A new tool to study plant cell biomechanics

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We know that within every living plant there are millions of cells working together in a wonderfully complex harmony. But what we don't know is, within each of these cells, what exactly is going on. Scientists have known for some time that cell biomechanics plays a significant role in plant development, but have lacked the tools to advance our knowledge. Researchers from the University of Vermont have developed a method that promises to shed light on single cell biomechanics—by capturing individual cells in microscopic gel beads.

The beads are no wider than a strand of hair, a mere sixty micrometers, but they allow researchers to manipulate the external environment of a single cell and study how the cell responds. They are made using agarose, a material that maintains a fluid state at warm temperatures and hardens as it cools.

"We're enthusiastic about this method being a useful tool for researchers interested in mechanical signaling at the cellular level," says Matthew S. Grasso, a graduate student working in Dr. Philip Lintilhac's laboratory in the Plant Biology Department. The new microbead protocol is available in a recent issue of *Applications in Plant Sciences*.

The first step in creating the microbeads is to prepare the protoplasts from plant tissue. For this study, Grasso used a tobacco cell line. Within a developed piece of plant tissue the cells would look much like a grid, with the grid lines being the cell walls. To get a close look at the mechanics within each cell, Grasso first strips the cells of their cell walls, creating a suspension of free-floating, membrane-enclosed plant

protoplasts.

"In the plant body, cells are subject to the mechanical forces generated by their own cell walls, as well as by the cells that surround them. Using individual protoplasts helps control these variables, making it easier to interpret how cells respond to a given mechanical stimulus," says Grasso.

Cells are constantly communicating with each other via signals that pass through cell walls. Recent studies have uncovered a bit about these chemical signals, but the micromechanics occurring within each cell, and the complex relationship between a cell and its cell wall, are yet to be understood fully.

To make the beads, Grasso assembled a multi-column microfluidic droplet system. In the system, warm liquid agarose from one column meets the plant cell suspension from another column. After the two fluids merge into one, microdroplets are generated, and gently fall into a pool of cooled mineral oil where they solidify (see Video). The system can generate around 130 beads per second, with 25% successfully carrying a protoplast.

"Unraveling the nuances of the droplet microfluidics system took some time. For a while, it was confusing as to what the different variables were, making it difficult to control them and achieve consistency," says Grasso.

Dr. Rachael Oldinski in the Mechanical Engineering Department at the University of Vermont provided assistance and specialized laboratory equipment for the development of the bead protocol.

"Dr. Oldinski has helped explain different aspects of forming hydrogel microbeads, as well as some variables of the droplet system that should be considered. Her knowledge of stimuli-responsive hydrogels may help

us manipulate the micromechanics of individual cells in a unique and highly controllable way," says Grasso.

Within twenty-four hours of bead formation, the membrane-enclosed plant protoplasts regenerate their cell walls. From there, the cells expand and multiply, bursting the beads open. Observing this regenerative ability of the cells under highly controlled conditions could reveal unprecedented knowledge of cellular function.

More information: Matthew S. Grasso et al, Microbead Encapsulation of Living Plant Protoplasts: A New Tool for the Handling of Single Plant Cells, *Applications in Plant Sciences* (2016). DOI: 10.3732/apps.1500140

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