series of high resolution images of onion microfibrils were taken to study the stress relaxation of individual fibrils after applying strain to the epidermis for the first time.

This success has been repeated to investigate the individual fibrils' nanoscale movement, or possible reorientation, after mechanical elongation to the onion epidermis.

In addition, sequences of images were used to investigate the ability of pectin to tether microfibrils or, perhaps, to support them as a filler. Other researchers have suggested that the pectin could inhibit the unfolding of Xyloglucan chains [1], which was studied by adding Pectate lyase to both onion, and celery epidermis wall to cut the polygalacturonic acid chains.

Materials and Methods:

One of the challenges for extending the AFM technique was using an intact cell wall which is considered an open structure for the imaging of plant cell wall microfibrils under the AFM tip. In order to achieve this goal, the onion and celery epidermal peels' primary cell walls were used. The benefit of using onion and celery epidermis was that it required a simple sample preparation. The onion and celery epidermis were bathed for 1 hour in 1x PBS (Phosphate Buffered Saline) solution with 0.05% Tween 20. The epidermal peels were then rinsed in 1x PBS. Due to a significant amount of Pectin in the celery cell wall, the epidermal tissues required bathing for more an additional 4 to 6 hours.

For the enzymatic interaction, Pectate Lyase (Pel10Acm) was used and diluted into 25, 50, and 100 µg/ml with 20mM HEPES buffer, pH 7.0. The enzyme was kept at four degrees Celsius before use, and added about 20 µl enzyme to cover the whole tissue for about 40 min.

Peak Force QNM Technique:

Peak Force Quantitative Nano-mechanical Mapping (Peak Force QNM)® is a new AFM technique [14] which uses tapping technology to record very fast force response curves at every pixel in the image while using the peak tip-sample interaction force as the feedback mechanism. Peak Force QNM® is able to simultaneously obtain quantitative modulus, adhesion, dissipation, and deformation data while imaging topography at high resolution. Also, by maintaining control of direct force to a very low level (pN), the scanning can limit indentation depths to deliver non-destructive and high-resolution imaging. Furthermore, material properties can be characterized over a wide range to address samples in many different research areas [14]. As explained in the previous chapter, several mechanical property parameters can be obtained from each force curve. The adhesion force represents the minimum force point when the tip starts to pull away from the sample. Energy dissipation is calculated by the hysteresis area between the approaching and retracting processes. This dissipation includes the work associated with adhesion and viscous or plastic deformations. When the set point of the peak force is set at or close to zero, the energy dissipation is dominated by the work of adhesion. Deformations here represent the total penetration depth, including elastic and plastic deformations [14].

Mathematical models used in PeakForce QNM:

Elastic Modulus:

To measure the elastic modulus of the system, DMT theory [15] has been used. The relation between adhesion force is as follows:

$$F - F_{adh} = \frac{4}{3} E^* \sqrt{R(d - d_0)^3} \quad \text{Equation 1}$$

Where, $F - F_{adh}$ is the force on the cantilever relative to the adhesion force, R is the tip radius, and, $d - d_0$ is the sample deformation, and E^* is the reduced modulus. The relation between the Young's Modulus of the sample, E_s , and E^* is as follows:

$$E^* = \left[\frac{1 - \nu_s^2}{E_s} + \frac{1 - \nu_{tip}^2}{E_{tip}} \right]^{-1} \quad \text{Equation 2}$$

Where ν_s and ν_{tip} are Poisson's ratio of the sample and tip respectively [14].

