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Biochemical Aspects of Cell Staining

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Author's contribution

Author TS solely conduct all the activity from designed the study, wrote the protocol and wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches.

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ABSTRACT

Aims: to revisited the biochemical aspects of basic, negative and positive staining by dyeing organic materials in the laboratory for the purpose of visualization, identification and even differentiation of object being analyzed.
Discussion: The best analysis for cells is by staining. Staining allows detailed visualization of the part of the cell being examined. In principle, dyes have a chromophore root that will bind to the protein content in cells. This is the basis for discussing negative staining, basic staining and positive staining. Unfortunately, its widespread use also increases the health risks of users.
Conclusion: Biochemical aspect of staining rely on the electric charged of the dye that bind the electronegative properties of proteins that build cell. Basic staining, negative staining and positive staining are widely used staining for research, clinical-laboratory service and also education. Unfortunately, some of these stains are hazardous with the potency of multiple health risk and continuous improvement in handling will prevent such risk to happen.

Keywords: Cellular imaging; dye; organic material; positive; negative; laboratory.

1. INTRODUCTION

Cells, live or dead, are ultimately better to be colored or stained to magnify microscopic visualization of the whole cell or certain components or area within the cell [1]. Cells are sometimes also stained to apotheosis crucial metabolic processes [2-5] or to differentiate

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between live and dead cells such as in the process of apoptosis or programmed cell death [6-8]. Normal living cells always have intact membranes [9] that exclude dyes while on contrary dead cells absorb and allow its permeabilization to dye substrate to enter its internal milieu [10]. A normally membrane-impermeable dye enters a cell only when its membrane has been compromised or in simple word the cell is dead [11]. This is the basis for a dye exclusion assay accustomed to ascertain the viability of cells being observed [12].

Cellular visualization by dyeing plays an increasingly important role in basic and applied biology [13] or even in clinical observation [14]. Cell staining is a technique that can be used to better visualize cells and cell components under a microscope. By using different stains, one can preferentially stain certain cell components, such as a nucleus or a cell wall, or the entire cell.

The aim of this review is to revisited the biochemical aspects of basic, negative and positive staining by dyeing organic materials in the laboratory for the purpose of visualization, identification and even differentiation of object being analyzed.

2. STAINING PRINCIPLE AND GENERAL PROCEDURE

This approaches simply facilitate analysis of the cell's target region that can be expressed in the context of their external, e.g., capsule staining using lipophilic dye [15] or when analyzing the structural, ultrastructural and cellular components of the cell wall [16] or internal portion where its organelle exists [17] and even go deeper to a complex environment regarding metabolic activity of the cell [18]. These long lists of possibilities for which subcellular localization can be aimed and directed in order to get the best cell image for further analysis, quantitatively [19] and qualitatively [20]. Staining the cell carefully is basically the methods to extract visual information from these potential vast and complex data sets [21]. Image analysis methods for cell, dead or alive, in particular required tissue or cell preparation and processing [22]. Before putting specific staining, tissue or cell samples must engage in preparation through the ensuing phases: Fixation, processing, embedding, sectioning, and sometimes antigen retrieval; steps that in modern histology laboratories, most of them are already automated [23].

The core of cell or tissue sample preparation for staining is undoubtedly the fixative engaged [24-26]. Its main purpose is to preserve the tissue's cellular structure and molecular constituents as authentically as possible; the way fixatives stabilizes the tissue through enzyme or proteins deactivation and through other vital components [27,28]. "Fixation consists of two major steps: cessation of normal life functions in the tissue (killing) and stabilization of the structure of the tissue (preservation). Fixation initially destructed the integrity of cell membranes and increased the permeability of intra- and extra-cellular molecules, it was permeabilization process that substantially induced significant loss in cellular mass density" [29,30].

Almost all tissue stains are performed on tissue that has been removed from the body [23]. Unfixated cells are prone object to rapidly demolish outside or apart from their main tissue. Those rapid changes in its morphology is due to the immediate activation of digestive organelle called lysosome, a membrane-enclosed organelle that mediate the intracellular degradation of macromolecules [31], that contain digestive enzyme digest and destroy all the structure of the cells, start from the inner part until complete lysis of the cell [32].

