The Application of Raman Spectroscopy in the Investigation of the Changes in Tissue Chemistry Caused by Variable Formalin Fixation Times

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Histology is routinely used in medicine for disease diagnosis. However, it involves poorly defined chemical procedures that were largely established in the early 20th Century [1]. For example, formaldehyde fixation is a routine step in histology for preserving excised tissues. Its protein cross-linking chemistry has only recently begun to be clarified by mass spectrometry [2]. Over/under-fixation has often hampered diagnosis [3-4], resulting in delays in diagnosis, psychological burden on the patients, and financial impacts on our healthcare system. Improving the understanding of formaldehyde fixation and optimising this crucial process will lead to improvement in histological section preparation.

Raman spectroscopy is a vibrational spectroscopic technique used to provide chemically rich information about the composition of analytes, and has proven to be a powerful tool for biological studies. This study was conducted to investigate the feasibility of using Raman spectroscopy to evaluate of the effects of varied fixation on rat liver tissue.

Rat liver biopsies were fixed for 0.5 hrs, 24 hrs and 65 hours, and were immediately processed using techniques resembling routine clinical histological laboratory practice. The resultant blocks were cut, mounted onto slides, de-waxed with xylene, and analysed by Raman microscopy. An inVia Raman microscope (Renishaw, UK) coupled to a 532 nm laser source was employed. StreamLine™ imaging and principal components were applied for data collection and analysis.

It was found that the spectra obtained from the samples fixed for 0.5 hours differed greatly from the spectra obtained from those fixed for 24 and 65 hours. A transition from relative lipid to protein dominance was one of the notable features as well as an overall reduction in signal intensity and spectral changes in the amide I bands, signifying protein secondary structural changes caused by fixation. It also became apparent that longer fixation time appears to fragment the DNA when compared against 0.5 hours of fixation. We conclude that additional protein structural information may well be available and preserved with fixed tissues. The information was hitherto not provided by mass spectrometry.

1. C. H. Fox, F. B. Johnson, J. Whiting and P. P. Roller, *J Histochem Cytochem* 33(8), 845–853 (1985).
2. B. W. Sutherland, J. Toews, J. Kast, *J Mass Spectrom* 43(6), 699–715, (2008).
3. K. B. Engel and H. M. Moore, *Arch Pathol Lab Med*. 135(5), 537-43 (2011).
4. T. Y. Leong, K. Cooper and A. S. Leong, *Adv Anat Pathol*. 17(6), 404-18 (2010).