Adhesion:

The next mechanical property that can be acquired by AFM is the adhesion force. The source of adhesion force can be any attractive force between the tip and the sample. In most cases the adhesion

force is the combination of electrostatic force F_e , Van der Waals

force F_{vdW} , the capillary, or meniscus, force F_{cap} , and the forces related to chemical bonds or acid-base interactions F_{chem} :

$$F_{adh} = F_{el} + F_{vdw} + F_{cap} + F_{chem} \quad \text{Equation 3}$$

In some cases, when the tip and the sample are not net charged or when the surfaces are saturated with chemical bonds, the electrostatic or chemical forces can be neglected. But Van der Waals force always contributes and, in some cases, is attractive. At ambient conditions, a water neck forms between the AFM tip and substrate by way of capillary condensation and adsorption of thin water films at the surfaces. This attractive interaction depends on the relative humidity and hydrophilicity of the tip and the sample [16]. This so-called meniscus, or capillary, force is caused by the pressure difference between the liquid and the surrounding vapor phase which is given by the Young-Laplace equation:

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \quad \text{Equation 4}$$

Where R_1 and R_2 are the two principal radii of curvature for the water meniscus. So, the capillary force between water on the plate and a sphere, with radius R and contact angles θ_1 and θ_2 , have been measured as follows [17]:

$$F_{cap} = 2\pi R \gamma (\cos\theta_1 + \cos\theta_2) \quad \text{Equation 5}$$

The area below the zero force reference (the horizontal line in the force curve) and above the withdrawing curve is referred to as “the work of adhesion.”

Dissipation:

The dissipation energy can be obtained by the product of force and velocity integrated over the period of the vibration [14]:

$$W = \int \vec{F} \cdot \vec{dz} = \int_0^T \vec{F} \cdot \vec{v} dt \quad \text{Equation 6}$$

Where W represents energy dissipated in a cycle of interaction, F is the interaction force vector, and dz is the displacement vector. For pure elastic deformation there is no hysteresis between the repulsive sections of the loading-unloading curve, resulting in very low dissipation. In this case, the work of adhesion becomes the dominant contributor to energy dissipation. Energy dissipation is presented in electron volts as the mechanical energy lost per tapping cycle [14].

Deformation:

The deformation corresponds to total penetration depth of the tip into the sample, including both elastic and plastic deformations. With the knowledge of the tip shape, the deformation can be converted to indentation hardness [18]. An accurate description

of tip shape can be derived by AFM measurement on a reference sample through morphological dilation [19].

AFM Cantilever Calibration:

Spring Constant Calibration:

During the movement of the cantilever, due to the forces acting on it, the optical lever performs the measurement through reflecting a laser off to the top of cantilever and towards a photo-detector at the cantilever's free end over certain time period. In the most Contact Mode and Tapping Mode applications, the cantilever deflection is small, which indicates a linear behavior of the cantilever free end shown by Hooke's law as:

$$F = -k \times h \quad \text{Equation 1}$$

where: F is the force on the tip (N), h is the vertical displacement of the cantilever (m), and k is the spring constant, which is a property of the cantilever and is the same number in air, water, and vacuum (N/m or in nanoscale work picon/pico m).

Knowing k , the measured cantilever deflections can be converted to an inferred tip/sample force, thus, the initial step for an accurate force determination is to find the k of the cantilever. There are several techniques to measure a cantilever spring constant including: thermal tuning (most preferred method due to less time consuming and accuracy), geometry, comparison, hydrodynamic model.

Measuring Thermal Noise:

While the cantilever is away from the sample, the measurement data consists of the time interval of the cantilever deflection signal in contact mode at the thermal equilibrium. During the sampling, the cantilever is impacted by the Brownian motion of surrounding molecules. The power associated with resonance is obtained from integrating the area under the resonant peak curve. The dynamics of the cantilever can be expressed as an oscillator by the systems' total energy, which the value for both potential and kinetic energy is defined as:

$$\frac{1}{2} k_B T \quad \text{Equation 8}$$

where T is the temperature (kelvins) and k_B is Boltzmann's constant (equal to 1.3805×10^{-23} joules/Kelvin).

The potential energy can also be defined as the following:

$$< \frac{1}{2} m \omega_0^2 z^2 > = \frac{1}{2} k_B T$$

where, $\omega_0 = \sqrt{\frac{k}{m}}$ (resonant angular frequency), m is the effective mass, and z is the displacement.

