A method of calculating the mechanical properties of nanoscopic plant cell wall components from tissue properties

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Biological tissues are made from nano-composite materials and given the recent interest in manufacturing synthetic nano-composites an analysis of natural nano-composites seems a worthwhile exercise. There is also potential for extracting natural nano-fibres and using them as reinforcements in other materials. In this paper a hierarchical mechanical model is formulated to describe potato tuber tissue and the model is used to back calculate the properties of cell wall nano-fibres. The model contains two structural levels, the cell structure and the cell wall structure. Material properties are assigned at the level of cell wall microfibrils (nano-composite fibres). Force deflection data from the compression of cubes of potato tissue were fed into the model and the properties of the cell wall microfibrils predicted. The modulus was found to vary with strain, but had a maximum value of 130 GPa, which is close to predictions from theoretical chemistry for the stiffness of cellulose microfibrils. At 8% wall strain (the value at which failures were suspected to begin), the stress was predicted to be 7.5 GPa which is also close to theoretical chemistry predictions for the strength of cellulose microfibrils. The large strains and decreasing stiffness indicate the influence of polymers other than cellulose.

1. Introduction

In the last decade there has been growing interest in the manufacture of composite materials that are reinforced with nano-fibres [1, 2]. However the fact that many biological tissues are nanocomposites is often over looked. Often there is a hierarchy of composite structure, down to a molecular scale. For example in plant tissues, at the nano-scale primary cell wall looks like a woven mesh [3]. Each strand of the mesh is composed of cellulose microfibrils, hemicellulose polymers or pectins, or mixtures of all three. The gaps between fibres are probably filled with pectin gel. This cell wall encloses a living protoplast, which regulates water uptake and is capable of exerting a turgor pressure on the cell wall. At the micro scale individual cells are connected to their neighbours to form tissues and several tissues may combine to form an organ. By analysing these tissues we may be able to learn a great deal about the construction of hierarchical nano-composite materials and we may also be able to extract and use natural nano-fibres [4]. Huge quantities of these nano-fibres are potentially available in the form of plant primary cell walls, often as waste products from agriculture.

However these tissues are difficult to analyse using standard techniques. The properties of the nanoscopic fibrous components cannot be physically measured without extracting them from the tissue, which may result in significant chemical or mechanical damage. An alternative is to back calculate their properties from the tissue properties. This is difficult because most biological tissues do not obey Hookean elasticity theory, instead exhibiting large deformation, non-linear, mechanical properties. Many techniques for describing this behaviour have been proposed [5–8]. However most of these neglect the complex hierarchical structure of the tissues, therefore the parameters in the equations do not have a physical significance [8–13]. Attempts have been made to produce structural models based on hyper elasticity, that explain biological material constants [14–16]. However most are based on linearly elastic components and it is likely that the component polymers have non-linear mechanical properties.

In this paper we will make use of the structural approach to develop a hierarchical description of plant tissue mechanical properties down to the level of cell wall components. We will then use this model to back calculate cell wall microfibril properties. Rate dependent behaviour will not be investigated in this paper.

2. Model

This model is based on the structure of potato tuber tissue in which the cells are well stuck together and there are few air spaces. This means that a living turgid piece of tissue (or one where the cells are at incipient

plasmolysis) should deform at constant volume, provided that the rate of deformation is sufficiently rapid to avoid significant fluxes of water out of the living cells (see results). Also in potato tuber tissue there is very little preferential orientation of the cells so that macroscopically large regions of tissue are almost isotropic. Therefore a cube of tissue should remain flat sided during compression provided that end effects are negligible (see results). Deformation of such a structure at constant volume can be described in the following way:

$$y \rightarrow y + \Delta y. \tag{1}$$

$$x \rightarrow \frac{x}{\sqrt{1 + \frac{\Delta y}{y}}}. \tag{2}$$

$$z \rightarrow \frac{z}{\sqrt{1 + \frac{\Delta y}{y}}}. \tag{3}$$

where x, y, z refer to the global cartesian co-ordinates of a volume of tissue. Assuming that every individual cell within the tissue also deforms at constant volume and that deformations are affine [14] (we have tested this assumption in a number of tissues including potato and found that it is valid down to a micrometre scale, Hepworth unpublished) it is possible to describe how a flat cell face at any orientation in space within the tissue will deform. Observations of deforming tissue have shown that these faces do remain flat during deformations (Hepworth, unpublished) i.e. they do not bend significantly (except when they are next to an air filled space). The change in dimensions ΔL_i of cell wall faces (Fig. 1) can be calculated from vectorial analysis of the cell wall faces based on the global x, y, z axes of the tissue as follows.

