Tissue issues

Millions of tissue samples have been collected and archived, but researchers wanting to explore them at the molecular level have found it tough going. Nathan Blow investigates the issues.

According to experts, there are more than a billion tissue samples archived in hospitals and tissue banks around the world, most of them formalin-fixed and paraffin-embedded (FFPE). Today, these samples present both an incredible opportunity and a huge challenge to researchers. FFPE tissue samples have been extensively annotated and well preserved, allowing detailed study of the progression of diseases such as cancer. But due to the method of preservation, obtaining biomolecules from these samples is proving difficult, to say the least.

FFPE was first described more than 100 years ago, and most hospitals still use this method today. But there has never been a set of standardized guidelines for processing FFPE tissue samples taken from patients to preserve tissue histology, let alone biomolecules. And although it works well for histology, the lack of standardized guidelines seems to have hampered the use of FFPE samples in molecular analyses. This may soon change, as pathologists are working towards standardizing FFPE sample preparation, and companies and researchers are developing the technology needed to isolate biomolecules and tap into the vast treasure chest of archived samples.

Although FFPE tissue preparation is simple in theory, many problems associated with downstream molecular applications — such as PCR or microarray analysis — can arise. "This is all about the fact that there has been no attention paid to uniformity of preparation," says David Rimm, a pathologist at Yale University. Between hospitals the time to tissue fixation and even the method of fixation can vary dramatically.

D. RIMM

David Rimm: time is key in tissue analysis.

"I would say that the biggest issue is time from ligation of circulation to fixation," says Rimm. During this period of ischaemia, molecular changes occur that cause problems in obtaining biomolecules. "Phosphorylation is very sensitive to ischaemic times. There seems to be promiscuous phosphotases in the cell that knock phosphates off tyrosines during this period," says Rimm. DNA and RNA can also suffer damage before fixation, with enzymes degrading and modifying both.

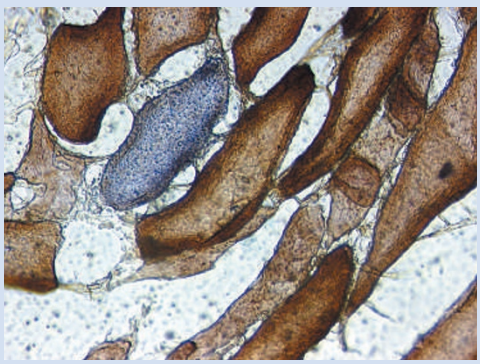
It seems obvious that rapid fixation is the answer, but no simple solution is in sight. Rimm says that for a researcher interested in only DNA or RNA, rapid fixation using quick freezing methods, instead of chemical fixation, is probably best. But if you are interested in proteins, freezing is problematic as the subsequent thawing process tends to break up and denature proteins and, like ischaemia, can lead to modifications.

Then there are pathologists who think that standardizing the time to fixation will prove to be a difficult task. "Standardization would be great, but I don't know how realistic it is across institutions or even within institutions," says Christine Iacobuzio-Donahue, a pathologist at Johns Hopkins University School of Medicine in Baltimore, Maryland. She believes that the best place to tackle the problems caused by ischaemia is actually downstream of fixation,

FROZEN IN TIME

Analysing proteins from tissue samples that have been formalin-fixed and paraffin-embedded (FFPE) can provide critical information about how cells function before fixation. "When you formalin-fix the protein, you fix it in time; it is not going anywhere," says Peter Tunon, vice-president of sales and marketing at Expression Pathology in Gaithersburg, Maryland. Researchers are taking advantage of this fact to explore the protein world in FFPE samples.

Expression Pathology was founded in 2001 by researchers from the US National Cancer Institute and the company Life Technologies (now Invitrogen) who had experience in studying gene expression in tissue and histology. "The company was founded on the fact that examining protein expression is crucial to understanding what is happening in cells," says Tunon. To this end, Expression Pathology has worked on ways of extracting and isolating proteins from FFPE tissue samples for analysis by mass spectrometry or reverse-phase dot blot arrays. The company developed a technology that integrates extraction of total proteins from FFPE samples with tryptic digestion so that finished samples are ready for mass spectrometry. To simplify the system further, all reagents are completely compatible with mass-spectrometry instrumentation, says Tunon, making it a good starting point for broad-based screening of proteins in FFPE samples. Other companies also think that protein isolation from FFPE tissue samples will provide valuable information. QIAGEN and EMD Chemicals, both based in San Diego, California, now offer systems that chemically reverse formalin crosslinking to isolate full-length proteins for applications such as western blotting and protein arrays.



LEICA MICROSYSTEMS

Caught on camera: a section of human muscle after dual histochemistry.

Surprisingly, post-translational modifications (PTMs) such as phosphorylation and acetylation, can also be observed when examining proteins isolated from FFPE samples. PTMs can be critical to the role of a protein in the cell, changing the function or localization. "We do see post-translational modifications that are preserved on the peptides, and they seem to be present in ratios that are similar to those we get when using fresh frozen tissues," Tunon says of work done by Expression Pathology.

Even much older archival samples of FFPE tissues do not seem to pose a problem for protein extraction. "We have worked with samples that are more than 15 years old and found no difference in the profiles compared to samples that were just a few weeks old," says Tunon. He is quick to add that he believes even older samples could be examined, but Expression Pathology has yet to test this idea. N.B.