Banff International Research Station

for Mathematical Innovation and Discovery

Multiscale Modeling of Cell Wall Mechanics and Growth in Walled Cells
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REPORT

The workshop was the first of its kind since it represented a venue that brought together people from very different backgrounds that typically know each other's publications but do not meet at scientific conferences as the respective fields are extremely diverse.

- Different scientific disciplines: mathematics, physics, engineering, biology
- Focus on biological organisms across kingdoms: bacteria, fungi, plants

Especially for the scientists working on bacteria, who essentially never attend meetings frequented by fungal or plant biologists, this meeting represented an opportunity to interact with a new set of colleagues with synergistic interests and expertise. Similarly, the representatives with mathematical and physical backgrounds rarely attend biological conferences and vice-versa. Collaborations typically take place at the individual level between single labs, whereas cross-pollination between highly different projects is rare. The most important comment by participants was therefore the appreciation for this opportunity to finally meet people that approach the topic 'cell wall' with similar or complementary strategies, but in different biological systems. By consequence, many of the workshop participants met for the first time at BIRS, and rapidly identified common interests and opportunities for interaction and collaboration. The following examples illustrate the variety of contributions and different structural scales at which the cell wall was discussed at the workshop:

Molecular structure of the cell wall

The cell wall is an envelope that surrounds the cells of most organisms except for those of animals. The molecular structure of cell walls differs significantly between different biological kingdoms. In bacteria the wall is built from a single type of proteoglycan, whereas in plants and fungi the wall is composed of multiple polysaccharidic polymers with a great variety of structure and biochemistry. Despite these differences the fundamental laws governing shape formation in cells is similar since independent of cell type this process relies on yielding of the wall under the force provided by the internal turgor pressure. The

following presenters gave detailed overviews of the properties of the different types of cell wall:

Olivier Ali (INRA Lyon) reported on force-driven polymerization of a hydrogel as a fundamental mechanism behind cell wall matrix expansion. While many molecular players involved in growth control have been identified in the past decades, it is often unknown how they mechanistically act to induce specific shape changes during development. Plant morphogenesis results from the turgor-induced yielding of the extracellular, load-bearing cell wall. Its mechano-chemical equilibrium appears as a fundamental link between molecular growth regulation and the effective shape evolution of the tissue. For animal cells, it has been well established that the dynamics of load-bearing structures, such as the cortical actin network, relies on their force-driven polymerization. The authors tried to determine if turgor-induced morphogenesis in plants originates from the same kind of force-driven polymerization of cell wall components.

Molecular interactions are modeled by **Michael Crowley** (National Bioenergy Center). The authors have studied the specific interactions in cellulose in its various forms and found evidence of how many of the structures find their stability and how conversions between structures occur. Further they are looking in detail at the molecular interactions and dynamical properties between cellulose and many of the other plant cell wall components. Using several approaches to atomic and molecular modeling, they found many interesting properties of cell wall components that are consequences of different kinds of stress on the polymers and polymer complexes.

Experimental tools for quantitative measurement of cell wall architecture and mechanics

Modeling the cell wall behavior requires detailed information on the structure, architecture and mechanical behavior of the material composing the wall. Novel experimental methods have recently contributed to enhancing the researchers' ability to visualize the cell wall and to measure its mechanical properties.

Bara Altartouri (University of Montreal) presented experimental tools used to visualize cell wall components. Insight into cellulose architecture can be obtained by field emission scanning electron microscopy and atomic force microscopy. While these techniques provide exquisite detail in terms of spatial resolution and mechanical properties of the cell wall, they only have limitated capability to follow the dynamic changes over time. The authors have begun exploring the feasibility of using optical microscopy to examine the orientation and anisotropy of cellulose at high spatial resolution.