Even though, if a cell dies, its remaining debris or ghost of the cells might probably available and can be seen. But the main reason for staining a cell is to visualize its morphology as similar to when the cell was still alive and functioning.

The normal tissue sections, without staining, actually appear colorless and different structures cannot be appreciated [33], e.g., as the fixed protein have the same refractive index as that of glass.

Dyes are used to impart color to the different components of the tissue for interpretation [34]. Staining the tissue sections by different colored dyes, having affinities towards certain components of the tissues [35] that simplify identification effort and also enhance their morphology appearance.

"Certain type of dye available. Basically, a chromogenic dye not only adds contrast by absorbs the light of a particular wavelength of the white light representing a specific color and emits the light containing the rest of the color, but also give distinctive color as well" [36]. "Therefore, observer see a colored light from the dye. The

dye can be classified on the basis of electrical charge or chemical structure" [35]. "The staining is the combination of a colored dye with the tissue that retains the dye after washing" [37]. "It is primarily a chemical reaction between the dye and the tissue and the common chemical reactions are covalent bond, electrostatic bond, hydrogen bond, Vander Waals attractions, hydrophobic bond and dye aggregations" [35-37,38].

"Staining itself is basically the artificial coloration of a substance to facilitate its examination by the use of a colored organic molecule called dye" [48] "The process can be technically performed progressively (the dye only interacts with the tissue until the proper stain is achieved) or regressively (the tissue is overstained and the excess of unabsorbed dye is afterwards removed)" [35]. At its simplest, the actual staining process may involve immersing the sample (before or after fixation and mounting) in dye solution, followed by rinsing and observation. Many dyes, however, require the use of a mordant: a chemical compound which reacts with the stain to form an insoluble, colored precipitate. When excess dye solution is washed away, the mordant stain remains.

In addition, with the purpose of identifying more than one cellular component in a sample; the staining can be achieved by either simultaneous or successive addition of dyes, which selectively stain certain structures [39]. Thus, double, triple and also multiple staining can be performed. The following section will continue to discuss several types of biological staining with emphasize on its biochemical aspect.

2.1 Type of Staining

Sometime, classification being made as simple staining (the procedure of using only one stain on the slide) and differential staining **(**a procedure where more than one dye is used to differentiate between different types of microorganisms on a slide. This type of staining helps to differentiate between cell types and cell structures). Due to the complexity of staining properties and its use, sometimes it also simply differentiates into negative staining, basic staining, and positive staining. Negative and positive staining are two types of staining techniques that are differentiated based on their ionic properties.

2.2 Negative Staining and Its Biochemical Aspect

"Negative staining colors the background surrounding the cell but not the cell itself. The result is a dark colored halo around a clear cell. Dyes used in this technique are acidic dyes, which have a negative charge. There is no need to prepare a bacterial smear or heat-fix the sample prior to staining. A simple staining method for bacteria or other organisms which is usually successful even when the "positive staining" methods detailed below fail, is to employ a negative stain" [40,41]. **"**Negative staining is one of the many staining techniques that can be employed for viewing of bacterial cell morphology and size. This can be accomplished simply by smearing the sample on to one side of the slide, then followed by an addition of nigrosine or Congo red or Indian ink" [40-44]. After drying or after the application of Indian Ink on to wet culture of the microorganisms (virus or bacterial or yeasts) may be viewed in light microscopy [45] or bright field microscopy [46] or transmission electron microscope [47] as lighter inclusions well-contrasted against the dark environment surrounding the organism being observed [40-44]. Negative staining is a mild technique which may not destroy the microorganisms therefore it is unsuitable and not recommended for studying highly contagious pathogens [48].

Negative staining employs the use of chemically specific acidic stain [41] and, due to repulsion between the negative charges of the stain and the bacterial or other microorganism surface (bacterial or microorganism surface charge is a critical characteristic of the cell's interfacial physiology that influences how the cell interacts with the local environment [49]), and this will cause the dye unable to penetrate the cell [40-42]. In negative staining, the results yield a clear cell with a dark background. The negative staining technique involves the use of an Acidic stain, for example, methylamine tungstate, Uranyl acetate, phosphotungstic acid, or Phosphomolybdic acid. Generally, an acidic stain Readily donates a hydrogen ion (proton) resulting in the Chromophore of the dye becoming negatively charged. As the cell surface of the majority of bacterial cells is Negatively charged, therefore, it would repel and block The penetration of the negatively charged chromogen of the acidic stain. This results in the unstained bacterial or yeast cells being easily distinguished against the Colored or darker background.