The cantilever spring constant k , is measured by simplifying the term $k = \frac{(k_B T)}{z^2} = \frac{(k_B T)}{P}$ placement as:

By integration of the area under the resonance in Power Spectral Density (PSD), and excluding the noise floor and shoulders to either side of the resonance peak, the power can be measured.

When measuring the cantilever spring constant by the thermal method, the accuracy highly depends on the isolation of nonthermal noise and the accuracy of the PSD and its magnitude. The typical nanoscope V controller sample deflection produces a signal every 16.5 seconds, which corresponds to the 64 kHz sampling rate. Accordingly, cantilever resonances with frequencies above 32 KHz can produce distorted to Power Spectral Density.

The thermal tuning method is mostly applicable to soft cantilever, mainly in fluids where cantilever resonance frequencies are significantly lower in air. The cantilevers can generate a smaller signal to analyze due to the magnitude of the PSD is proportional to the mean cantilever displacement.

Calibrating a Cantilever:

Measurement of Deflection Sensitivity in Force Mode:

Every time the laser beam path changes, the deflection sensitivity must be recalculated due to laser alignment, photodetector adjustment and probe change. The deflection sensitivity calibration is done on the hard and stiff surface.

Below are the procedures for deflection sensitivity measurement:

Engage on the hard surface, like silicon or glass, then switch to ramp mode. Obtain a force diagram, which represent the

interaction with the hard surface, then display deflection error vs. Z sensor drag the ramp mode, or force mode, in from the left and/or right edge and displace as far as possible while they are still in the contact region of the force diagram. Figure 1 shows the tip deflection error vs. Z sensor on a glass slide in water. The update sensitivity was calculated using software and repeated for five times to calculate the average number for the deflection sensitivity.

The deflection sensitivity is measured based on the assumption of that tip and the test sample do not deform. During the process of measuring the deflection sensitivity, the static force is applied to the end of the cantilever. However, during the thermal energy measurement, the cantilever is oscillating at resonance at only one end instead of two.

Every time the laser beam path changes, the deflection sensitivity must be recalculated due to change in laser alignment, photodetector adjustment, and probe change. The deflection sensitivity calibration is done on a hard and stiff surface.

Below are the procedures for deflection sensitivity measurement:

Engage the hard surface, like silicon or glass, then switch to ramp mode. Obtain a force diagram which represents the interaction with the hard surface and then display deflection error vs. Z sensor drag during the ramp mode, or force mode, in from the left and/or right edge and displaced as far as possible, while they are still in contact region of the force diagram.

Figure 1: Deflection sensitivity determination using force mode in liquid (water).

Measurement of Cantilever Spring Constant by Thermal Tune:

The following is the procedures of determining cantilever constant:

To choose the least square fit the data click on LORENTZIAN (AIR) or SIMPLE HARMONIC OSCILLATOR (FLUID).

The following equation (Simple Harmonic Oscillator is used to fit the filtered data

$$A(v) = A_0 + A_{DC} \cdot \frac{v_0^2}{\sqrt{(v_0^2 - v^2)^2 + \frac{v_0^2 v^2}{Q^2}}}$$

where: $A(v)$ is the amplitude as a function of frequency (v), A_0 is the base line amplitude, A_{DC} is the amplitude at DC with zero frequency, v_0 is the center frequency of the resonant peak, and Q is the quality factor.

Figure 2: The thermal tune panel

Normally, the markers are located where the spectrum rises from the noise floor. The curve fit is not sensitive to the minimal power contributed from these frequencies, which make the precise placement unnecessary. The process of the test was explained in this section. The first step was to choose the best fit of the data. As shown in Figure 2, the curve fit along with the acquired data displayed (in red). In order to make sure the data is consistent with the best curve fit at the thermal peak, the marker position was adjusted, then the cantilever temperature and calculated spring constant (K) were entered.

