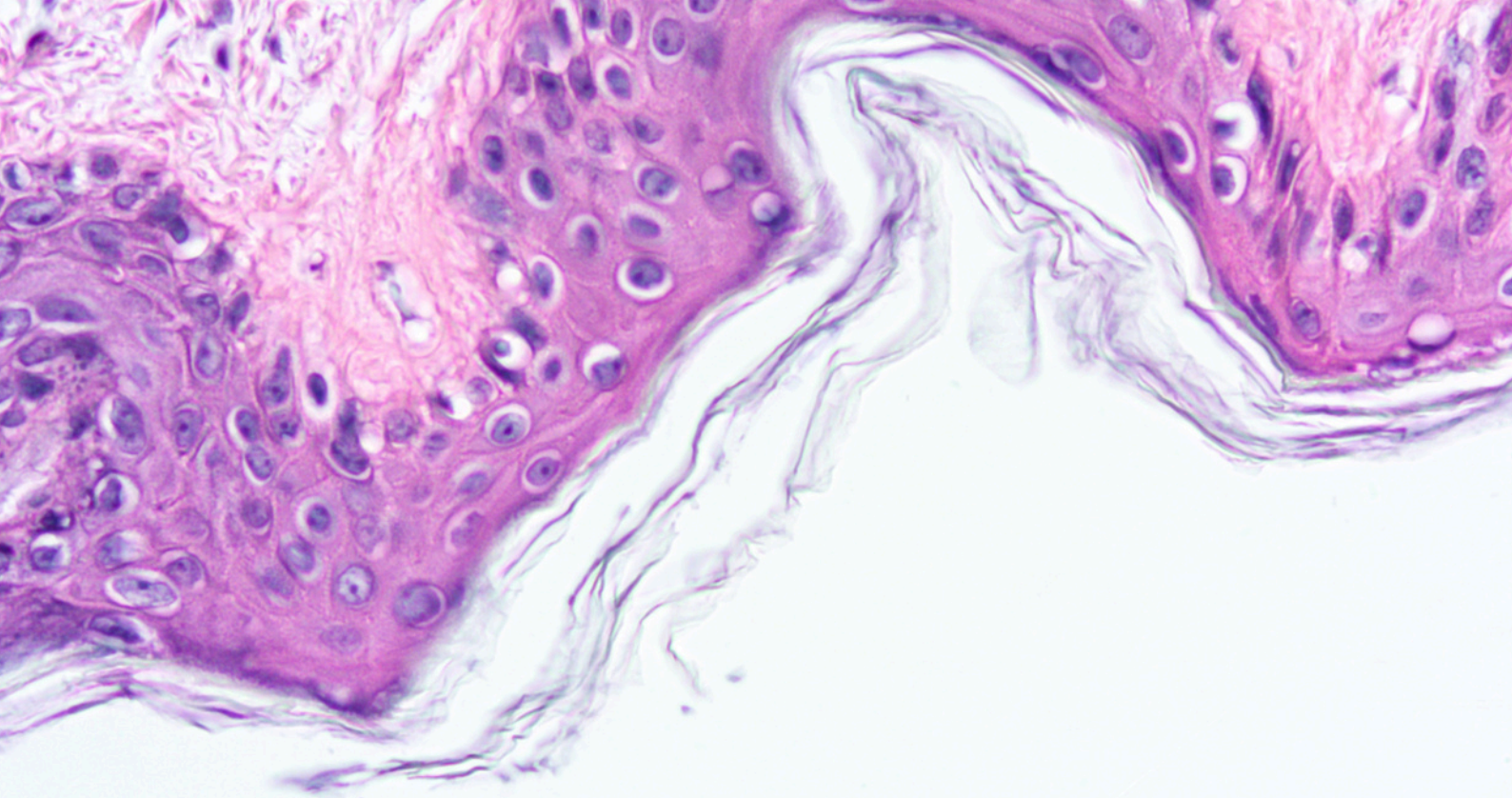

Clay: An innovative tool to understand the complex 2 dimensional histology

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Abstract

Introduction: It is often difficult to understand two dimensional hispathology representing a three dimensional biopsy tissue during the initial days of training as a pathologist. So we tried using clay to understand the artefactual phenomenon like connective tissue cores within epithelium and explain phenotype of cells with different nuclear to cytoplasmic ratio. Methodology: Mainly blue and red coloured clay were used. To explain sections at tissue level, blue clay represented epithelium and red clay connective tissue. At cellular level, blue represented nucleus and red cytoplasm. The clay was modelled as overlying epithelium with reteridges and connective tissue. The model was cut inlongitudinal and tangential way using an old disposable microtome blade. Results: In tangentially cut section only we could see connective tissue cores and epithelial islands within the connective tissue, proving that tangential cutting was responsible for such artefacts. Conclusion: Clay can be an excellent tool for explaining complex two dimensional histology or histopathology.

