Experiment: Microscopic Observation of Cell Tissues

Aim: To observe and analyze the microscopic structure of cell tissues from a selected specimen using staining techniques to enhance visibility.

Principle: The microscopic observation of cell tissues involves the preparation of thin tissue sections, staining them to highlight cellular components, and examining them under a light microscope. Staining enhances contrast, allowing different cellular structures to be distinguished more clearly.

Materials:

- Specimen tissue (e.g., plant leaf, animal liver)
- Fixative solution (e.g., formalin)
- Microtome
- Glass slides
- Coverslips
- Stains (e.g., Hematoxylin and Eosin)
- Mounting medium (e.g., Canada balsam)
- Light microscope
- Staining jars
- Distilled water
- Ethanol series (70%, 90%, 100%)
- Xylene or toluene
- Forceps and scissors
- Pipettes
- Timer

Methodology:

1. **Fixation:**
    - Fix the tissue in 10% formalin for 24-48 hours to preserve cellular structure.
2. **Dehydration:**
    - Pass the fixed tissue through an ascending ethanol series (70%, 90%, 100%) to remove water.
3. **Clearing:**
    - Immerse the tissue in xylene or toluene to remove ethanol and make the tissue transparent.
4. **Embedding:**
    - Embed the cleared tissue in paraffin wax to provide support for sectioning.
5. **Sectioning:**
    - Use a microtome to cut thin sections (5-10 µm) of the embedded tissue.
6. **Staining:**
    - Deparaffinize the sections by immersing in xylene.
    - Rehydrate sections by passing through descending ethanol series (100%, 90%, 70%) to distilled water.
    - Stain with Hematoxylin for 5-10 minutes, rinse in running tap water.
    - Differentiate in 1% acid alcohol for a few seconds.
    - Blue in running tap water for 10 minutes.
    - Stain with Eosin for 1-5 minutes, rinse in running tap water.

7. **Dehydration:**
    - Dehydrate sections by passing through ascending ethanol series (70%, 90%, 100%).
8. **Clearing:**
    - Clear sections by immersing in xylene.
9. **Mounting:**
    - Mount the sections on glass slides using a drop of mounting medium and cover with a coverslip.
10. **Observation:**
    - Examine the prepared slides under a light microscope at various magnifications (e.g., 10x, 40x, 100x).

Procedure:

1. **Tissue Preparation:**
    - Carefully excise a small piece of tissue (approximately 1 cm^3) from the specimen.
    - Place the tissue in the fixative solution (formalin) for 24-48 hours.
2. **Dehydration and Clearing:**
    - Transfer the fixed tissue through a graded ethanol series: 70% ethanol for 1 hour, 90% ethanol for 1 hour, and 100% ethanol for 1 hour.
    - Immerse the tissue in xylene for 30 minutes to clear the ethanol.
3. **Embedding and Sectioning:**
    - Embed the tissue in molten paraffin wax and allow it to solidify.
    - Using a microtome, cut 5-10 µm thick sections and place them on glass slides.
4. **Staining Process:**
    - Deparaffinize the sections by immersing in xylene for 5 minutes.
    - Rehydrate sections by passing through 100% ethanol (2 minutes), 90% ethanol (2 minutes), 70% ethanol (2 minutes), and rinse in distilled water.
    - Stain with Hematoxylin for 5-10 minutes, rinse in tap water.
    - Differentiate in 1% acid alcohol for a few seconds, rinse in tap water.
    - Blue in tap water for 10 minutes.
    - Counterstain with Eosin for 1-5 minutes, rinse in tap water.
    - Dehydrate sections by passing through 70% ethanol (2 minutes), 90% ethanol (2 minutes), and 100% ethanol (2 minutes).
    - Clear sections in xylene for 5 minutes.
5. **Mounting:**
    - Place a drop of mounting medium on the section and cover with a coverslip.
    - Allow the slide to dry.
6. **Microscopic Observation:**
    - Examine the prepared slide under the light microscope starting at low magnification (10x) and increasing to higher magnifications (40x and 100x) to observe detailed cellular structures.

