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UNDERSTANDING THE MECHANICAL PROPERTIES OF MICROALGAE USING ATOMIC FORCE MICROSCOPY

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Abstract

From consumer productions to energy production, algae is used in many industrial processes. Understanding the mechanical behavior of algae is important to optimize these processes. To obtain a better understanding of algae cell response, we mechanically characterized single, dried *Scenedesmus dimorphus* cells. To accomplish this, we used atomic force microscopy (AFM) to image *S. dimorphus* cells, which enabled us to map the AFM measurements to a location on the individual cells. We were then able to perform force measurements on the AFM to determine the Young's modulus of *S. dimorphus*. These findings enable a more detailed understanding of the mechanical properties of a single *S. dimorphus* cell, which may be useful in many applications.

Introduction

Optimization of algae processing is desired because of algae's widespread usage in many products, such as consumer goods, biofuel, and animal feed. Algae is attractive in comparison to other plants because (a) it does not compete with American food supply; (b) it does not compete for water or land resources because it can grow in areas that are considered unusable for other crops; (c) it has a higher productivity than any of the other crops; and (d) it can be used in the sanitation industry to capture carbon dioxide and treat waste water [1].

Many of the algae-based products require processing to separate the algae's internal components from the algae cell wall. The process to obtain these components is very complex. Besides selecting the correct strain of algae and providing it with the necessary conditions and nutrients to grow, the algae also has to be harvested and dewatered. Then, the useful components of the algae can be separated and processed for specific uses. All steps within this process must be optimized for algae to be efficiently utilized in industrial processes [2]. It is believed that obtaining a better understanding of the mechanical behavior of algae can optimize such processes. In this work, Atomic Force Microscopy (AFM) is used to obtain the mechanical properties of algae to assist with the optimization of algae processing.

Methods

The microalgae strain selected was *Scenedesmus dimorphus* from the University of Texas in Austin (UTEX) collection. *S. dimorphus* was selected based on its ease of growth in comparison to other algae strains. A protocol was developed to grow healthy algae that would prevent the algae from clumping, one of *S. dimorphus* complications. Hindering the growth of the algae, clumping occurs because of the dense nature of the microorganism.

Obtaining the mechanical properties of algae requires equipment with high resolution on the micro-scale level and the ability to perform force measurements on the nano-scale. To do so, a Park Systems AFM was used. The main advantage of using an AFM over other technologies is that the AFM allows one to view living cells in real-time at high resolution while taking various measurements [3]. With the Park Systems AFM, two cantilever tips were used. The first was an AppNano aluminum/silicon pyramidal ShoconA cantilever/tip

whose spring constant is 0.16 N/m. The second cantilever used was MikroMasch NSC36, which has three separate tips. The NSC36B tip was used, which has a spring constant of 1.75 N/m. Of the various scanning modes, contact mode was utilized which allows the cantilever to glide across the sample recording changes in cantilever deflection. After the image is acquired, force measurements were taken with the acquired image as a reference. The elastic modulus was calculated using the XEI post-processing software that accompanies the Park Systems AFM. The Young's modulus is a function of the tip geometry, indentation depth, and loading force. Based on the types of cantilever tips utilized, the ShakonA and NSC36B, both the pyramidal, and conical shapes are used, respectively, within the XEI program to determine the Young's modulus. The results of these actions are described in the following section.

Results and Discussion

Utilizing the AFM, the images in Fig. 1 were produced based on the topography (top) and error signal data (bottom). Within the error signal images, the lighter areas on the cell represent a higher topography which is believed to be caused by the cell matter within the membrane that have not completely been broken down. The error signal images provide us with a better idea of what the cells' outer membranes might be. One can clearly see the outline of the cells in both the topography and error signal images. The left and right images are 15 x 15 um and the middle image is 25 x 25 um. No further image processing was done to these images. These images show that we can utilize the Park Systems AFM for further data.

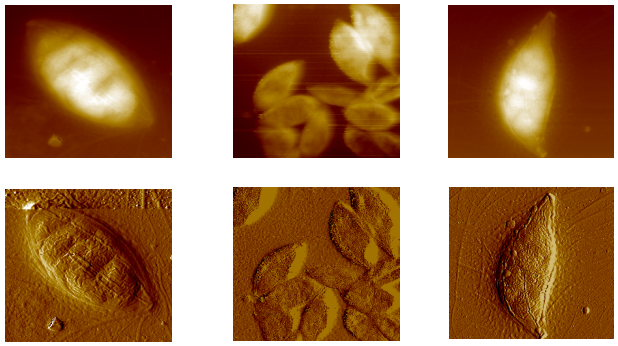

Fig 1. Park AFM topography (top) and error signal (bottom) images of algae strain *Scenedesmus dimorphus*.

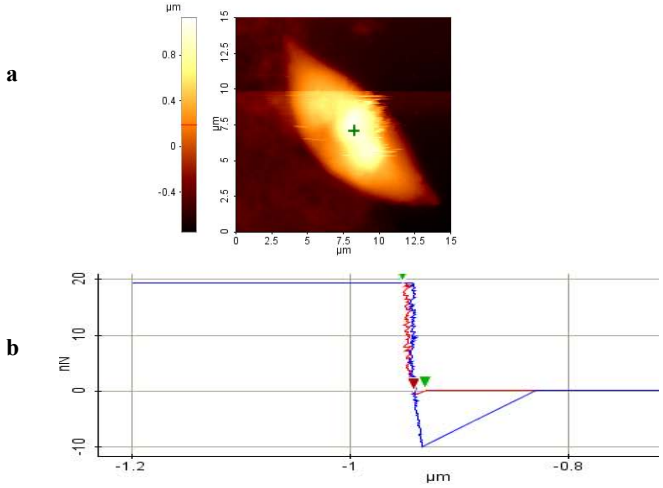

Fig 2. ShakonA AFM Data a) Topography reference image and its respective b) Force/Distance (F/D) Curve for a dried Scenedesmus dimorphus cell.

To determine the Young's modulus, both AFM tips were used and the resulting force curves were generated using the topography as a reference. In Fig. 2a, the small green cross in the middle of the cell is where the measurement was taken on what was thought to be the cell's nucleus. Below the cell topography reference image is the Force/Distance (F/D) curve for the cantilever tip, Figure 2b.

After conducting force measurements on various cells, the resulting average Young's modulus as shown in below in Table 1. With these Young's modulus values, *S. dimorphus* in its dried state has a similar elasticity as a protein crystal [4].

Table 1 AFM Tip Type and its Respective Young's Modulus Range for *S. dimorphus*

Tip Type	Range (MPa)
ShakonA	87.73 – 908.82
NSC36B	14.62 – 358.07
TOTAL	14.62 – 908.92

These are acceptable values for dried biological samples based on the literature [4], [5], [6], as well as the dried state of the algae cells. The broad range may be an effect of measuring various algae cells in multiple areas over a large span of time as the cells dry out more over time.

Conclusions:

The mechanical properties of *Scendesmus dimorphus* were examined. *S. dimorphus* was grown and characterized using a Park Systems AFM. The cell topography was mapped and a force measurement approach to determine the Young's modulus was used from the topography reference image. For *S. dimorphus*, the Young's modulus is between 14.62 and 908.92 MPa, which is similar to the elasticity of protein crystals. We believe that this work will have implications in a diversity of fields including cell mechanics, biomaterials, and membrane biology.

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