$$\Delta L_i = \sqrt{\Delta L_X^2 + \Delta L_Y^2 + \Delta L_Z^2}. \tag{4}$$

where L_X, L_Y and L_Z are the components of dimension, with respect to the x, y and z co-ordinate axes of the tissue.

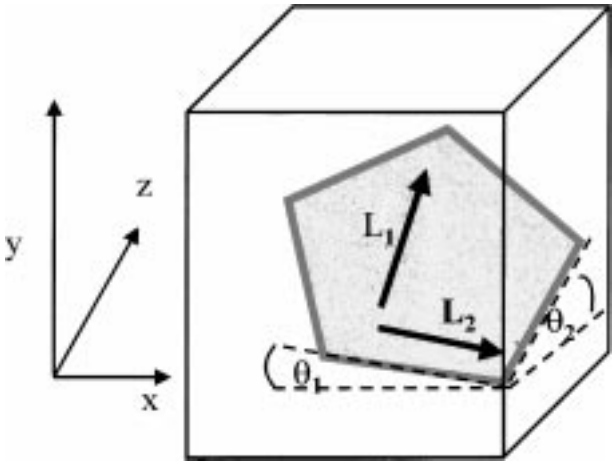

Figure 1 Depiction of a cell wall face in three-dimensional space.

The total work done (W) within the solid wall material, as a result of the deformation, will be equal to the sum of the energy stored in every cell wall, 1 to n [14];

$$W = \sum_1^n \int_0^{\Delta L_i} f(\Delta L_i)\, \mathrm{d}L_i \tag{5}$$

where $f(\Delta L_i)$ is the force, calculated from a function of the change in wall dimensions. Compressive strains of thin cell walls probably require negligible force. At the nano scale the cell wall looks like a loosely woven fabric [3] and such a structure would require very little force to compress even if the gaps between fibres are filled with polysaccharide gels. Therefore the deformation energy stored in the solid elements is due to tensile strain and is balanced by the action of a fluid pressure (through electro chemical interactions and hydrogen bonding). In many tissues there will be a fluid pressure and thus a pre-stressing of the components in the unloaded state. This will have to be taken into account, so that the potential energy equations for the individual components are started from the appropriate level of pre-stress. During a deformation the increase in fluid pressure (P) will be equal to the applied stress.

If we treat the cell wall as a material then in order to evaluate the stress and hence the modulus (slope of the curve) from $f(\Delta L_i)$ we have to make assumptions about the form of $f(\Delta L_i)$. For example we could assume that the cell wall behaves as an incompressible neo-Hookean material and follow the analysis of Chaplain [8] to define a strain energy function for cell wall material. However the problems highlighted in the introduction will then be introduced.

There is a different solution, which is to resolve each cell wall face into its constituent fibrous components. We will define a microfibril as a composite fibre of cellulose, hemicellulose and pectin, with its long axis parallel to the direction of the cellulose. Each cell wall face is modelled as a mesh of composite microfibrils. These microfibrils are orientated at particular angles within the cell wall faces. Each microfibril occupies a certain volume and is separated from its neighbour by a space, which may be filled with water and polysaccharide gel. The deformations of the cell wall faces described in Equation 4 will result in stretching, rotation or compression of these microfibrils depending upon the angle at which they lie within the wall and the angles that the wall makes with the global axes. The deformation that a particular microfibril develops when the surface area of the face is changed can be calculated as in Equation 4, by describing the dimensions of the microfibrils, relative to the 2-D co-ordinate axes of the cell wall faces.