Results:

- The stained tissue sections will display various cellular components with different colors: nuclei (blue/purple) stained by Hematoxylin, and cytoplasm and extracellular matrix (pink) stained by Eosin.
- Detailed observations of cell shapes, sizes, and arrangements will be possible.

- Differences between cell types and tissue organization will be visible, providing insights into the microscopic anatomy of the specimen.

Observation of Stages of Mitosis and Meiosis

Aim: To observe and identify the different stages of mitosis and meiosis using prepared slides of plant and animal cells.

Principle: Mitosis and meiosis are two types of cell division processes. Mitosis results in two daughter cells each having the same number and kind of chromosomes as the parent nucleus, typical of ordinary tissue growth. Meiosis, on the other hand, results in four daughter cells each with half the number of chromosomes of the parent cell, typical of gamete formation.

Methodology:

1. **Materials Required:**
    - Prepared slides of onion root tips (for mitosis) and grasshopper testis (for meiosis)
    - Compound light microscope
    - Staining reagents (e.g., Aceto-orcein, Aceto-carmine)
    - Distilled water
    - Dropper
    - Microscope slides and cover slips
    - Forceps
    - Scalpel or razor blade
2. **Preparation of Onion Root Tip Slides (Mitosis):**
    - Grow an onion bulb in water until roots are 1-2 cm long.
    - Cut the root tips (about 1 cm) and place them in a fixative solution (e.g., Carnoy's fixative).
    - Rinse the root tips in distilled water.
    - Stain the root tips using aceto-orcein or aceto-carmine for 10-15 minutes.
    - Place the stained root tips on a slide, add a drop of water, and cover with a cover slip.
    - Gently press the cover slip to squash the root tips, spreading the cells in a single layer.
3. **Preparation of Grasshopper Testis Slides (Meiosis):**
    - Dissect a mature grasshopper to remove the testes.
    - Place the testes in a fixative solution for a few hours.
    - Rinse in distilled water and stain with aceto-carmine for 10-15 minutes.
    - Place the stained testes on a slide, add a drop of water, and cover with a cover slip.
    - Gently press the cover slip to squash the tissue and spread the cells.

Procedure:

1. **Observation of Mitosis:**
    - Place the prepared onion root tip slide under the microscope.
    - Start with the lowest magnification to locate the root tip.
    - Increase the magnification to observe the different stages of mitosis: prophase, metaphase, anaphase, and telophase.
    - Draw and label the observed stages.
2. **Observation of Meiosis:**
    - Place the prepared grasshopper testis slide under the microscope.

- Start with the lowest magnification to locate the meiotic cells.
- Increase the magnification to observe the different stages of meiosis: prophase I, metaphase I, anaphase I, telophase I, prophase II, metaphase II, anaphase II, and telophase II.
- Draw and label the observed stages.

Results:

1. **Mitosis:**
   - **Prophase:** Chromosomes condense and become visible, nuclear membrane starts to disintegrate.
   - **Metaphase:** Chromosomes align at the metaphase plate.
   - **Anaphase:** Sister chromatids are pulled apart to opposite poles.
   - **Telophase:** Nuclear membrane reforms around the two sets of chromosomes, which de-condense.
2. **Meiosis:**
   - **Prophase I:** Homologous chromosomes pair up and exchange segments (crossing over).
   - **Metaphase I:** Paired homologous chromosomes align at the metaphase plate.
   - **Anaphase I:** Homologous chromosomes are pulled to opposite poles.
   - **Telophase I:** Two haploid cells form, each chromosome still consists of two sister chromatids.
   - **Prophase II:** Chromosomes condense again.
   - **Metaphase II:** Chromosomes align at the metaphase plate.
   - **Anaphase II:** Sister chromatids are finally separated and move to opposite poles.
   - **Telophase II:** Four haploid daughter cells result, each with a single set of chromosomes.

Mitosis in Onion Root Tip Cells by Squash Method

Aim

To observe and study the stages of mitosis in the root tip cells of an onion (Allium cepa) using the squash method.

Principle

Mitosis is the process of cell division in eukaryotic cells that results in two daughter cells each having the same number and kind of chromosomes as the parent nucleus. The root tip of an onion is an ideal material for studying mitosis because it is a region of active cell division. By staining the cells and using the squash method, the different stages of mitosis can be observed under a microscope.

