

Nearly all plant cells are surrounded by a wall made of cellulose, long fibers made of glucose joined by 1-4 bonds. Cellulose fibers are laid down in a precise array such that 36 individual fibers twine to form a cable, and the deposition of the cable is guided by microtubules underlying the plasma membrane. Celluose therefore serves as a reinforcing fiber that determines the direction in which plant cells can elongate. Primary cell walls also contain large amounts of hemicelluoses which serve as crosslinkers joining individual cellulose cables, and pectins and proteins which fill in the gaps. Once a cell has finished elongating it starts synthesizing secondary cell walls by secreting phenolic precurosrs into the cell wall which then polymerize to form lignin. Today we will observe various cell types to look at their cell walls, and we will compare the ease with which cell walls from various plant tissues can be digested.

**Experimental**

We will first set up the enzymatic digestions, then periodically check on them. In the meantime we will observe various prepared slides illustrating different types of cell walls.

**Preparing protoplasts**

Each of you please pick a separate plant tissue:

* For leaves, cut two punches, slice a few times with a razor blade, then place in a 2 ml microcentrifuge tube and add 0.5 ml enzyme mix containing 1 % cellulase, 0.5% hemicellulase and 0.2 % pectinase dissolved in 400 mM mannitol as osmoticum.
* For roots, onion bulbs, beets and carrots cut small segments slice a few times with a razor blade, then place in a 2 ml microcentrifuge tube and add 0.5 ml enzyme mix containing 1 % cellulase, 0.5% hemicellulase and 0.2 % pectinase dissolved in 400 mM mannitol as osmoticum.
* Observe at hourly intervals and note whether any are releasing protoplasts. If we find any tissues releasing protoplasts please make a drawing of a protoplast next to a normal cell.

**Looking at cell walls**

1. Parenchyma: this cell type is widely distributed throughout the plant body. It usually constitutes the major portion of the cortex, pith, and mesophyll, and has relatively simple cell walls. Obtain a prepared slide of a Coleus stem and identify parenchyma cells in the pith region. Draw two cells in the top half of the circle on your datasheet, emphasizing the junction between them.
2. Collenchyma. This cell type is characterized by irregularly thickened primary cell walls, with the thickenings in the corners of the cells. In addition, the cells are elongate and are usually found aggregated into stands or cylinders just within the epidermis. Obtain a prepared slide of a Medicago (alfalfa) stem and identify collenchyma (it should be at the corners). Draw two cells in the bottom half of the circle on your datasheet, emphasizing the junction between them.
3. Sclerenchyma. This type of cell provides mechanical support or serves to make plant tissues hard. They may be either sclereids or fibers, which differ greatly in shape but both have thick lignified secondary cell walls, and they usually die soon after the cell reaches maturity so that the cell persists solely as cell wall.
   1. Sclereids: these are usually found in the hardest parts of a plant, such as the seed coat, but they are also scattered through the tissue of a pear fruit. Obtain a slide of a pear fruit or of a nymphea leaf and draw a sclereid in the top half
   2. Fibers: these are for support and have very thick walls with almost no interior space. Obtain a slide of a Tilia stem cross section, and identify and draw a fiber cell in the bottom half of the circle. You find them in the phloem.
4. Phloem is a complex tissue that contains sieve tube elements which conduct the sap, small companion cells next to them that control the loading and unloading, and often fibers and parenchyma. Identify then draw a phloem element from any of your stems in the top half, identifying sieve elements, companion cells, parenchyma and fibers if they are present.
5. Xylem is a complex tissue composed of tracheids, vessels and often fibers and parenchyma. Tracheids and vessels are both dead hollow tubes that conduct the xylem fluid, but tracheids are much smaller in diameter than vessels. The fibers are there to provide mechanical support, while the parenchyma is there to help with metabolism and as a source of water. Identify then draw a xylem element from any of your stems in the bottom half, identifying vessels and tracheids, parenchyma and fibers if they are present.
6. Obtain a slide of an older Quercus stem, and draw a few cells representing different cell types.
7. Pollen. During pollen development at the four-cell stage the pollen grains are held together by a wall made of callose, which is later broken down to release the individual grains. Obtain a prepared slide of a lily anther showing pollen tetrads and draw a tetrad of pollen grains and a few tapetum cells in the top half. Mature pollen grains are protected by a thick outer wall called the exine which is primarily made of sporopollenin, a biopolymer that is poorly characterized because it is very resistant to degradation (so pollen grains can survive for thousands of years in sediments). Obtain a prepared slide showing various pollen grains and draw three different types in the bottom half.