Introduction

Have you observed lots of connective tissue cores in a hyperplastic epithelium or in a wrongly embedded tissue and different shapes and sizes of phenotypically similar cells on the same slide? These differences are explained by tangential cutting of the specimen. But as inexperienced students of pathology, it is difficult to imagine and correlate these artifactual findings.

Tissue from in vivo to the slide under the microscope

Did you not think, how was the epithelium defined as a simple or stratified or pseudostratified and how was a connective tissue defined as fibrous or myxoid or dense. Who defined or described all the features? These questions should take us to the annals of practice of histopathology, where after a lot of trial and error. It was discovered that tissue when treated with specific chemicals (formalin as fixative, alcohols for dehydration, xylene as clearing agent). Then embedded in paraffin, cut into thin sections and finally coloured with stains (Hematoxylin and eosin mainly) and visualized under a higher magnification using a microscope.

In short histopathology technique is based on producing an artefact which literally means producing art.

But this word is loosely used to describe unwanted features in a slide owing to faulty laboratory

techniques. As the slides were viewed by our ancestral pathologist, individual features were recorded and as the number of tissues seen by them increased and the repeating features in the slides were recognized. These features were recognised as pathognomic for certain lesions. They also correlated the clinical findings to the histopathological features and disease morphology and processes were recorded. Thus the lengthy and elaborate textbook of histology and pathology were written. Today we recognise different shapes of cells, their nuclei and also appreciate any atypia/abnormality associated with such cells. The different patterns of arrangement of the cells are also learnt. With this background, if we start looking at the tissue and cells on the slide and expect variation in morphology because of the pre-analytical steps in the laboratory, our analytical procedures (case reporting) would be easier with our educated guess than

wild guess about a feature that we see on the slide.

Histopathology interpretation is an Art and a Science.

Imagination is a virtue that has not been blessed to everyone. Imagination and correlation of the tissue section seen on a slide to the vital tissue in the body is essential for a good or a great histopathologist. As a student of histopathology, I found it greatly difficult to understand the nuances of reporting a slide, it was difficult to understand to recognise the cells, their shape and size which varied but were told me that it was the same cell. It baffled me when I saw bits of connective tissue in between the epithelium, how can it be this way? I often questioned myself. On questioning my teachers, I would get answers as difference in plain of sectioning, tangential sections etc. As I thought and analysed I realised that the problem was when we fix a tissue or when we process the tissue and when we embed it, we are appreciating the only at a macroscopic level. Only after the section is stained we look under the microscope. So I taught lets enlarge the microscopic picture of tissue to the level that we can appreciate what's happening when we are grossing.