Materials Required

- Fresh onion
- Scalpel or sharp blade
- Beaker
- 1N Hydrochloric acid (HCl)
- Acetic orcein or Aceto-carmine stain
- Microscope slides
- Coverslips
- Filter paper
- Compound microscope
- Distilled water
- Forceps
- Burner or hot plate
- Watch glass
- Needle or teasing needle

Methodology

1. **Preparation of Onion Root Tips:**
    - Select a healthy onion and place it in a container with water such that the root end is submerged.
    - Allow the onion to sprout roots (about 1-2 cm long).
2. **Pretreatment:**
    - Cut 1-2 cm of the freshly grown root tips early in the morning (this is when cell division is most active).
3. **Fixation:**
    - Place the root tips in a beaker containing 1N HCl and heat gently for about 3-5 minutes or until the root tips become soft. Alternatively, root tips can be placed in a fixative solution (such as acetic acid and ethanol mixture) for 24 hours if heating is not preferred.
4. **Staining:**
    - Transfer the softened root tips to a watch glass containing acetic orcein or Aceto-carmine stain and let them sit for 10-15 minutes to stain the cells.

5. **Squash Preparation:**
    - Place a stained root tip on a clean microscope slide.
    - Add a drop of stain on the root tip.
    - Gently cover it with a coverslip.
    - Use a piece of filter paper to press down on the coverslip gently, squashing the root tip to spread out the cells in a single layer. Avoid excessive force to prevent breaking the coverslip.
6. **Microscopic Examination:**
    - Observe the slide under a compound microscope starting with low magnification (10x) to locate the meristematic region where cell division occurs.
    - Switch to high magnification (40x or 100x) to observe the different stages of mitosis.

Procedure Summary

1. Grow onion roots.
2. Cut 1-2 cm root tips.
3. Soften root tips in 1N HCl.
4. Stain root tips with acetic orcein or Aceto-carmine.
5. Place root tip on a slide, add a drop of stain, cover with a coverslip.
6. Gently squash the root tip and observe under a microscope.

Results

Under the microscope, you should be able to identify and distinguish between the different stages of mitosis in the onion root tip cells:

1. **Interphase:**
    - Nucleus appears intact, chromatin is not yet condensed, and nucleolus may be visible.
2. **Prophase:**
    - Chromatin condenses into visible chromosomes.
    - Nuclear membrane starts to disintegrate.
3. **Metaphase:**
    - Chromosomes align at the metaphase plate (equatorial plane).
    - Spindle fibers attach to the centromeres of the chromosomes.
4. **Anaphase:**
    - Sister chromatids are pulled apart to opposite poles of the cell.
5. **Telophase:**
    - Chromatids arrive at the poles.
    - Nuclear membrane re-forms around each set of chromosomes.
    - Cytokinesis begins, leading to the formation of two daughter cells

Experiment: Arrest and Observation of Chromosomes after Colchicine Treatment in Onion Roots

Aim

To arrest and observe the chromosomes in mitotic cells of onion root tips after treatment with colchicine.

Principle

Colchicine is an alkaloid that inhibits microtubule polymerization by binding to tubulin, thereby preventing the formation of spindle fibers during cell division. This results in the arrest of cells at metaphase, where chromosomes are most condensed and visible. This property is utilized in cytogenetic studies to observe chromosome structure and number.

Materials

- Onion bulbs
- Colchicine solution (0.05% or 0.1%)
- Distilled water
- 1N Hydrochloric acid (HCl)
- Aceto-orcein or aceto-carmine stain
- Glass slides and coverslips
- Microscope
- Forceps
- Scalpel or razor blade
- Beakers
- Petri dishes
- Watch glass
- Dropper

Methodology and Procedure

1. **Preparation of Onion Root Tips:**
    - Place onion bulbs in a container with water, ensuring only the base of the bulb is submerged.
    - Allow the roots to grow to about 1-2 cm in length, which usually takes 3-5 days.
2. **Treatment with Colchicine:**
    - Transfer the onion bulbs to a beaker containing 0.05% or 0.1% colchicine solution.
    - Incubate the bulbs in the colchicine solution for 3-4 hours at room temperature. This treatment will arrest the cells at metaphase.
3. **Fixation:**
    - After colchicine treatment, rinse the roots thoroughly with distilled water to remove any residual colchicine.
    - Cut the root tips (about 1-2 cm) and place them in a watch glass containing freshly prepared 1N HCl.

