Cellular pathology: dyes and stains

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Introduction

In the study of tissues, histological staining is important in order to study cellular structures, intracellular and extracellular substances at the microscopic level (Stevens and Lowe, 1997). Staining is an auxiliary technique used in microscopy to enhance contrast in images obtained and to highlight structures. Stains may be used to define and examine bulk tissues, cell populations or organelles within individual cells; histological features useful for biological research and/or diagnosis in medicine (Bancroft and Cook, 1994). The importance of dyes in identifying normal and abnormal histological features of tissues is herein discussed.

Medical and biological research is underpinned by knowledge of the normal structure and function of cells and tissues as well as the organs and structures they make up (histology) Understanding disease in the context of structure-function relationships (histopathology) enables differentiation between normal tissues and abnormal tissues in a particular disease state (Cook, 2008). The differentiation of these enabled by the identification and understanding of the divergence of normal and abnormal histology is highly beneficial in disease diagnostics and therapeutics (Bancroft and Gamble, 2008).

Such essential study disciplines are based on a thorough understanding and ability to recognise basic tissue types which combine to form the different organs of the body. Understanding normal structure of tissues is essential to the identification of altered structure (Lakhani, et al., 1998). With knowledge of normal histology, one can see the types, location and scope of cells involved in disease, whether their intrinsic morphology is impacted indicating

cellular dysfunction, and whether higher order tissue structure is impaired which indicates organ dysfunction (Stevens and Lowe, 2000). Histopathology, on the other hand, encompasses the means to verify accurate models of particular diseases based on understanding the visual picture of molecular mechanisms differentiated from normal (Kiernan, 1999).

In the normal healthy state, cells and other elements of tissue are arranged in regular recognisable patterns. Tissues usually have particular defining characteristics such as surface structure and shapes and formations of constituent cells which are used in their identification and assessment of function (Stevens and Lowe, 1997). Changes in these patterns can be induced by a wide range of chemical and physical influences such as microbial infection and cell malignancy in cancer are reflected by structural alterations at the microscopic level (Lakhani, et al., 1998). Many diseases such as Cancer are also characterised by typical structural and chemical abnormalities which vary the normal pattern of tissues (Lakhani et al., 1998). This is the basis of microscopic examination of specimens.
Examination of various specimen and differentiation of structures is challenged as tissue sections or smears obtained from biopsies or aspirations appear dull and less detailed when viewed in light microscopy. This is because the fixed materials in the preparation have a similar refractive index and have a similar grey colour which makes it difficult to identify the structure of the tissues (Kiernan, 1999). It is essential to stain the cells/tissues to enable better visualisation of the different structures in contrasting colours (Bancroft and Cook, 1994). Staining is most commonly carried out through the use of histological dyes which are coloured organic

compounds obtained from natural sources or from synthetic production that selectively bind to or concentrate in various cell and tissue structures (Kiernan, 1999). Dyes contain auxochromes which are chemical components that enable attachment to tissue such as the ionisable –OH group, and chromophores which are substances added to absorb visible light responsible for the colour observed. Colour arises when an attached chromophore molecule absorbs certain wavelengths of visible light (Bancroft and Gamble, 2008). Most modern dyes such as the Haematoxylin and Eosin stains commonly used are synthesised from simpler organic molecules, usually benzene or one of its derivatives (Kiernan, 1999).

Stains are generally aimed as special probes, which possess variable specificity depending on the unique ionization or chemical reaction with tissue structures and components (Stevens and Lowe, 1997). Staining does not result in a random colouring of the tissue specimens, but rather exploits the differences in the chemical structure of the tissue. This is shown by colour variation depending on which dye is bound. Colours acquired reflect the nature of the tissues and their properties and proffers an advantage in the revelation of specific parts or areas (Cook, 2008). This enables detailed visualisation of structures including cell structures such as the cytoplasm, nucleus and organelles, as well as extra-cellular components. Additionally, under certain conditions such as glycogen storage diseases, staining (in this case using the Periodic acid-Schiff (PAS) to detect carbohydrates) can reveal molecular compounds and differences associated with pathological conditions (Lakhani, et al., 1998).

Enhanced capacity for visualisation and identification of structures is the primary advantage for the use of dyes in staining of tissue specimen. Tissue

staining therefore plays a critical role in tissue-based diagnosis and research allowing the visualization of tissue morphology and histological features, and in distinguishing normal and abnormal histological features (Cook, 2008; Stevens and Lowe, 1997; Kiernan, 1999). These observations are sufficient to allow analysis of tissuehealthand diagnosis of disease.

Histological dyes commonly used for staining in light microscopy include the Haematoxylin and Eosin stain (H&E), Van Giessen, Masson's Trichrome, and Periodic acid-Schiff (PAS), among others. The H&E stain is the most commonly used stain for light microscopy in histology and histopathology. It is routinely used as it provides a very detailed view of the tissue achieved by staining cell structures staining the nuclei a dark blue or purple, and the cytoplasm and connective tissue in shades of pink (Cook, 2008). Staining using these and other dyes forms a critical part of thediagnosticpicture given the sufficient contrast obtained for the display of tissue morphology (Stevens and Lowe, 1997).

In conclusion, staining is an essential process in histology and histopathology with its primary advantage being the enhancement of contrast between different components of the tissue specimen, particularly as seen in light microscopy. The overall objective of histology is to acquire knowledge of normal tissues and organs, which is essential to understanding the altered structure and function of diseased cells, tissues and organs. There is no doubt that the use of dyes to allow for differentiation between normal and abnormal tissues is fundamental to our understanding of this.

References

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