Mohammad Shafayet Zamil (University of Montreal) used micromechanical testing combined with finite element modeling to assess the mechanical behavior of the substance responsible for the adherence between the walls of neighboring cells. The authors have taken an onion outer epidermal cell wall profile as representative of a multicellular material system

and developed a framework of multiscale finite element method (FEM) computational model to scale-up mechanical properties from subcellular to tissue scale. A 3D repetitive volume element (RVE) was developed in a way so that if arrayed in X and Y directions will produce an idealized onion epidermal wall tissue patch. In a RVE, wall fragments from four adjacent cells are attached by a distinct continuous layer also known as middle lamella (ML), and therefore the RVE contains both subcellular and extracellular scale parameters. By changing the material properties of the ML and the anisotropy of cell shape (width to length or WL ratio of a cell), their respective contributions were investigated from subcellular to RVE scale. The RVE mechanical responses were one more time scaled up to a 7.2X2.7 mm tissue scale. It was observed that from subcellular to tissue scale the ML mechanical properties have little to no impact on overall mechanical responses. The shape factor was found to have contribution on mechanical responses both from subcellular to RVE and from RVE to tissue scales. Form subcellular to RVE scale, the anisotropy in modulus values decreases as the WL ratio approaches 1, i.e., become isotropic in shape. However, from RVE to tissue scale the shape factor does not change the anisotropy in modulus value. An interesting finding was that the anisotropy in modulus value trend was reversed from subcellular-RVE to RVE-tissue scale. The tissue scale modulus values and their anisotropy were validated by experimental results at similar length scale, which were in good agreement with the computational estimations.

Mechanics of cell growth

Cellular growth in walled cells is governed by completely different physical principles compared to growth and movement in animal cells. Modeling of the growth process necessitates a mechanical representation of the cell wall and its deformation under the effect of load application by turgor.

Adelin Barbacci (INRA Nantes) reported on his model of growth that integrates mechanical properties and biosynthesis of the wall. Expansive growth of plant cell is conditioned by the cell wall ability to extend irreversibly. Growth is then possible thanks to two processes. The first process consists in the development of a tensile stress in the cell wall caused by turgor pressure and the modulation of its mechanical properties through chemorheological processes. The second process is the biosynthesis and the assemblage to the existing wall of new cell wall elements. Despite its central aspect for expansive growth, evolution of mechanical properties of cell wall remains poorly understood. Recent results emphasizing the complexity of the relations between cell wall polysaccharides and emerging properties justify the development of models. In a previous work, the autho proposed a model, based on irreversible nonequilibrium thermodynamics, considering cells as dissipative structures. In this first proposition, mechanical properties of the cell wall and stress tensor were coarsely described to focus on coupling between temperature, turgor pressure and polysaccharides biosynthesis. The present work aims at modeling kinetics of mechanical properties of the cell wall in function of chemorheological process. The development of the model is based on Gibbs-Duhem relations and kinetic relations acting on generalized non-equilibrium forces relative the reshuffling of the cell wall.

Plant cell wall growth remains a complex problem as documented by **Daniel Cosgrove** (Penn State University). In the context of plant cell growth, wall extensibility refers to the ability of the cell wall to extend irreversibly. Plant cell walls are more extensible at low pH, a phenomenon called 'acid growth' and known to be mediated by expansins. The action of expansins on wall mechanics will be contrasted with that of family-12 endoglucanases such as Cel12A. Mutagenesis of Cel12A gives novel insights into the nature of 'acid growth'. The results suggest a new paradigm for how plant cells regulate the activities of cell wall enzymes and also imply that meso-scale features of cell wall architecture may play a crucial role in such regulation.

Fission yeast (*Schizosaccharomyces pombe*) displays multiple cellular shapes as discussed by **Fred Chang** (Columbia University). How do these cells form rods of a certain shape and size, and how do they grow and divide? Chang has embarked on studying the role of the cell wall and cell mechanics. He finds that after cell division, a mechanical process is responsible for producing the rounded shape of the new end, in which turgor pressure simply pushes out the cell wall of the septum in the absence of cell growth. He is also interested in developing models for how turgor pressure and cell wall insertion produces the rounded shape of cell ends while they are growing. New results suggest that softening of the cell wall by itself is able to initiate cell growth, suggesting the presence of mechano-sensory pathways involved in regulation of cell polarity.