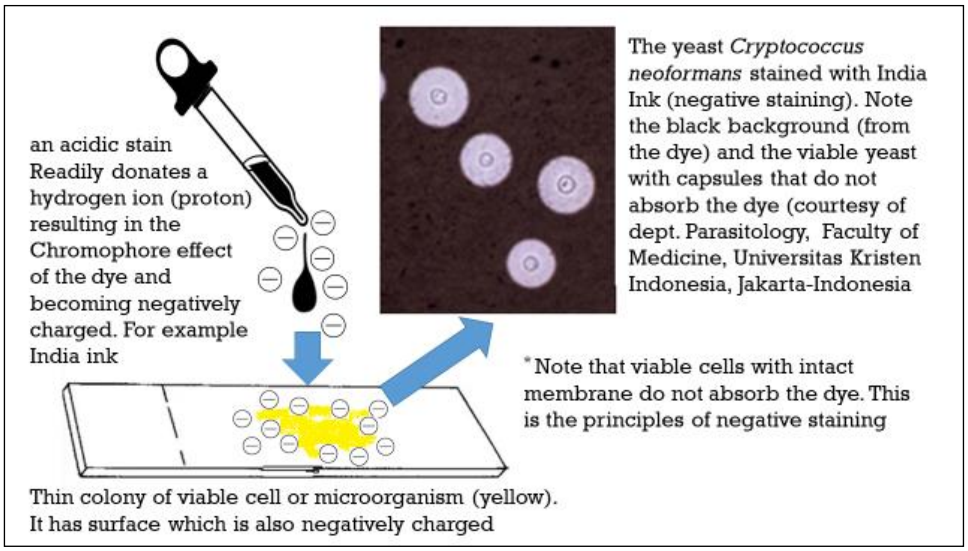

Fig. 1. Shematic illustration of negative stain. Photo of the yeast *cryptococcus neoformans* stained with India ink (negative staining) was generously provided by dr Forman Erwin Siagian of the dept. Parasitology, Faculty of Medicine, Universitas Kristen Indonesia, Jakarta, Indonesia

This technique will remain an important tool for the analysis of very small sized living organisms, e.g., the assembly and architecture of virus [50] or the appraisal of biological/biochemistry macromolecules, e.g., reconstruction of smooth muscle thin filament images [51] or analysis of fraction of tight junction, which was first delineated as membrane fusion near the apex of epithelial and endothelial cells [52].

“Due to its ability to produce higher and better visual contrast on the section/portion being analyzed, a large amount of small-scale molecules can be better visualized by negative staining” [42]. “Also, while molecules prepared by vitrification (the full or partial transformation of a substance into a glass, that is to say, a non-crystalline amorphous solid) which usually adopt random orientations in the amorphous ice layer, beneficial effect of negative staining tends to persuade selected orientations of the molecules on the carbon support film”, as reported by Sahiro et al [53]. Combining negative staining with image classification techniques [42,54] facilitate researchers to accommodate with very heterogeneous molecule populations [55,56], which are difficult or even impossible to analyze by just employing vitrified specimens.

2.3 Basic Staining

The main constituents engrossed in cell staining are dyes and the proteins constituent of the cell being analyzed [57-60]. The most essential step is the involvement of the chemical bonding between part of carboxyl groups of one side and part amino groups of the other side [37]. The most common bonds involved are ionic bonds, where it exploits electrostatic attraction between ions of opposite charge, one of which is fixed in the tissue and the second of which is in the dye [60]], although there are exceptions especially in the case of nuclear staining of DNA which usually hydrogen bond [35,61,62,63].

The application of coloring substance aimed to visualize, identify and analyze an organelle or other separate components of cell element or even tissue segments is accomplished primarily by the addition of chemical dyes. although other effort is occasionally used. Dyes, however, are the largest group that can be engineered, molecularly [64] or structurally [65].

