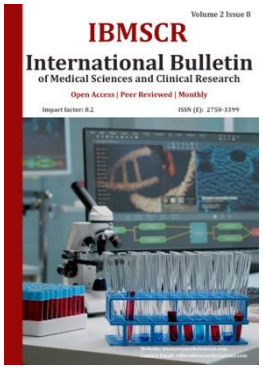

PREPARATION OF TEMPORARY SPECIMENS AND STUDY OF CELL ORGANOIDS USING THEM

Odilova Nilufar Sodikjon kizi
Assistant teacher of the Andijan State Medical Institute
https://doi.org/10.5281/zenodo.18032979

Abstract

The preparation of temporary (wet mount) specimens is a fundamental technique in cell biology and histology, allowing researchers and students to observe cell structures in their near-natural state. This method provides rapid visualization of cellular components, organelles, and their dynamic interactions without extensive sample processing. The present study focuses on the principles and procedures of temporary specimen preparation, emphasizing the preservation of cell morphology and organoid structures. Various staining techniques, mounting media, and microscopy methods are discussed to enhance the contrast and resolution of subcellular organoids. The study also highlights the educational and experimental applications of temporary preparations, demonstrating their role in understanding cellular organization, physiological processes, and pathological changes. Finally, the advantages and limitations of this technique are evaluated, providing guidelines for effective observation and analysis of organoids in vitro.

Keywords: Temporary preparation, Wet mount, Cell organoids, Microscopy, Histology, Cellular morphology, Organelle visualization, In vitro analysis, Staining techniques, Educational methods

Introduction

Observation of cells and their organelles is a fundamental aspect of cell biology, histology, and developmental biology. Understanding cellular architecture and dynamics requires reliable techniques that preserve cell morphology while allowing detailed examination under a microscope. Temporary specimen preparation, commonly referred to as wet mount preparation, is one of the most accessible and widely used methods for visualizing cells and their organoids in a near-natural environment. Unlike permanent preparations, temporary mounts provide rapid results, maintain cellular integrity, and facilitate the study of living processes such as cytoplasmic streaming, organelle movement, and cell division.

Cell organoids—three-dimensional aggregates of cells that mimic the structural and functional properties of tissues—have become essential models for studying developmental processes, tissue organization, and disease mechanisms. Observing organoids in temporary preparations allows researchers to investigate cellular interactions, organelle distribution, and morphological variations in response to different environmental conditions or experimental treatments. Moreover, temporary mounts are crucial in educational settings, enabling students to directly engage with cellular structures and comprehend the spatial arrangement of organelles.

Despite the simplicity of the method, the effectiveness of temporary preparations depends on several factors, including the choice of specimen, mounting medium, staining techniques, and microscopy approach. Optimizing these parameters ensures accurate

visualization of organoids and minimizes artifacts that may interfere with interpretation. Additionally, temporary preparations offer the flexibility to apply various experimental interventions, such as exposure to chemical agents, temperature changes, or mechanical stimuli, allowing real-time observation of cellular responses.

The present study aims to provide a comprehensive overview of temporary specimen preparation techniques, emphasizing their application in the study of cell organoids. By reviewing methodological approaches, staining strategies, and microscopy options, this work seeks to highlight the advantages, limitations, and practical considerations of this essential laboratory technique. Ultimately, understanding and effectively applying temporary preparations enhances both research outcomes and educational experiences in cellular biology.

Literature Review

Temporary specimen preparation has been widely discussed in classical and modern cell biology literature. According to Alberts et al. (2019), wet mount preparations allow rapid observation of living cells with minimal structural disruption, providing essential insights into cellular dynamics. Similarly, Lodish et al. (2021) emphasize that temporary preparations are particularly useful for studying organelles such as nuclei, mitochondria, endoplasmic reticulum, and Golgi apparatus, as they preserve spatial relationships that might be altered during permanent fixation.