Bacterial cell wall was also discussed by **Simon Foster** (Sheffield University). Bacterial cell wall peptidoglycan is essential for the life of most bacteria. It determines cell shape, and its biosynthesis is the target for many important antibiotics. The fundamental chemical building blocks of peptidoglycan are conserved: repeating disaccharides cross-linked by peptides. However, despite this relatively simple chemistry, how this is manifested into the myriad bacterial shapes and how this single macromolecule remains dynamic permitting cell growth and division has largely remained elusive. The advent of new microscopy approaches is beginning to revolutionize our understanding of the architecture of this polymer and to reveal novel insights into its biosynthesis and hydrolysis. Atomic force microscopy has demonstrated a complex, nanoscale peptidoglycan architecture in diverse species, which meets the challenges of maintaining viability and growth within their environmental niches by exploiting the bioengineering versatility of the polymer. The application of super-resolution fluorescence microscopy, coupled with new chemical probes has begun to reveal how this essential polymer is synthesized during growth and division.

Sun Sean (Johns Hopkins University) described how combination of physical and chemical processes are involved in determining the bacterial cell shape. In standard medium, gram-negative Escherichia coli cells are rod-shaped, and maintain a constant diameter during exponential growth. The authors demonstrate that by applying precisely controlled compressive forces to growing rod-shaped *E. coli*, cells no longer retain their rod-like shapes but grow and divide with a flat pancake-like geometry. The observed deformation is reversible: deformed cells can recover back to rod-like shapes in several generations after

compressive forces are removed. During compression, the cell elongation rate, proliferation rate, DNA replication rate, and protein synthesis are not significantly different from those of the normal rod-shaped cells. They find that during the observed shape transition, peptidoglycan synthesis takes place along the entire envelope of the cell rather than constrained to the lateral cell wall. Quantifying the rate of cell wall growth under compression reveals that the cell wall growth rate depends on the local cell curvature. MreB not only influences the rate of cell wall growth, but also how the growth rate scales with cell geometry. They discuss a mechanochemical model of *E. coli* cell wall growth and morphogenesis, and suggests an active mechanical role for MreB during cell wall growth.

There is a feedback between cell wall and cellular growth, as reported by **Ethan Garner** (Harvard University). Rod shaped bacteria elongate by the action of cell-wall synthetic complexes that move circumferentially around the cell width. These motions that are thought to reflect the insertion of new cell wall material. These synthetic complexes, located on the outside of the cell, are linked to MreB filaments bound to the cytoplasmic membrane surface. Each filament/enzyme complex moves around the rod independently, with adjacent complexes moving in opposing directions. To understand how the independent, disconnected motions of these filament/enzyme complexes are able to orient their motions 90 degrees to the long axis of the bacteria, the authors observed their dynamics as they deformed and reformed bacteria from rods into spheres. To cause this transition, the authors modulate the levels of teichoic acids and PBP2, each titrating the width and the rigidity (straightness) of the rod shaped *Bacillus subtilis*. They find that as they decrease cell rigidity, the cellular width increases, up to a point at which rod shape fails. They find that the motions of MreB are circumferentially organized in rods of all widths, yet become anisotropic and disorganized in spheres. By confining these disorganized cells in chambers near the width of normal cells, they authors see that MreB aligns its motion along this externally imposed axis, indicating that the elongation system can sense the aspect ratio of the cell. This orientation also occurs within confined spheroplasts, and MreB assembled inside liposomes, indicating this orientation is dependent upon filaments alone. The authors can watch how this system reorganizes as they convert spheres back into rods by suddenly increasing the magnesium or teichoic acid levels. In isolated cells, they observe the orientation and shape transitions occur by spheres emitting rods from one point, suggesting a local, not global, reorganization of growth, a result corroborated with FDAA staining. The initially emitted rods are near the normal bacterial width, suggesting that this machinery is tuned to both sense and propagate a given cellular radius. These results suggest that the elongation machinery encodes an intrinsic sensor of cell width, one that creates rod shape by orienting their motion of synthesis along given curvatures. The authors suggest that feedback between curvature, orientation of synthesis, and cell wall stability provides a robust mechanism to initiate and maintain rod shaped growth, independent of the pre-existing shape or cell wall organization. This mechanism would allow cells to form and grow as rods based on local rules rather than long-range organization.

Fungi have a very different molecular composition of the cell wall compared to plants and bacteria. **Amir Jafari Bidhendi** (University of Montreal) reported on fungal spores, which are spherical structures with extremely thick and resistant walls. Nevertheless, the spores of mycorhyzal fungi that are produced commercially as biological fertilizers can get damage during the production process. The authors therefore wanted to characterize the biomechanical properties of the spore walls. *Glomus intraradices* spores are formed both inside and outside the root, representing two very different environments. Using microindentation the authors examined the spore stiffness and its correlation with size, wall thickness and origin of the spores. A finite element model of the micro-indentation procedure was developed to analyze the influence of different geometrical and material constants on deformation behavior of the spores. To relate the mechanics to the biochemical composition of the wall, they determined the subcellular localization of the structural cell wall components chitin, β(1,3) glucan and glomalin using confocal laser scanning microscopy.