- Incubate the root tips in HCl for 5 minutes at 60°C (use a water bath if available) to soften the tissue.

4. **Staining:**
   - Remove the root tips from HCl and rinse with distilled water.
   - Place the root tips in a watch glass containing aceto-orcein or aceto-carmine stain.
   - Stain the root tips for 10-15 minutes.
5. **Slide Preparation:**
   - Place a stained root tip on a clean glass slide.
   - Add a drop of the stain on the root tip and gently tease apart the tissue with a scalpel or needle.
   - Cover with a coverslip.
   - Gently press the coverslip to spread the cells into a single layer, taking care not to break the coverslip. You can use a piece of blotting paper to press.
6. **Microscopy:**
   - Observe the prepared slide under a microscope starting with low power (10x) and then moving to high power (40x or 100x).
   - Look for cells in metaphase with well-defined and condensed chromosomes.

Results

- **Expected Observations:**
  - Chromosomes should be visible and arrested in metaphase. They will appear as distinct, condensed structures within the cell.
  - Well-prepared slides should show a number of cells with clear metaphase chromosomes.

Blood Smear Preparation and Identification of Cells

Aim

To prepare a blood smear and identify different types of blood cells under a microscope.

Principle

A blood smear is a thin film of blood spread on a microscope slide and stained to allow for the examination of blood cell morphology. The differential staining of cellular components allows for the identification of various types of blood cells, including red blood cells (RBCs), white blood cells (WBCs), and platelets. Common stains used are Wright's stain or Giemsa stain, which help differentiate between the different types of cells based on their morphology and staining characteristics.

Materials

- Microscope slides
- Cover slips
- Sterile lancet or needle
- Alcohol swabs
- Blood sample (usually from a finger prick)
- Wright's stain or Giemsa stain
- Staining buffer (usually phosphate buffer)
- Distilled water
- Immersion oil
- Microscope
- Personal protective equipment (gloves, lab coat, safety glasses)

Methodology

1. **Preparation of Blood Smear:**
    - Clean a microscope slide with alcohol and allow it to dry.
    - Prick the fingertip with a sterile lancet or needle to obtain a small drop of blood.
    - Place the drop of blood near one end of the slide.
2. **Spreading the Blood:**
    - Hold a second slide (spreader slide) at a 30-45 degree angle to the slide with the blood drop.
    - Touch the spreader slide to the drop of blood and allow it to spread along the edge of the spreader.
    - Quickly push the spreader slide forward, spreading the blood across the main slide to create a thin film.
3. **Drying the Smear:**
    - Allow the blood smear to air dry completely without using heat.
4. **Staining the Smear:**
    - Place the slide on a staining rack.
    - Cover the smear with Wright's stain for 1-3 minutes.

- Add an equal amount of staining buffer and mix by gently blowing to create a uniform film.
- Let it stand for 5-10 minutes.
- Rinse the slide gently with distilled water to remove excess stain.
- Allow the slide to air dry.

5. **Microscopic Examination:**
   - Once the slide is dry, place a drop of immersion oil on the stained smear.
   - Place a cover slip over the smear.
   - Examine the slide under the microscope using oil immersion lens (100x objective).

Procedure

1. **Collection and Preparation:**
   - Ensure all materials are prepared and that the work area is clean.
   - Use alcohol swabs to clean the fingertip and allow it to dry.
   - Prick the fingertip with a sterile lancet to obtain a blood sample.
   - Transfer the blood drop onto a clean microscope slide.
2. **Smearing:**
   - Use the spreader slide to create a thin, even blood smear.
   - Allow the smear to air dry completely.
3. **Staining:**
   - Cover the dried smear with Wright's stain.
   - Add the staining buffer and mix gently.
   - Rinse off the stain with distilled water.
   - Let the slide air dry.
4. **Examination:**
   - Place a drop of immersion oil on the dried smear.
   - Cover with a cover slip.
   - Examine under the microscope at 100x magnification.