Basic stain is a simple method of staining. Simple staining embraces directly adding the dye to the thin colony of microorganism [41]. The negatively charged cell wall of this organisms will be strongly stained with an electropositive dye in order to visualize its properties in detail, can be its outer portion, its cell wall or its inner part/organelle [57,58]. This is different to negative staining where the bacteria remain in its previous condition unstained and only the background stained with the dye [40,41].

Table 1. Difference between acidic stain, basic stain and neutral stain

Type of Stain	Acidic stain	Basic stain	Neutral stain
Electric charge Used to/attracted to	Electronegative stain cytoplasm and other acidophilic (acid-preference) cellular structures in tissues.	Electropositive negatively charged molecules in the cell including nucleic acids (DNA & RNA) and some proteins	Contain both Electronegative and Electropositive In the case of neutral red staining, has been used to stain living cells and fixed tissue. It can be used as a general-purpose stain, a pH indicator (turning from red to yellow, as the medium becomes alkaline), or a nuclear stain.
Name of staining	Nigrosin, Eosin, Carbol Fuchsin, India Ink, Malachite green, etc	Crystal violet, Methylene blue, Safranin, etc	Giemsa, Leishman, Wright, etc

Basic stains are strongly attached to the existence of electronegative molecules in the cell, including nucleic acids (DNA and RNA) and some proteins [35]. Basic stains are often more preferred for bacterial staining than acidic stains due to its effectiveness in coloring; its benzene properties are unable to impart color to acidic stains, but readily imparts color to basic stains, as can be seen in Gram stain where basic fuchsin stains gram-negative organisms more intensely than safranin [59].

Negatively charged cellular elements that contain protein, e.g., the cell walls and DNA, will strongly attach to and receive the color suspension which electropositive charged chromogen (a coloring molecule which often a benzene derivative) of a basic stain [2]. Basic stains fluoresce under the light microscope [9], making cellular components readily visible to examiner [66]. Basic stains are anionic and the electronegative charged stain is readily taken by the electronegative charged of components of the cell [67]. Different from negative staining dyes (e.g. nigrosine), acidic stains will demolish the organisms being analyzed by way of damaging the cells (except for acid fast bacteria) [68] due to the its low pH properties [69].

Basic stains include methylene blue, crystal violet, malachite green, basic fuchsin, carbolfuchsin, and safranin. Acidic stains include eosin, acid fuchsin, rose bengal, and Congo red.

2.4 Positive Staining

Dissimilar to negative staining, positive staining applies basic dyes to color the clinical specimen (cell or tissue) against some clear, bright surroundings [47]. While chromophore is used for both negative and positive staining alike, the type of chromophore used in this technique is an electropositive charged ion instead of a negative one [2]. Because common cells being analyzed typically consists of cell walls which is electro negatively charged, the positive chromophores in basic dyes tend to strongly attaches to that type of cell walls; and because of this bond, it then considered as electropositive stains.

Widely used basic dyes such as crystal violet [70], methylene blue [71], basic fuchsin [72], malachite green [73] and even safranin [74] typically serve as positive stains. Positive staining colors the whole body of the cell that absorb the dye. Dyes used in this technique are basic dyes, which have a positive charge. Before staining, the bacteria are smeared onto a slide and heat-fixed. Example of famous and widely used positive stain are Gram staining or Crystal Violet staining

Some of the stains mentioned previously are synthetic formulated stains. It may have engineered in such a way that after becoming a dye, it might be harmful to human health [75]. Continuous and regular exposure to them possesses serious health risk to human and also environment [76]. Many of the synthetic stains are found to be carcinogenic [77], mutagenic-cytotoxic [78], genotoxic [79] an even immunotoxin [80]. Continuous improvement in safe handling and disposal is very important from the point of view of health and environmental [81].

3. CONCLUSION

Biochemical aspect of staining relies on the electric charged of the dye that bind the electronegative properties of proteins that build cell. Basic staining, negative staining and positive staining are widely used staining for research, clinical-laboratory service and also education. Unfortunately, some of these stains are hazardous with the potency of multiple health risk and continuous improvement in handling will prevent such risk to happen.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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