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APPLICATIONS OF MICROSCOPY IN BIOLOGY AND MEDICINE: REVIEW ARTICLE

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ABSTRACT

In today's scientific world community, microscope is commonly used instrument in the area of biology and medicine.Recent advances related to microscopy have led to such remarkable improvements in fluorescence based microscopies for contrast and sensitivity that researchers can now engagedinidentifying and detect various signals at the single molecule level.Although much more expensive, electron microscopes have their place in high-throughput studies that are neededfor extreme magnification. New histology advancements or techniques, commonly used stainsand dyes in the form of flurochromes areutilized to enhance its clearness of biological specimens that are continually evolving to upgrade the value of the light microscope and maintained the gap between the light microscope and upgraded microscopes in terms of magnification for observing living biological specimens. The development of electron microscope in terms of magnification has been projected by humans pertaining to explore and understand the world around us. In this review, discuss about the applications of microscopy in different aspects of biology and medicine.

KEYWORDS: microscope; fluorescent; applications; biology; medicine.

INTRODUCTION

As per the literature, organisms exist in the universe as single cells or made up of billions or trillions of cells whereasin case of complex organisms, cells group together with similar type of cells to form tissues and ultimately to produce organs and perform various biological processes carried out with in the cells that are necessary to maintain the life in the cell.[1,2] Like all living things, humans are also composed of cells but these cells should be observed under the ordinary microscope in the form of smear on glass slide but the organelles that exists within the cells that are so small[3] and cannot be able to resolve (even at high power 40X) with our light microscope.[4]

The term microscope was first coined by members of first scientific community (Academia dei Lincei; includes Galileo). In 1590, Scientists (Hans and Zacharias Janssen, Middleburg, Holland) produced first compound microscopes and Marcello Malpighi circa (1660), first microscopists, considered the father of embryology and histology whereas Robert Hooke (1635-1703) published a book Micrographia in the year 1665 and mentioned about his studies related to microscopy using thin slices of cork.[5,6,7,8] In contrast, earliest microscopes come across and reported bacteria, free living and parasitic microscopic protists, sperm cells, blood cells, microscopic nematodes etc.

In 1730, Chester More Hall observed that flint glass (newly made glass) dispersed colours much more than crown glass (older glass) using concave lens next to a convex lens which could realign all the colours and was considered as first achromatic lens.[9] At that time, George Bass was the lens-maker that actually made the lenses, but he did not disclose the secret for 20 years later on John Dolland who copied the idea in 1759 and patented the achromatic lens.[10] Giovanni Battista Amici (1827) produced high quality microscopes and introduced as well as established the first matched achromatic microscope.[9,10] In 1813, Giovanni Battista Amici outlined or designed reflecting microscopes using curved mirrors rather than lensesand recognized the importance of coverslip thickness and developed as well as learned the concept of water immersion.[11] In addition, Abbe and Zeiss developed oil immersion systems by making oils that matched the refractive index of glass and able to fabricate a numeric aperture to the maximum i.e. 1.4 allowing light microscopes to rectified two points distanced only 0.2 microns apart (theoretical maximum resolution of visible light microscopes).[12] During early 20th Century, Professor Köhler developed the method of illumination called as Kohler Illumination[13] and recognized that using shorter wavelength light (UV) could improve resolution.[13] In terms of resolution and magnifications, these

microscopes showed various applications in the area of biology and medicine.

- **Cancer stem cells**

Awareness of stem cell biology and its molecular basis is now appropriate paramount importance in cancer research. In this study, cancer stem cells has the potential to control their fate by maintaining themselves in undifferentiated state or by self-renewing to geometrically expand their number. Firstly, asymmetric division which gives origin to two different daughter cells i.e. first progeny is devoted to expansion by multiple cellular division and differentiation to produce the entire heterogeneous cell population and then formed tumor. Secondly, cancer stem cells does not enter the cell cycle (G, S and M phase) and rests as a reservoir i.e. able to self-renew. A typical stimulus involving the stem cell compartment expansion i.e. response to injury, e.g. repopulation of damaged or disrupt bone marrow in case of hematopoietic stem cells. In this case, stem cells giving rise to two new cells with identical stem cell fate and proliferation potential (split in a perfectly symmetric manner). In this regard, we need to look possibly living cellsuses fluorescence microscopy and confocal microscopy that are valuable instrument in the study of stem cells.[14,15,16]

Stem cell technologies are included as acentre of attention in regenerative medicine and it involves culturing along with differentiation of stem cells and generate tissue-specific cells. Those tissues that can be introduced into the body to help repair organs or to express vital proteins. Similarly, pluripotent stem cell should remain undifferentiated during sub culture but it has the capability to differentiate any type of tissue induced by specific cell culture conditions. Identification, association and characterization of these stem cells in culture and in tissue can be carried out with microscopes i.e. stereomicroscopes (high zoom); microscopes (confocal, multiphoton and super-resolution) that enable imaging with molecular resolution.[15,16] The most familiar example i.e. human embryonic stem cells (hESC e.g. cell lines like LRB008, LRB010, etc.) were used to derived cell lines cultured in a humidified atmosphere consisting of 5% CO_2 and 37ºC temperature. Now, these cell lines should be harvested for transmission and scanning electron microscopy (SEM).[17]