Results

- **Red Blood Cells (RBCs):** Appear as biconcave, disc-shaped cells with a pale central area.
- **White Blood Cells (WBCs):**
  - **Neutrophils:** Multi-lobed nucleus with granules in the cytoplasm.
  - **Lymphocytes:** Large, round nucleus with minimal cytoplasm.
  - **Monocytes:** Large cells with kidney-shaped nucleus and abundant cytoplasm.
  - **Eosinophils:** Bi-lobed nucleus with red/orange granules in the cytoplasm.
  - **Basophils:** Bi-lobed nucleus with dark blue granules in the cytoplasm.
- **Platelets:** Small, purple-stained fragments of cells involved in clotting.

Total RBC count

Aim

To determine the total red blood cell (RBC) count in a given blood sample.

Principle

The RBC count is a measure of the number of red blood cells in a unit volume of blood. Red blood cells are counted using a hemocytometer under a microscope. The blood is diluted with an isotonic solution to preserve the integrity of the cells while making them easier to count.

Materials and Equipment

1. Blood sample
2. Hemocytometer with cover slip
3. Microscope
4. RBC diluting fluid (usually isotonic saline or Hayem's solution)
5. Pipettes (capillary and micropipette)
6. Test tubes
7. Rubber bulb or aspirator
8. Personal protective equipment (gloves, lab coat, safety goggles)

Methodology

Sample Preparation

1. **Collecting the Blood Sample:**
    - Use aseptic techniques to collect a venous blood sample.
    - Anticoagulated blood is preferred (e.g., EDTA tube).
2. **Diluting the Blood Sample:**
    - Mix the blood sample thoroughly.
    - Using a micropipette, draw 20 µL of blood.
    - Add the blood to 4 mL of RBC diluting fluid in a test tube to achieve a 1:200 dilution.
    - Mix the diluted sample gently to ensure even distribution of cells.

Using the Hemocytometer

1. **Preparing the Hemocytometer:**
    - Clean the hemocytometer and cover slip with 70% alcohol and let it dry.
    - Place the cover slip over the counting chamber.
2. **Loading the Hemocytometer:**
    - Using a capillary pipette or micropipette, draw the diluted blood sample.
    - Carefully charge the hemocytometer by placing a small drop of the diluted blood at the edge of the cover slip. The sample will be drawn into the chamber by capillary action.
    - Allow the cells to settle for 2-3 minutes before counting.

Counting the RBCs

1. **Microscope Settings:**
   - Place the hemocytometer on the microscope stage.
   - Focus on the grid under low power (10x objective) to locate the cells.
   - Switch to high power (40x objective) to count the cells.
2. **Counting Procedure:**
   - Count the RBCs in the five small squares (four corner squares and the central square) of the large central square on the hemocytometer grid.
   - Count the cells touching the top and left borders of the square but not those touching the bottom and right borders to avoid double counting.

Calculation

1. Calculate the total number of RBCs using the formula:

   Total RBC count(cells/µL)=Number of cells counted×dilution factorvolume of the ch amber counted\text{Total RBC count} (\text{cells/µL}) = \frac{\text{Number of cells counted} \times \text{dilution factor}}{\text{volume of the chamber counted}}Total RBC count(cells/µL)=volume of the chamber countedNumber of cells counted×dilution factor

   - Dilution factor = 200
   - Volume of the chamber counted = 0.02 mm30.02 \, \text{mm}^30.02mm3 (since each of the 5 squares is 0.004 mm30.004 \, \text{mm}^30.004mm3)

   Thus, the formula simplifies to:

   Total RBC count=Number of cells counted×2000.02\text{Total RBC count} = \frac{\text{Number of cells counted} \times 200}{0.02}Total RBC count=0.02Number of cells counted×200

Result

- Report the total RBC count in cells per microliter (cells/µL).

