Tissue artefacts caused by sponges

We read with interest the short report by Farrell and colleagues on tissue artefacts caused by sponges.1 We note that they have resorted to wrapping some specimens in transparent paper prior to processing. Likewise, Rossi suggests the use of tea-bag paper to wrap small fragments of tissue that would otherwise go through the cassette perforations.2 A logical extension of these suggestions is to try empty tea-bags.

We were recently supplied with a quantity of empty, sealed, perforated tea-bags (CWS Ltd, Crewe, England) and investigated whether these would provide a viable alternative to synthetic specimen bags which are already on the market (Shandon UK).

Both were subjected to scanning electron microscopy and neither was found to have sharp spikes, as seen with foam pads, on which tissue might be transfixed (figure). The tea-bag had pores of variable size, but even in the regions of the perforations the largest holes were of the same order of magnitude as the larger holes in the synthetic bags (Brooke Bond tea-bags filter particles of the order of 250 μm).

Both bags can be easily loaded by pouring formalin and biopsy specimens through a funnel into the bags. This is particularly useful for fragmented specimens because there is no need for further manipulation for forceps and the process is less time consuming than picking out tiny bits of tissue from a pot. A bowl left under the bag catches the waste formalin which is discarded. The synthetic bag can be folded in half into the cassette while the tea-bag requires three folds for a near perfect fit.

Both bags survive processing. At the embedding stage the synthetic bag was easily opened and the contents retrieved while the tea-bag was less easily opened and very small pieces of tissue were difficult to separate from the wax impregnated paper.

Synthetic bags cost over 9 pence each while an empty tea-bag can be manufactured for about 2 pence.

Small biopsy specimens are likely to remain a major part of the workload in histopathology. They need to be handled efficiently and safely while minimising damage to the tissue. Specimen bags offer one solution but are rather expensive. A standard square tea-bag would require modification of size and its seam before widespread use but could potentially be cheaper.

Our thanks to Steve Carpenter (Department of Metallurgy and Materials, University of Birmingham) for the scanning electronmicrographs.


Non-nuclear staining of thick tissue sections

Thick sections (1 mm deep or more) of the breast and other organs have been examined histologically since the turn of the century.1 Hitherto, exclusive use has been made of nuclear stains for sections more than 300 μm thick. As a result of our interest in the comparison between radiographs and histological examination of breast tissue we have found, by experimentation, that this restriction is unnecessary. Counterstaining with eosin and direct staining of calcific deposits is possible using such thick sections of tissue, either directly processed from formol-saline,3 or after “back-processing” from archival paraffin wax embedded tissue.4

Pilot studies were performed on mamoplasty resections of excessive breast tissue (macromastia) or breast tissue identified as having mammographically suspicious microcalcification in the United Kingdom national breast screening program.

In order to apply eosin to thick sections after nuclear staining with Mayer’s haematoxylin5 the following schedule proved successful. Once differentiation of the nuclear staining has been performed with acid alcohol and washed in tap water the thick section (1 mm or deeper) is placed in 0-1% aqueous eosin Y (Shandon, Runcorn, Cheshire), used diluted 1 part to 9 in 85% methanol for 10 minutes. It is washed again in tap water, dehydrated, and cleared for mounting in methyl salicylate (fig 1).3,6

To show the presence of calcium deposits by cationic complexing with alizarin red S (BDH, Poole) this dye is applied instead of haematoxylin nuclear staining. After the tissue has been partially rehydrated from paraffin wax,4 or dehydrated from formol-saline3 the staining procedure described below is followed.

The tissue is left for 12 to 24 hours in 95% alcohol and transferred to an aqueous 1% solution of KOH for 12 hours, and thereafter into a solution of 1 mg alizarin red S in 100 ml of 1% aqueous KOH for 24 hours. Subsequent dehydration and clearing is as described before,3,4 and the tissue is viewed once mounted in methyl salicylate (fig 2).

One of the reasons for the previous relative neglect of thick section histological examinations has possibly lain in the lack of attempts to stain tissue constituents other than nuclei. The original methods of examining thick sections merit expansion to include study of different tissue components.7 Such methods are, as we indicate in this letter, both entirely feasible and merely require adjustment to the increased depth of the tissue under study—often achieved by judicial dilution of the reagents used for 5 μm sections and by increasing the time of staining. The new-found ability to select specific lesions from conventional 5 μm sections for examination in thick sections should motivate the development of further methods.
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