Normally, living hESCs often appear equivalent undifferentiated in their colony formation, as noticed as well as observed in phase contrast microscopy but researchers noted various differentiation events occur after every passage (5-7 days to maintain hESCs both at ultra-structural and light microscopic level). In addition, group of cells that directly connected with culture medium formed a complex i.e. linkage of adjacent cells through junctional complexes contained tight adherens junctions as shown by electron microscopy and immunohistochemistry. In addition, it also provides some information about central compartment containing cells comprises of undifferentiated embryonic stem cells morphology and indicates its long term retention of an environment permissive of undifferentiated cell growth, regardless of surrounding peripheral differentiation. Finally, these microscopic based observations are helpful in morphological undifferentiated hESCs that are grown for prolonged period of time (two weeks or more) and is normally observed in the central and basal compartments which are in direct communication with bottom of the culture dish without tight junctions underneath layers of more differentiated cells on top of the colony.[15,16,17]

- **Immunology and virology**

Applications in different aspects of Immunology as well as virology based studies (cell lines and animal models) that are totally reliedon fluorescence based tools i.e.cell based assays using fluorochromes e.g. flow cytometry, luminex, fluorescence microscopy etc.[18] The advantage of these fluorescence tools is their inherently greater sensitivity and range in comparison to methods based upon changes in optical density or chemiluminescent emission and the latter or final process emits one photon per molecule in comparison to hundreds to thousands of photons emitted by one fluorochrome.[18,19]

For the last ten years, flow cytometry has contributed greatly in the field of immunology and virology applications including some diverse areas i.e. characterization of cell populations and the selection of hybridomas using different fluorochromes. But this instrument showed detection and quantification of cell surface antigens but it does not showed any qualitative characteristics (distribution and locations of constituent molecules/fluorochrome and cannot be extended to in vivo experiments). Due to these features, fluorescence microscopy showed some importance in the area of immunological and virological applications related to structural analysis of immune cells and tissues.[18,19,20]

In virology, virus particles are detected and identified using electron microscopy on the basis of morphology including magnification (around 50,000 is normally used). This electron microscope is recurrently used for diagnosis and detecting some viruses in clinical samples e.g. rotavirus, adenovirus, astrovirus, calicivirus, Norwalk-like viruses etc.[19,20,21] In addition, light microscopy also be used for histological studies related to virus replicating in infected cells either in nucleus or cytoplasm. In short, histological studies serves as competent adjunct related toidentification and recognition of viral pathogens.[20,21]

- **Entomology**

The majority of entomological studies are done throughmicroscopy using low power (typically stereo zoom or digital) microscopes. Most of the entomologists also desire to arrest high resolution images of their specimens.[22] The most commonly used types of microscopes for entomological studies as shown below-

- Stereo zoom microscopes- Used for dissection, identification and photography.
- Dual magnification stereo microscopes- optics for dissection andidentification.
- Biological upright microscopes – used for studies of slide-mounted micro-insects and parts.
- Digital microscopes- used for viewing and recording insects.
- Portable field microscopes - Study insects in their natural environment

- **Limnology/Microbiology**

The main goal of limnological studies is to identifythe biological specimens (e.g. planktons, benthic micro-organisms etc.) and these were examined through microscopy techniques. The first objectiverelated to limnological studies is to studied various physic-chemical properties of water bodies (lakes, ponds and rivers) and second one is to recognized various organisms in water bodies e.g. benthic micro-organisms, planktons etc.The microscopic evaluation techniqueinvolves identification, size and percentage population estimates of microorganisms (autotrophic organisms e.g. algae; fresh water plankton e.g. Animalia, monera etc.; arthropods- segmented exoskeletons) and organic or inorganic debris found in water.[23]

Microorganisms incorporates the members of plant kingdom, protozoa, bacteria, and fungi. These organisms are totally different in terms of shape and size. Most of these organism are observed underthe microscope e.g. bacteria (gram positive or negative) are available in virtually every type of environment and are reported in plenty in all aquatic system but these were identified on the basis of giemsa staining and observed under the microscope. In addition, aquatic fungi are microscopic called as hypomycetes (most abundant and important); protozoa (microscopic, single celled and growing in colonies) etc.[24]

CONCLUSION

Microscopy is the biggest tool for researchers pertaining to reveal the mystery and beauty of unseen world. In other words, microscopes are of great importance in the study of micro-organisms and biomolecules. The most valuable applications in the field of biology and medicine is to analysed live blood cells, stem cells and other biological specimens using different fluorochromes etc. In contrast, electrons, x-rays and infrared rays are used by far more sophisticated and expensive microscopes to detect even smaller and smaller structures.

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