New, sharp ScanAsyst Fluid+ probes were used in this research which had the combination of sharpness of silicon tip with low spring constant and high sensitivity of silicon Nitride cantilever necessary for this application. This test was conducted for an unprecedented level of high resolution and force control on samples in fluid. The tip height is about 2.5 - 8.0 μm , with the radius of about 2-3 nm. The spring constant is between 0.35-1 N/m.

Results and discussion:

As previously discussed, in order to accurately measure the relative elastic modulus of individual components of a cell wall on the nanoscale, the QNM package of the ICON AFM was used in

this study. This calibrated technique accounts for water, allowing for intact cells to be examined. The AFM tip and cantilever were scanned across a section of the sample while recording non-destructive force indentations at each pixel. As a result of the force distance curves, the elastic modulus at each point was calculated. The software package accounted for the contributions of water, isolating the mechanical information of the specimen. The data obtained can be used in form of chromatic images for which the contrast of the image can represent the range of elastic modulus within the image. Under tension, sequential nano-elastography measurements showed the evolution of elastic modulus as stress were distributed throughout the cell wall components.

These experiments provided significant, new information to the field and, for the first time in research analysis, quantified the distribution of stress resulting from tensile loading throughout cell wall components as evidenced by change in elastic compliance. When the tensile load was applied, each component's elastic modulus should have been altered proportionally to its contribution to load bearing. Such information revealed how the mechanical interactions between cellulose microfibrils and other cell wall matrix materials attribute strength during loading.

Figure 3: Horizontal tensile test stage suitable for AFM

Mechanical Test and Nano level effect on individual Fibrils:

One of the main challenges in this research was the suitability of the tensile tester stage in the AFM method. The configuration of the AFM required a very thin (less than 2 inch), horizontal tensile stage allowing for easy, quantitative adjustment of the applied tensile force. As shown Figure 3, the size and configuration of the stage was addressed. The remaining efforts were focused on applying a quantitative measure of applied tensile force. That was a technically challenging area based on the inequality in elastic compliance that arises between the need for a stationary (solid) sample holder and the relatively weak (soft) sample.

As showed in Figure 3, the tensile test stage included two horizontal plexiglass sheets, one of which was fixed and the other connected to a fine-gauged micrometer. The knob is graduated in μm , where each grade of fine-adjustment knob was about $2.5 \mu\text{m}$, and rotation of coarse-adjustment knob was $12.5 \mu\text{m}$. The knob provides a maximum separation distance of 5 mm between the plexiglass sheets, which translates to the greatest possible displacement applied to the sample. However, without the micrometer, it was possible to add more separation distance between glasses. The sample was mounted on glass slides and clamped on both sides. The side wall of the epidermal tissue was blocked by regular, adhesive tape to make a small channel for holding water during scanning. As stated earlier, Peak Force QNM method were used in this study which was represented a quantitative nano-mechanical method using ultra low force (picoNewton) for tapping the cantilever during the scanning process. This method allowed nondestructive indentation for scanning of a sensitive, intact sample in water, such as plant cell wall microfibrils. The first step was to take an image before the applied load, and then carefully cut the sides of the epidermis with sharp blades to apply load, and used tape to block the sides.

The first set of experiments was conducted in various cells at various positions of the sample. Based on observation, there was drastic change in microfibrils' orientation before and after stretching. In order to obtain a consistent and reliable result, the sequence of images must be taken in the same cell. At this stage, locating the exact position was almost impossible, even with marking the position, however at least the results can be visible, if not the same area. Prior experiments conducted, for this study, evidenced the orientation of the microfibrils in the same cell is consistent, regardless of position in the cell.

