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Finite element modelling of the mechanical behaviour of onion epidermis with incorporation of nonlinear properties of cell walls and real tissue geometry

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ABSTRACT

In the field of agricultural sciences, numerical modelling has proven to be a valuable tool in finding solutions to practical and scientific issues. In the present study a computational model of plant tissue that incorporates micro-scale geometrical features was developed to provide qualitative and quantitative predictions of the mechanical properties of onion (*Allium cepa*) epidermis. The simulations of cellular structure behaviour under various mechanical load conditions were carried out using the finite element method (FEM). The models were validated against experimental data from a tensile test of the real tissue strips. The models showed capabilities of simulating large strains (up to 25%) with nonlinear behaviour and produced force-strain curves that closely matched the experimental data. Also, a new insight into the relationship between microstructure and mechanical behaviour was provided for this type of material. The incorporation of the real microstructure resulted in a qualitative improvement of the mechanical characteristics obtained from FEM models of plant tissues. The ability to directly simulate the impact of changes in turgor pressure was limited due to the semi-three dimensional representation of tissue. The general behaviour of tissue resulting from the changes in turgor was approximated using the semi-solid protoplast that replaced the cells liquid. On the basis of a sensitivity analysis it was concluded that the validity of the results provided by the model is largely dependent on accurate measurements of cell wall thickness.

1. Introduction

Along with increasing knowledge on complex hierarchical microstructures of biological materials, a multi-scale approach has become the key to understanding the resulting constitutive relations for mechanical behaviour at macroscopic scales (Verboven et al., 2008). Multi-scale modelling helps with solving physical problems, which have important features at multiple scales. This approach involves building a hierarchy of sub-models, which describe mechanical behaviour at different spatial scales. The output data obtained from one scale is reused as the input data at other simulation scales.

However, with a decreasing scale of simulation, difficulties in building a suitable model increase rapidly. The development of micro-scale models has been constrained by the lack of technology available for conducting reliable measurements at such scales and also by the hardware limitations and the lack of efficient computational methods. Early attempts at micromechanical modelling of plant tissue involved mechanistic approaches, using simplified geometrical models of tissues or single cells (Nilsson et al., 1958; Pitt, 1982; Pitt and Chen, 1983; McLaughlin and Pitt, 1984; Lin and Pitt, 1986). Most often, tissues were described as uniformly stressed structures, with symmetry and uniformity assumptions on cell shape and cell wall deformations.

One of the first applications of FEM in modelling plant micromechanics was aimed at the analysis of the response of a single cell under uniaxial compressive loading. For this purpose Pitt and Davis (1984) modelled a potato parenchyma cell as a thin-walled, fluid-filled sphere and cylinder. A more realistic approach (Wu and Pitts, 1998) involved a three-dimensional finite element model based on typical apple cell geometry. Further development of the single cell models allowed for consideration of large deformations, cell wall permeability and nonlinear constitutive relationships in cell wall material (Smith et al., 1998; Wang et al., 2004; Dintwa et al., 2011). These models were very successful and provided a new insight into the mechanical behaviour of this type of materials. The progress in modelling single cell micromechanics has significantly contributed by the development of new measurement techniques. Up to now a few techniques have been developed to investigate the biomechanics of single cells. Mechanical properties (stiffness) can be investigated in situ using compression between two parallel

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Nomenclature

σ	stress [Pa]
ε	strain [arbitrary units]
E_1	Young's modulus of onion epidermis tissue [Pa]
E_2	modulus of strain hardening of onion epidermis tissue [Pa]
σ_{pl}	yield strength of onion epidermis tissue [Pa]
ε_{pl}	yield strain of onion epidermis tissue [arbitrary units]
$E_{1\mathrm{exp}}$	Young's modulus of tissue cell wall [Pa]
$E_{2\mathrm{exp}}$	modulus of strain hardening of tissue cell wall [Pa]
$\sigma_{pl\mathrm{exp}}$	yield strength of tissue cell wall [Pa]
$E_{1\mathrm{mod}}$	Young's modulus of virtual tissue cell wall [Pa]
$E_{2\mathrm{mod}}$	modulus of strain hardening of virtual tissue cell wall [Pa]
$\sigma_{pl\mathrm{mod}}$	yield strength of virtual tissue cell wall [Pa]
ν	Poisson's ratio of virtual tissue cell wall [arbitrary units]
F	tensile force [N]
E_p	Young's modulus of protoplast [MPa]

Abbreviations

APW	artificial pond water

plates (Mashmoushy et al., 1998) or micro- and nano-indentation methods (Geitmann, 2006).