The sum of potential energies in Equation 5 can now be re-written with a second hierarchical level of k to l microfibrils.

$$W = \sum_1^n \left(\sum_k^l \int_0^{\Delta L_m} f(\Delta L_m)\, \mathrm{d}L_m \right) \tag{6}$$

The total potential energy of the piece of tissue after a compressive deformation is the sum of the tensile deformation of every composite microfibril in every cell wall.

Using Equation 6 the function $f(\Delta L_m)$ for microfibrils can be mapped out as follows. Many small increments of tissue deformation are performed and for each increment the average change in deformation of the microfibrils is calculated (total change in deformation/number of microfibrils with positive strain). Also at each step the average increase in work is calculated (total increase in W/number of microfibrils with positive strain). Thus the stress/strain curve of an average microfibril at this particular rate of deformation, can be back calculated form the energy function of the whole tissue.

For this simulation the cell wall was constructed of 4 layers of microfibrils, each one being 0.25 μm thick. In one layer the microfibrils are aligned along the 1 axis, in another they are aligned along the 2 axis and in the other two layers they are aligned at 45° to these axes. Each microfibril was defined as being 225 nm^2 in cross sectional area and separated from the next microfibril in the same layer by 70 nm (based on measurements from electron micrographs provided by M. McCann at the John Innes Institute). This is a larger cross sectional area than for individual cellulose microfibrils, which have a width of 5–10 nm [3] and therefore a cross sectional area of 20–100 nm^2. However the unextracted microfibrils in the images examined were probably coated in hemicellulose and there are many points where two or more microfibrils lie very close together and may be physically joined to act as a single mechanical unit. Therefore we have taken this larger cross sectional area of 225 nm^2 as representative of a composite microfibril that may contain more than a single cellulose microfibril and also hemicellulose polymers. If individual cellulose microfibrils were taken as single mechanical elements then both the cross sectional area and the average distance between microfibrils would be less than the value used here, but the calculations would not be affected by using smaller microfibrils but more of them.

For a given deformation along the Y axis, values of ΔL_i and thus also for ΔL_m were calculated, using a matrix, for all walls at different orientations in the system. Values of change in tissue potential energy were obtained, from force deflection experiments carried out on cubes of potato that had been equilibrated in a very slightly hypertonic solution (so that the cell walls were not pre-stressed).

3. Methods

The variety of potato used was Maris Piper. Commercially grown tubers were stored on trays at 5°C for a maximum of one week before being used. Only tubers with an average diameter between 6 and 8 cm were used and these were selected to be as near spherical as possible. This should help to reduce the small amount of anisotropy [17] which is probably correlated with differences in the x, y, and z-axes lengths. From these, 2 cm by 2 cm by 3 cm blocks of tissue were cut. The axes of the blocks were always kept the same and specimens were compressed in the y direction. The blocks of tissue were placed in buffered 0.2 M manitol solution [18]. This is a very slightly hypertonic solution [18], therefore the cells should be just below incipient plasmolysis. This can be detected in the force deflection curves as a small initial toe region of very little increase in force with deformation. It was also checked using a pressure probe and a measurement of zero pressure was made. Above incipient plasmolysis this toe region disappears. Blocks were left to equilibrate for 24 hours. Prior to testing the blocks were re-cut into smaller cubes using small guillotines with mounted razor blades. The re-cutting was designed to eliminate surface tissue that may have begun to degrade during the equilibration.

Force deflection data were recorded during compression tests using a Davenport Nene DN10 testing machine. Cubes or cylinders were placed on a flat metal base on the test machine, covered in buffered manitol with a pipette and then compressed using a flat plate attached to the load cell. The loading rate was 10 mm/min and the maximum duration of a test was 1 minute. For the calculation of tissue volumes during testing the machine could be momentarily halted and the width or diameter (for cylinders of tissue) measured using electronic callipers.

Because of the isotropic nature of the tissue and the variable shapes of the cells, an even distribution of cell wall face orientations, over all possible orientations, was assumed. This assumption was corroborated by measuring the projected angles of cell walls in a section of tissue using light microscopy and image analysis [19]. The dimensions of the cells were also measured.