Example Calculation

Suppose you counted 500 cells in the five small squares:

Total RBC count=500×2000.02=5,000,000 cells/µL\text{Total RBC count} = \frac{500 \times 200}{0.02} = 5,000,000 \, \text{cells/µL}Total RBC count=0.02500×200 =5,000,000cells/µL

SEPARATION OF CELL ORGANELLS

Aim

To separate and isolate various organelles (nuclei, mitochondria, lysosomes, and microsomes) from a cell homogenate using differential centrifugation.

Principles

Differential centrifugation separates cell components based on their size and density. By subjecting the cell homogenate to increasing centrifugal forces, organelles will sediment at different rates, allowing their separation into distinct fractions.

Materials

- Fresh tissue or cultured cells
- Homogenization buffer (e.g., sucrose buffer: 0.25 M sucrose, 10 mM Tris-HCl, 1 mM EDTA, pH 7.4)
- Centrifuge and appropriate rotors (low-speed and high-speed)
- Centrifuge tubes (various sizes)
- Homogenizer (Dounce or Potter-Elvehjem)
- Ice and ice bucket
- Pipettes and pipette tips
- Vortex mixer
- Refrigerated microcentrifuge

Methodology

1. **Preparation of Cell Homogenate**
    - Harvest cells from culture or dissect fresh tissue.
    - Wash cells/tissue with ice-cold PBS to remove contaminants.
    - Resuspend cells/tissue in homogenization buffer.
    - Homogenize the suspension on ice using a homogenizer until ~90% of cells are lysed.
2. **Differential Centrifugation Steps**
    - **Low-Speed Centrifugation (Nuclei)**
        - Centrifuge the homogenate at 600 x g for 10 minutes at 4°C.
        - Collect the supernatant and pellet. The pellet contains nuclei and unbroken cells.
    - **Medium-Speed Centrifugation (Mitochondria and Lysosomes)**
        - Centrifuge the supernatant at 10,000 x g for 20 minutes at 4°C.
        - Collect the supernatant and pellet. The pellet contains mitochondria and lysosomes.
    - **High-Speed Centrifugation (Microsomes)**
        - Centrifuge the supernatant at 100,000 x g for 60 minutes at 4°C.
        - Collect the supernatant and pellet. The pellet contains microsomes (endoplasmic reticulum and Golgi apparatus).
3. **Collection and Storage**
    - Resuspend each pellet in an appropriate buffer for downstream applications or analysis.

- Store fractions at -80°C if not used immediately.

Procedure

1. **Homogenization**
   - Place the cells or tissue on ice.
   - Add homogenization buffer (10 mL per gram of tissue).
   - Homogenize using 10-15 strokes with a Dounce homogenizer.
2. **First Centrifugation (Nuclei)**
   - Transfer the homogenate to centrifuge tubes.
   - Centrifuge at 600 x g for 10 minutes at 4°C.
   - Carefully remove the supernatant to a new tube. Save the pellet (nuclei).
3. **Second Centrifugation (Mitochondria and Lysosomes)**
   - Centrifuge the supernatant at 10,000 x g for 20 minutes at 4°C.
   - Remove the supernatant to a new tube. Save the pellet (mitochondria and lysosomes).
4. **Third Centrifugation (Microsomes)**
   - Centrifuge the supernatant at 100,000 x g for 60 minutes at 4°C.
   - Remove the supernatant and save the pellet (microsomes).
5. **Resuspension and Storage**
   - Resuspend each pellet in a small volume of buffer.
   - Store at -80°C or proceed with further analysis.

Expected Results

- **Nuclear Fraction**: The pellet from the first centrifugation contains nuclei and possibly unbroken cells.
- **Mitochondrial/Lysosomal Fraction**: The pellet from the second centrifugation contains mitochondria and lysosomes.
- **Microsomal Fraction**: The pellet from the third centrifugation contains microsomes, including fragments of the endoplasmic reticulum and Golgi apparatus.

Analysis

Each fraction can be further analyzed by:

- **Protein Assays**: Determine the protein content of each fraction.
- **Enzyme Activity Assays**: Assess the presence of specific enzymes to confirm the identity of organelles.
- **Electron Microscopy**: Visualize organelles to confirm successful separation.
- **Western Blotting**: Detect specific organelle markers.