Although there has been a significant increase in the complexity of single cells models, the literature is still lacking in complex models of the whole tissue involving real spatial heterogeneity. Loodts et al. (2006) developed a 2D model of onion epidermis derived from a discrete element approach. Cells in the tissue were described by sets of nodes that formed rectangular shapes. Ho et al. (2009, 2010) presented a two-dimensional microscale model of gas-exchange that featured virtual tissue geometry based on light microscopic images of fruit. Models used in further studies incorporated the actual 3-D microstructure of the tissue based on data obtained by means of synchrotron radiation X-ray tomography (Ho et al., 2011). By summarising the literature it is evident that there is lack of mechanical models of tissue, which consider the real structure of the tissue. Development of such model is necessary, since many properties could depend, for example, on a local stress distribution originating from tissue heterogeneity.

Therefore, the general goal of this study is to create computational model of plant tissue deformation that incorporates the actual geometrical features of tissue structure at a microscopic scale. Such model is necessary to understand the structure related properties of plants, like fruits and vegetables, and brings, in the next step, possibilities of extension by nano-structural features (like cell wall composition). This knowledge will be useful for engineering and improve fruits and vegetables quality. Onion epidermal tissue has been chosen as a simpler and well-defined system for model validation. The finite element method was chosen due to its computational efficiency, flexibility and ability to incorporate geometric nonlinearities. Tissue structure was reproduced in an FEM environment on the basis of processed microscope images. The attempt aimed at quantitative and qualitative validation of the created model using experimental data.

2. Materials and methods

2.1. Samples of plant tissue

The proposed technique of the simulation of micromechanical cellular systems was demonstrated in a case study of onion (*Allium cepa* L.) upper epidermis, which was chosen due to its simple single-layer structure, lack of intercellular spaces and ease of sample preparation. Samples of tissue were isolated from the equatorial region of a bulb, from beneath the first outer scale, parallel with the vascular bundles. Epidermal strips were from 1.5 to 2 mm in width and from 20 to 30 mm in length.

2.2. Turgor manipulation

To control turgor pressure, samples were immersed overnight in 0.1 M, 0.3 M and 0.5 M solutions of mannitol in an APW matrix (0.0001 M of KCl, 0.0001 M of $CaCl_2$, 0.05 M of mannitol, 0.1 M of Tris and 0.1 M of Mes solution on the basis of deionized water). The highest concentration of mannitol was used to induce plasmolysis in the protoplast. Ten samples of onion epidermis were chosen for each mannitol concentration. During soaking samples were kept at room temperature.

2.3. Mechanical tests

Before mechanical testing, samples were protected from sliding and deformation by placing them between two layers of waterproof paper with one side covered with glue, as is shown in Fig. 1. Tissue strips were placed under a microscope to record the initial microstructure. Then stripes of onion epidermis were subjected to uniaxial tensile testing using a miniature tensile stage (Deben Microtest, Suffolk, UK). The initial gap between grips was equal to 4 mm. After clamping the samples, the supporting paper leaves were cut across. Mechanical testing was carried out up to a strain of 50% with a deformation speed of 0.5 and 1.5 mm/min.

2.4. Image acquisition and analysis

A confocal scanning laser microscope CSLM (OLYMPUS FluoView300, Olympus Corporation, Tokyo, Japan) was used for imaging the onion epidermis before mechanical deformation. Images were acquired using a UPlanSApo 4x/0.16 objective. The image size was equal to 1024×1024 pixels, which corresponded to a field of observation of $752.64\ \mu m \times 752.64\ \mu m$. The images were segmented using a protocol developed using MATLAB® R2010a (MathWorks, U.S.A.) and image analysis tool pack. The segmentation procedure was based on morphological reconstruction, using the eroded image as the marker and the original image as the mask, followed by another morphological reconstruction, using the dilated image from the previous reconstruction. The resulting image was segmented using the watershed method (Wojnar and Kurzydlowski, 2000). As a result of segmentation, skeletons of the cellular structure were obtained.

2.5. Data analysis

Tensile force and elongation of the sample were recorded. Both values were converted into stress and strain respectively. Stress was determined using two approaches depending on the turgor state of samples. For all samples the cross sectional area was defined as width x thickness of the tissue strip. In the case of samples immersed in the 0.5 M solution of mannitol, the cross-section area was additionally multiplied by the cell wall fraction and this value was called the effective cross-section area (as it takes into account only the portion of area occupied by the cell wall). Additional calculations were based on the observation that the solution with the highest concentration of mannitol caused separation of the proto-