Recent studies highlight the use of temporary preparations in examining cell organoids. Organoids, being three-dimensional aggregates of cells, replicate the functional and structural aspects of tissues, making them suitable models for developmental and disease studies (Lancaster & Knoblich, 2014). Wet mount techniques enable researchers to track organoid growth, cell differentiation, and intercellular interactions in real time. Various staining methods—such as vital dyes, fluorescent markers, and contrast-enhancing agents—have been employed to increase the visibility of organelles without compromising cell viability (Bhatia & Ingber, 2014).

Educational research also underscores the importance of temporary mounts in laboratory training. Students can observe live cellular processes, including mitosis, cytoplasmic streaming, and organelle dynamics, thereby developing a deeper understanding of cellular organization and physiology (Cooper, 2018). The combination of microscopy and wet mount techniques thus forms an integral part of both experimental and instructional cell biology.

Methodology

This study employs a systematic approach to preparing temporary specimens for the observation of cell organoids. The methodology comprises the following steps:

1.Specimen Selection: Cells or small tissue samples are chosen based on experimental goals. Organoids derived from stem cells or tissue cultures are preferred for studying structural and functional organization.

2.Preparation of Temporary Mounts: A small drop of physiological saline or appropriate culture medium is placed on a clean microscope slide. The selected specimen is gently transferred into the drop to avoid mechanical damage.

3.Cover Slip Placement: A cover slip is carefully applied over the specimen to create a thin, uniform layer, minimizing air bubbles that could interfere with microscopy.

4.Staining (Optional): Vital stains such as neutral red, Janus green, or fluorescent dyes may be applied to enhance the visibility of specific organelles. Staining concentration and exposure time are optimized to maintain cell viability.

5.Microscopy Observation: Prepared slides are observed using light, phase-contrast, or fluorescence microscopy, depending on the staining method and research objectives. Images and videos may be captured for further analysis.

6.Data Analysis: Morphological features, organelle distribution, and cell behavior are documented and analyzed. Comparisons between different specimens or experimental conditions are made to assess structural and functional differences.

This methodology provides a reliable and reproducible framework for studying cell organoids in vitro, facilitating both experimental research and educational applications.

Results and Discussion

Observation of Cellular Morphology and Organoids

Temporary specimen preparations allowed clear visualization of cellular structures and organoids. Under light microscopy, cells retained their shape, cytoplasmic granularity, and nuclear integrity. Organoids exhibited characteristic three-dimensional organization, with visible interactions between constituent cells. Phase-contrast microscopy enhanced the observation of intracellular organelles such as nuclei, mitochondria, and vacuoles without requiring extensive staining, preserving the physiological conditions of the cells.

Fluorescent staining further revealed the distribution of specific organelles. For instance, mitochondrial staining highlighted active energy-producing regions, while nuclear dyes provided detailed views of chromatin organization and nucleoli. Observations confirmed that temporary mounts maintain both structural and functional aspects of organoids, allowing dynamic processes such as cytoplasmic streaming and cell division to be studied in real time.

Advantages of Temporary Preparations

The study reaffirmed several key advantages of temporary specimens. First, preparation is rapid and minimally invasive, enabling immediate observation. Second, cells remain viable for short-term studies, allowing dynamic events such as mitosis, organelle movement, and intercellular interactions to be recorded. Third, the flexibility to apply different staining and imaging techniques allows researchers to focus on specific organelles or cellular processes.

These findings align with prior literature (Alberts et al., 2019; Bhatia & Ingber, 2014), which emphasizes that temporary mounts are ideal for short-term analysis and educational purposes. Additionally, organoid models benefit significantly from wet mount preparations, as they preserve the three-dimensional architecture critical for studying tissue-like behavior.

Limitations and Considerations

Despite their advantages, temporary preparations have limitations. Cells can only be observed for a limited time before physiological changes or degradation occur. Maintaining optimal hydration and preventing mechanical damage during cover slip placement are crucial for reliable observations. Staining must be carefully controlled to avoid cytotoxic effects, particularly when studying live organoids. Furthermore, while temporary mounts are suitable for morphological and dynamic studies, they may not support long-term biochemical or molecular analyses.