4. Results

The following average measurements of cell dimensions were obtained on 100 cells from 5 tubers. The diameter was 212 μm (s.d. 22 μm), the number of sides in a cell cross section was 5.5 (s.d. 0.9 μm), the length of a side was 115 μm (s.d. 47 μm) and the thickness of cell walls was 1 μm (s.d. 0.5 μm).

When 1 cm cubed pieces of potato tissue were compressed, the faces remained flat and end effects were minimal. This means that there was little friction between the tissue and the metal plates (probably because of fluid lubrication). Measurement of the volumes of 15 cylinders of potato tissue undergoing compression showed that on average there is a small but significant decrease of 16 mm^3 in volume at 22% compression (single factor analysis of variance, $P < 0.05$). However there were damaged cells on the cut surfaces of the potato cylinders. Compression of these will lead to a small reduction in volume. This was calculated to be 15.3 mm^3 (assuming that each damaged layer is the same thickness as the average radius of cells). This accounted for the volume reductions up to 22% compression. Beyond this level of compression the volume significantly decreased.

The force deflection curves for 5 cubes of potato tissue (all 1 cm cubed) are shown in Fig. 2. From this a

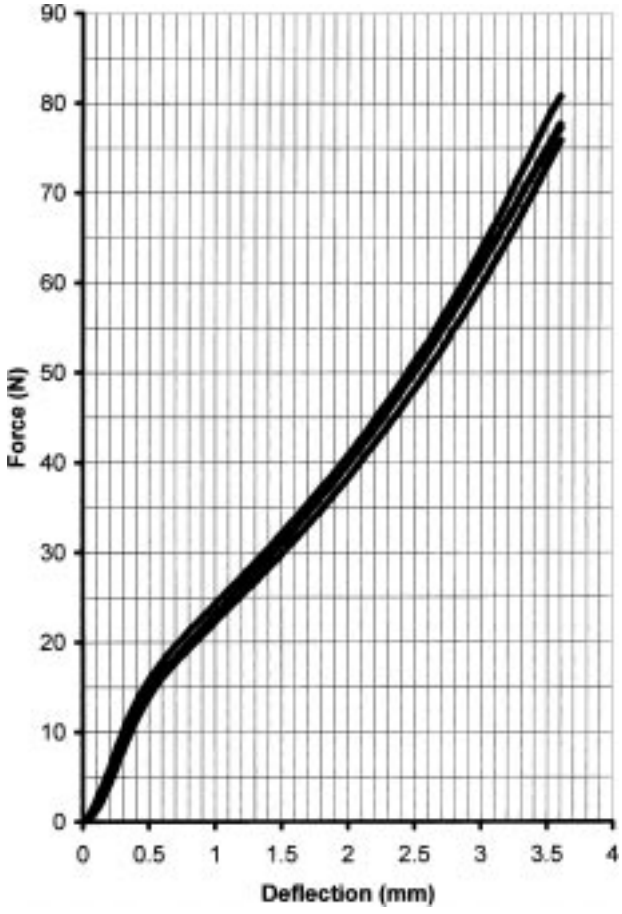

Figure 2 Compression curves for 5 cubes of potato tissue in 0.2 M manitol.