For the first time in the area of AFM research, the relaxation phenomena of individual microfibrils were imaged at identical positions to find how each individual component changed their angle, or orientation. Figure 4 shows the high resolution images of onion microfibrils at the same position. The top image shows the onion cell epidermis, including cell profiles, with the AFM tip on the exact spot of interest, shown as a red cross, which represents the exact position of scanning. The right side image is the topography, which is the height information of the sample. The left side image shows the Peak Force Error which is called the error signal of deflection image and represents the subtraction of the set point force from detector signal (actual deflection). In general, the deflection image shows the edges of features in the topography image which, in soft materials, subsurface structures were clearer than the topography image. If the error signal was too large (which was not in our case) it is interpreted as the tip not tracking the sample surface very well.

If the tip is calibrated the unit for PeakForce error signal images show variation of contrast based on force unit. As seen in the top right image, the highest deflection error force is about 1.3 nN. As explained in tip calibration section, Scan Asyst Fluid + tips were used in this study. Several force distance curves were gathered at different positions on a smooth, glass slide in water, and the deflection sensitivity was recorded for each. The calculated average

Figure 4: Topography (left) and Peak error (right) images of onion microfibrils of 24mm Onion Epidermis. A) After 6mm stretching with Tensile Test Stage T1=0. B) 24mm Onion Epidermis after 6mm stretching with Tensile Test Stage T2=15

deflection sensitivity was approximately 17.814nm/v. Based on this parameter, a spring constant of 0.859 N/m was calculated with AFM software for this specific tip.

For the epidermal tissue with the length of 24 mm and width of 2.5 mm, a displacement of 6 mm was applied. This displacement was based on a Dynamic Mechanical Analysis result applying of 2N of force (which will be discussed in following chapter). The average modulus, average adhesion force, and the average dissipation energy in this image were 1.74 MPa, 1.40 nN, and 1.7 keV, respectively, which was calculated by Peak Force QNM data processing software. As showed in Figure 4, the first set of images corresponded to the time when the microfibrils have been subjected to a displacement of 6 mm. The second set of images show how each individual component reacts to the applied load after 15 minutes. For example, the microfibril labeled number 1 changed its angle from 72° to 101° .

For the next step, the investigation of the effect of mechanical load on individual microfibrils before and after applying load at the same cell and position was attempted. To achieve this goal, an 18 mm onion epidermis tissue was fixed by clamps and taped on its side walls. Note that the inside tissue was free and intact during scanning process. Figure 5 shows high resolution of onion

microfibrils image before applying load. The top image shows the onion cell epidermis including cell profiles, AFM tip on the exact spot of interest with a red cross that shows the exact position of the scanning. The bottom right is the topography, and the bottom left image is Peak Force Error or deflection. The Peak force setpoint was selected for this set of experiments which remained constant (1.101 nN) during the experiments. Scan Asyst Fluid and tips were used and calibrated before the experiments. Several force distance curves were taken at different positions on the smooth, glass slide in water and deflection sensitivities were recorded. The calculated average deflection sensitivity was 37.66 nm/v and, based on this parameter, the spring constant of 0.9184 N/m was calculated using AFM software for this specific tip. The average modulus, the average adhesion force, and the average dissipation energy were 1.15 MPa, 500 pN, and 1.2 keV, respectively, which was calculated by Peak Force QNM data processing software.

As shown in Figures 6, 7, and 8, the onion epidermis are displaced for 2mm in the +X direction corresponds to a force range of 0.3-0.5 N, was measured using the Experimental Dynamic Mechanical analysis (DMA) method.

As already explained, all above images were taken in the same cell profile and the same spot. As seen in the first set of images (Figure

Figure 5: 18mm Onion Epidermis Before stretch, average modulus is 1.15 Mpa, $T_0=0$

Figure 6: A) 18mm Onion Epidermis Before stretch Ave Modulus: 1.2 Mpa , $T_1= 6\text{min}$. B) 18mm Onion Epidermis After 2mm stretch. average modulus: 1.22 Mpa, $T_2=11\text{min}$

Figure 7: A) 18mm Onion Epidermis After 2mm stretch. Ave Modulus: 1.2 Mpa , T3 = 18 min. B) 18mm Onion Epidermis After 2mm stretch. Average modulus: 1.2 Mpa , T4= 25 min