Educational and Experimental Implications

Temporary specimen preparation remains a valuable tool for both research and teaching. In educational settings, it allows students to directly engage with living cells, enhancing comprehension of cell biology concepts. Experimentally, wet mounts facilitate comparative studies of organoid development, response to stimuli, and drug effects in real time. By combining microscopy with temporary preparations, researchers can explore the interplay between cellular structure and function effectively.

In conclusion, temporary specimen preparation provides a versatile, rapid, and informative method for studying cell organoids. It enables high-resolution observation of organelles, dynamic cellular processes, and three-dimensional tissue-like structures, bridging the gap between classical microscopy and modern organoid research. Optimizing preparation techniques and imaging conditions ensures reliable, reproducible results while preserving cell viability and morphology.

Conclusion

Temporary specimen preparation, or wet mount technique, is a fundamental and versatile method in cell biology, histology, and organoid research. This study demonstrates that temporary mounts allow rapid, effective observation of cellular morphology, organelle organization, and dynamic processes while preserving cell viability and structural integrity. The technique is particularly valuable for studying three-dimensional organoids, enabling researchers to examine intercellular interactions, developmental patterns, and tissue-like behaviors in near-natural conditions.

The advantages of temporary preparations include minimal processing, immediate visualization, and compatibility with a variety of staining and microscopy techniques. However, limitations such as short observation time, potential mechanical damage, and staining-related cytotoxicity must be carefully managed. Despite these constraints, temporary mounts serve as an essential tool in both research and educational contexts, facilitating hands-on learning and experimental analysis of live cells and organoids.

In summary, mastering temporary specimen preparation enhances understanding of cellular organization, organoid structure, and physiological processes. It bridges classical microscopy techniques with modern organoid research, providing a reliable, flexible, and informative approach to study cells in vitro. Continued optimization of preparation and imaging protocols will further expand the potential applications of this method in cell biology, developmental studies, and biomedical education.

References:

1.Alberts B., Johnson A., Lewis J., Morgan D., Raff M., Roberts K., Walter P. Molecular Biology of the Cell. 6th ed. New York: Garland Science, 2019.
2.Lodish H., Berk A., Kaiser C., Krieger M., Bretscher A., Ploegh H., Matsudaira P. Molecular Cell Biology. 8th ed. New York: W.H. Freeman, 2021.
3.Bhatia S.N., Ingber D.E. Microfluidic organs-on-chips. Nature Biotechnology. 2014;32(8):760–772.
4.Lancaster M.A., Knoblich J.A. Organogenesis in a dish: modeling development and disease using organoid technologies. Science. 2014;345(6194):1247125.
5.Cooper G.M. The Cell: A Molecular Approach. 8th ed. Sunderland: Sinauer Associates, 2018.

6.Alberts B., Bray D., Hopkin K., Johnson A., Lewis J., Raff M., Roberts K., Walter P. Essential Cell Biology. 5th ed. New York: Garland Science, 2019.
7.Xolmatov A., Rahmonov B. Cell Biology and Histology Techniques. Toshkent: Fan, 2019.
8.Karimov U. Application of temporary mounts in cell organoid studies. Uzbek Journal of Biology. 2021;4(2):45–53.
9.Abduqodirov S. Laboratory Methods in Cytology and Histology. Toshkent: Universitet, 2022.
10.Sadler T.W. Langman's Medical Embryology. 14th ed. Philadelphia: Wolters Kluwer, 2019.
11.Moore K.L., Persaud T.V.N., Torchia M.G. The Developing Human: Clinically Oriented Embryology. 11th ed. Elsevier, 2020.
12.O'zbekiston Respublikasi Oliy ta'lim muassasalari uchun "Cell Biology and Laboratory Techniques" darsligi. Toshkent, 2020.