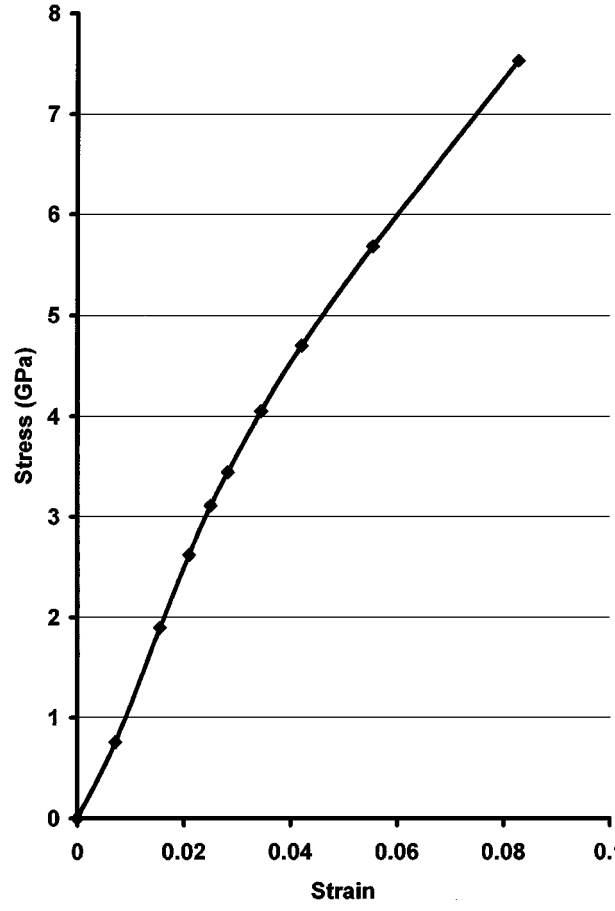

Figure 3 Calculated stress strain curve for cell wall composite microfibrils.

force deflection curve for individual microfibrils was calculated (Fig. 3). The highest value of stiffness along this curve of 130 GPa is very similar to the value of 135 GPa predicted for cellulose microfibrils [20, 21]. The maximum microfibril strain was chosen as the value after a tissue deformation of 22%. This is the maximum tissue deformation at which the constant volume assumption is applicable at this particular rate of tissue deformation. The value of microfibril stress at this level of deformation was calculated as 7.5 GPa, which is also similar to the value of 7–8 GPa predicted for the failure of cellulose microfibrils by chain scission [20]. At large strains the modulus falls significantly. This may indicate the influence of polysaccharides such as hemicelluloses on the composite microfibril properties.

5. Discussion

We believe that this is the first attempt to back calculate the properties of primary plant cell wall components from tissue properties. It may also be the first time that non-linear properties of nanoscopic fibrous components have been analysed in biological tissues. Obviously the potential errors in this type of analysis are large, but we think that by using potato tissue, with its isotropic structure, we have kept them to a minimum. The measurements could be improved with a much more detailed analysis of cell wall face shapes and orientations. However we are encouraged by the fact that the predicted properties of the microfibrils are similar to those calculated from theoretical chemistry for cellulose microfibrils. The predictions for the properties of cell wall components could also be checked by using the equations in reverse on other plant tissues i.e. inputting microfibril properties (assuming that they are similar) and predicting tissue properties. If these predicted properties are correct then plant cell wall microfibrils have great potential for re-inforcing man made materials. Potentially they can absorb large amounts of energy. From the plants point of view it makes sense to make the cell walls from a high performance material because very little of it is then required to produce an effective structure (less than one percent of potato tissue is cell wall material).

By treating the cell wall as a structure, many of the problems associated with the engineering analysis of materials that show large elastic deformations, are avoided. There are two levels to the structural hierarchy, the tissue level and the cell wall level. At the tissue level there is constant volume deformation up to 22% compression, during which the cell walls are reorientated, stretched and compressed according to their orientation in space. At the cell wall structural level the fibrous elements which make up the cell wall appear to have the theoretical stiffness of cellulose microfibrils. The predicted failure strains and hence strengths of microfibrils are large when compared to the failure strains of cellulose in secondarily thickened fibre cells such as flax. However these secondarily thickened

cells tend to be compromised by pitts and other structures that may significantly reduce the strength. They often have a multipurpose function to play, which may include the conduction of nutrients up and down the stem. Stiffness rather than toughness may be more important for secondarily thickened cells if part of their function is to increase the bending stiffness of stems. Also it is common for the strength of synthetic fibres to increase as the diameter is reduced [22] and natural fibres probably follow the same trend. Further experimentation would be required to determine the true failure strain of the microfibrils and the influence that polymers other than cellulose have at these large strains.

The experiments and calculations performed in this paper relate to potato tissue, which had initially been equilibrated to just below incipient plasmolysis. If the tissue was initially turgid then the cell walls would already have been loaded and the microfibrils stretched. This means there would be a pre-stress and strain that would have to be added into the matrix calculation. If the cells were flaccid then upon deformation of the tissue, re-orientation of cell wall material would be able to occur without the microfibrils being stretched. The deformation would then be described by standard theories for thin walled foams [23], until the volume was reduced to that of the fluid contents, at which point the above model could be used.

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