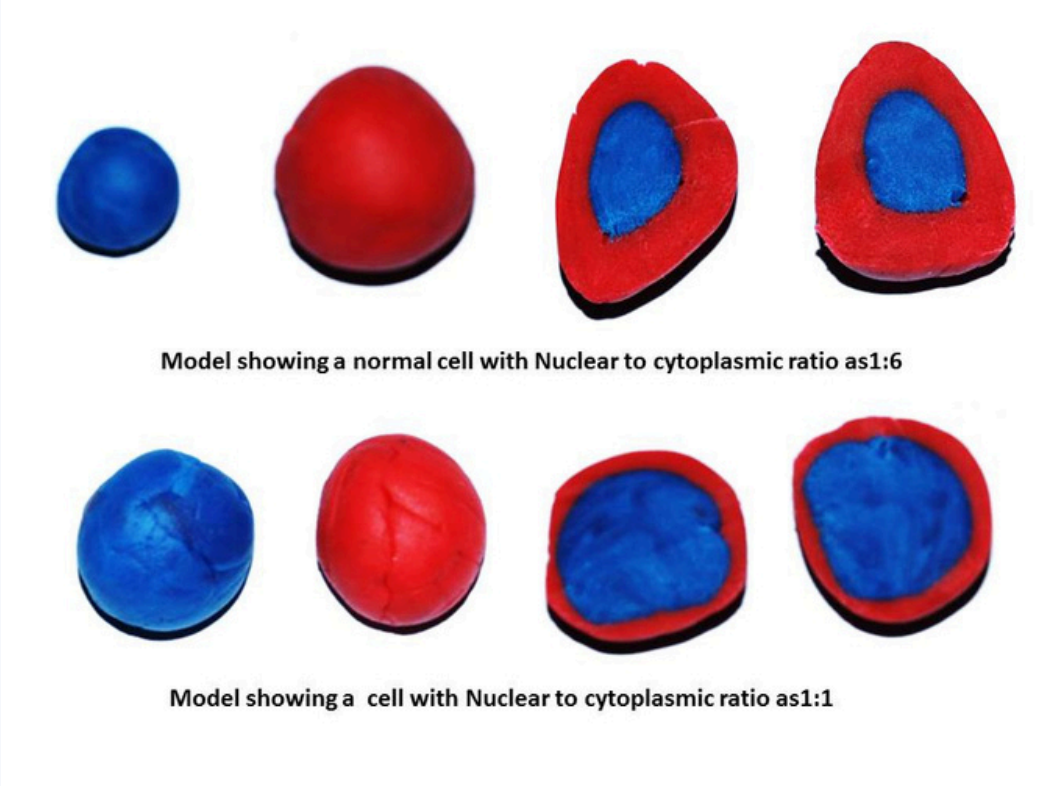

Image 1: Blue reteridges and red connective tissue papillae are shaped and arranged appropriately and a flattened blue clay sheet is stuck to it to represent the superficial epithelial layer and red clay represents deeper connective tissue layer. Then the completed tissue model with epithelial and connective tissue component is cut perpendicularly and tangentially. Note the epithelial island and connective tissue cores in the tangentially cut sections.

The Solution

The obvious choice was use of clay, which is mouldable and available in various colours. We used two colours of clay, blue for the epithelium and pink for the connective tissue when we wanted visualise/appreciate the features on a scanner view/at the tissue level. We used the blue for nucleus and pink for the cytoplasm when we wanted to visualize cytological features.

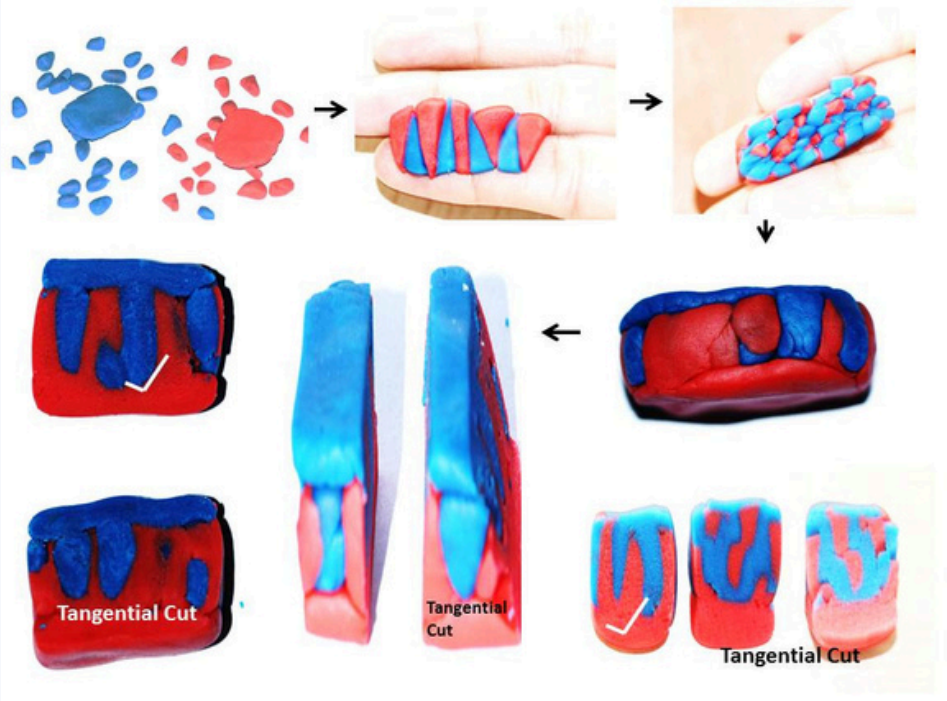

Image 2: Blue clay represents the nucleus, red one the cytoplasm. Cytological variations are often explained by the nuclear to cytoplasmic ratio. In the following image, this phenomenon is explained where 1:6 and 1:1 nuclear to cytoplasmic ratios are compared.

Connective tissue cores or Epithelial islands within the connective tissue

The blue coloured clay was shaped into epithelial reteridges and red coloured connective tissue papillae are arranged next to them and a sheet of blue clay was attached to the reteridges and it represented the superficial epithelial layer. A sheet of red clay was attached beneath the red connective tissue papillae to represent the rest of connective tissue. Then a perpendicular section was made and a tangential cut were made. Section cut tangentially show connective cores within the epithelium while the other section show no such cores. Refer Image 1.

Nuclear to cytoplasmic ratio

The normal ratio is about 1:6 and it is often spoken off in terms of volume and in dysplasia and malignancy it is told that the ratio increases upto 1:1. As a student it was difficult to imagine such differences but with use of clay such proportional comparison can be easily envisioned.

Conclusion

The profession of a diagnostic pathologist is a challenging, and years of training and experience can make a proficient pathologist. There are few important skills that we acquire through experience and few by learning. But with innovation in education like using clay to explain the phenomenon of variation can help the student in his/her learning and equip him/her to be a good diagnostic histopathologist.