Figure 8: A) 18mm Onion Epidermis After 2mm stretch. Ave Modulus: 1.2 Mpa T5=33 min, B) 18mm Onion Epidermis After 2mm stretch. Average modulus: 1.2 Mpa, T6= 40 min

6), the fibrils after six minutes showed evidence of the applied stress and rearranged, and then returned to the original position with a looser configuration. All of the images together show how individual microfibrils relax and become loose after applied force. The average modulus decreased to 1.2 MPa during stress relaxation. These systematic investigations showed the changes in angles and directions of microfibrils during nondestructive scanning of the intact plant cell wall in water.

Enzymatic treatment and Nano level effect on individual Fibrils:

Sequences of images were used to investigate the ability of pectin to tether microfibrils or, perhaps, support them as filler. A study has

suggested that pectin could inhibit the unfolding of Xyloglucan chains [1]. Abasolo et al. studied the role of pectin by adding Pectate lyase to the onion epidermis wall to cut the polygalacturonic acid chains. As higher concentrations of Pectate lyase were reached, images showed the microfibrils eventually collapsing with 3 μL of Pectate Lyase in 300 μL of buffer (Figure 9). Unfortunately, quantitative nanomechanical measurements were not made in this study, therefore, the units of images were not calibrated and not converted to force units.

Conclusion and Future work:

The following conclusions were summarized the outcome of this research.

Figure 9: From left to right:

- (A) Microfibrils, before adding pectate lyase to the wall to cut the polygalacturonic acid chains. (B) After treatment—medium (1 μL of the original pectate lyase in 1000 μL of buffer). (C) After treatment—strong (3 μL of the original pectate lyase in 300 μL of buffer): pectate lyase causes microfibrils to collapse completely. Note that some of the horizontal lines, especially in figure (C) are due to vibration noise.

1) For the first time in the area of AFM research, the relaxation phenomena of individual microfibrils were imaged and showed how each individual component changed its angle or orientation as a result of applied stress.

2) The AFM images showed the orientation of the microfibrils in the same cell were consistent, regardless of position of the cell.

3) For an onion epidermal tissue size of 24 mm x 2.5 mm, elastic modulus, adhesion force, and the dissipation energy of microfibrils were measured as 1.74 MPa, 1.40 nN, and 1.7 keV, respectively, using AFM.

4) For an onion epidermal tissue size of 18 mm x 2.5 mm, the average modulus, average adhesion force, and average dissipation energy were calculated as 1.15 MPa, 500 pN, and 1.2 keV, respectively, using AFM.

5) The enzymatic treatment showed the microfibrils eventually collapsing with 3 μL of Pectate Lyase in 300 μL of buffer.

Future Work:

Using AFM force measurements and functionalized tips on plant cell walls:

For future step functionalized AFM tips could be used to explore

the roles of matrix polymers in the primary cell wall. The binding energy between a single molecule of XyG and a cellulose microfibril can be measured. In specific, synthetic composite materials can be used as cell wall analogues to provide baseline comparisons to natural specimens of similar composition (i.e., varying degrees of XyG and Pectin with cellulose microfibrils). Mutant and enzyme treated samples can be used, for instance, to provide accompanying imagery of reorientation of microfibrils in natural plant cell wall samples with normal and deficient amounts of XyG or other matrix polymers. This may provide new insight as to how the mechanical strength of the cell wall can be maintained in XyG deficient samples (perhaps, the orientation of micro fibrils begins and/or evolves differently).

Another set of challenges will be extending the above technique to other intact cell wall samples of interest. The anticipated challenge is the preparation of samples to be imaged. For instance, to date the technique has been used on onion primary cell walls, for which a simple sample preparation can reveal the primary cell wall structure. However, other potential samples of interest may require more complicated treatments in order to expose the internal cell wall structure, for example cotton fibers.

Conflict of Interest disclosure:

The authors declare that there is no conflict of interest regarding the publication of this paper.

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