Cell growth and supply of cell wall material

Sustained growth of walled cells requires the supply and assembly of new cell wall material. The regulatory role of this process is rarely incorporated in mechanical models of growth, but various approaches to do so point at future avenues in this field.

Salomon Bartnicki-Garcia (Centro de Investigación Científica y de Educación Superior de Ensenada) discussed fungal cell wall morphogenesis powered by vesicle delivery as predicted by computer modeling. Hyphal wall synthesis is highly polarized. It originates from a sharp gradient of synthesis centered on the dome of the hyphal tip. It has been recently found that the enzymes that make the two principal polymers of most fungal walls, chitin and beta-1,3-glucan, are transported in different vesicles. In most fungi, the vesicles congregate in a structure called the Spitzenkörper. An exercise in computer simulation of hyphal morphogenesis led to the realization that the Spitzenkörper functions as a vesicle distribution center whose linear advance generates the gradient of cell-making vesicles necessary to produce a continues tubular cell. The present challenge is to elucidate the mechanisms by which vesicles move in out of the Spitzenkörper.

Rishi Bhalerao (Umeå Plant Science Center) investigates vesicular trafficking in ethylene and auxin mediated differential growth. Differential growth across tissue layers is mediated by asymmetric auxin distribution. The authors are using apical hook development, bending of hypocotyl immediately after seed germination, as a model to investigate how differential growth is achieved. Asymmetric auxin distribution essential for apical hook development is mediated by the concerted action of auxin influx- and efflux carriers many of which localize to the plasma membrane (PM). The abundance of auxin carriers at the PM is dynamically regulated through vesicle trafficking processes such as endocytosis, recycling and secretion.

Mechanics of multicellular tissues

The behavior of plant tissues is complex since plant cells adhere to each other and influence each other's growth behaviour, necessitating study of how these interactions affect tissue mechanics.

Arezki Boudaoud (Ecole Normale Superieure de Lyon) discussed how the mechanical properties of plant tissues determine how they grow into well-defined shapes. Little is known on the mechanics of internal tissues in developing organisms, making it difficult to assess their contribution to morphogenesis. Here the authors investigated the mechanics of internal cell walls in plants, more specifically in *Arabidopsis*. They combine confocal imaging of tissues in 3D, nano-indentation, and mechanical models. Their results provide support to the pressurized shell model of the shoot apex and reveal the differences between the shoot apex, the hypocotyl, and the root, shedding light on the contrast in organogenesis between the root and the shoot apex.

The presentation by **Siobhan Braybrook** (Sainsbury Lab) emphasized that pectin is a mechanically important component of the plant cell wall. Recent literature highlights a key role for an overlooked component of the cell wall, the pectin homogalacturonan (HG), in plant development. The authors have been exploring the role of pectin in plant cell and organ growth using *Arabidopsis thaliana* cotyledons and hypocotyls. They combine immunohistochemistry, growth and shape analysis, and mechanical measurements to understand how these organs and their cell shapes are generated. They have also begun exploring how pectin gels themselves behave mechanically as a result of chemical alteration, and furthermore what effect these changes have in cell wall material mimics. Their results indicate that HG pectin modification controls the magnitude of plant growth through precise chemical mechanisms.

Tissue mechanics determines tissue failure under stress, as studied by **Douglas Cook** (NYU Abu Dhabi). Cook's group studies stalk failure, focusing primarily on crop stalk biomechanics. In maize and other crops, failure often initiates in or near the meristematic tissue above each node, and almost always occurs due to compressive stresses. The localized buckling of individual cell walls is one hypothesized mode of failure propagation: (i.e. failure in the first cell leads to increased stresses in neighboring cells and so forth). The authors have conducted mechanical tests and imaging studies to try to determine where failure initiates, but this approach is problematic. If failure initiates on the surface of the stalk it may be visible using one of several optical techniques, but failure that initiates at any internal location is not visible optically until it is manifest on the surface. Is there a way to identify the location of initial failure? Could cell wall modeling provide an avenue for studying failure initialization and perhaps lead to insights that are not possible through purely experimental approaches? This presentation represented an excellent case study for the modelers in the room who immediately brainstormed on the problem.

Rosemary Dyson (University of Birmingham) presented that the arrangement, and dynamic rearrangement, of cell wall components lead to emergent anisotropic mechanical

properties which govern the directional growth of individual plant cells and hence the tissues these cells combine to form. Growth is therefore inherently a multiscale process, with the microstructure determining the mechanical properties of a cell wall segment, which in turn governs the expansion of individual cells and hence the whole tissue. However, this resultant tissue-level growth can also produce active rearrangement of the cell wall microstructure, which will again alter the macroscale mechanical properties. Understanding this interplay is essential to determining the mechanical origins of three-dimensional macroscale plant root behaviour, for example twisting and tropic responses. The author discussed her current work incorporating these macroscale effects on the microscale structure into mathematical models of plant root growth. She focused in particular on the effects of the current macroscale state of the tissue on the deposition, dispersion and reorientation of cellulose microfibrils, and how this manifests as changes to the evolving cell wall mechanical properties. This in turn determines the directional growth of cells and tissues to produce observable changes in plant phenotype.

Henrik Jönsson (Lund University) emphasized that physical forces have been suggested to contribute in generating form in plant tissue, and more recently act as main input to patterning processes themselves. Plants can guide their pressure-driven growth into anisotropic shapes by strengthening the walls by laying out cellulose fibers in specific directions. He discussed different efforts modeling morphodynamics in plants. Focus was on how stress and strain can provide input to elastic properties and plastic growth, and when these signals fail. He showed how plants' orientation of cellulose fibers represent an energy-minimizing principle similar to topology optimization.

Tissue and organ development are intimately linked as discussed by **Przemyslaw Prusinkiewicz** (University of Calgary). Early leaves of the model plant *Physcomitrella patens* consist of a single layer of cells. Although cellular patterns are thus easily observable, live imaging of developing *Physcomitrella* leaves has remained technically challenging. To describe and understand *Physomitrella* leaf development from the initial apical cell to the mature leaf form, they constructed a computational model integrating the information gathered from microphotographs of leaves in different stages of development and from short live-imaging sequences. A particularly puzzling observation was the transformation of diagonally-oriented cells near the leaf apex into staggered files of longitudinally-elongated rectilinear cells closer to the leaf base. To explain it, the authors considered two hypotheses: (1) diagonally-oriented cells are rotated to their final rectilinear position by a medio-lateral gradient of growth rates, and (2) diagonal cells undergo shape changes caused by inhomogeneous expansion of different wall segments. A biomechanical model implementing the second hypothesis produced cellular patterns consistent with the observations of real leaves. In addition to characterizing *Physcomitrella* leaf development at the cellular level, the model points to a possibly broad morphogenetic role of the growth-tensor discontinuities in symplastic development.

Richard Smith (Max Planck Institute for Plant Breeding Research) explained various modeling tools specifically developed for plant tissue modeling. This is especially true for mechanical models, where non-intuitive behavior often arises even from very simple assumptions. Although the field of mechanical modeling with the finite element method (FEM) is well developed, growth is typically not included, and many methods are not well suited for modeling plant cells. He presented FEM models he developed specifically for 3D plant cells. He uses the models for quantitative work to interpret force measurements from experiments, as well as qualitative models for growing 3D cellular plant tissues.

Joseph Turner (University of Nebraska-Lincoln) employs similar modeling strategies to analyze time-dependent material response of plant cell walls. One very importance aspect for growth is the time-dependent (viscoelastic) nature of the cell wall that allows the wall to relax and expand. In this presentation, the role of viscoelasticity and its impact on measurement interpretation and modeling are discussed. In particular, the computational modeling of cell wall behavior must include an appropriate time-dependent component in order for force-displacement responses to have physical relevance. In addition, model inputs are often derived from scanning probe microscope experiments (e.g., nanoindentor, atomic force microscope) that are now being applied to plants. However, data analysis is often made under the assumption of a quasi-static material response for which the loading rate is presumed unimportant. Here, the impact of such assumptions was discussed and quantified using several examples.