Pretreating Epoxy Thin Sections With Sodium Periodate Prior To Immunostaining

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Pretreatment of epoxy thin sections with strong oxidizing agents such as hydrogen peroxide, sodium methoxide, and sodium m-metaperiodate facilities the location of antigens with immunostaining procedures. Etching, or pretreatment, of sections unmasks antigenic sites on glutaraldehyde fixed and postosmicated tissue, partially removes osmium bonds, temporarily decreases the hydrophobicity of the epoxy surface layer of the section, reduces the electron density of the tissue and increases resistance to heavy metal poststaining[1,2,3].

Pretreatment of sections is generally restricted to epoxy embedded specimens. Acrylic embedding monomers such as LR White bond through tissue components, and not with them as epoxide monomers do[2]. Also, acrylic embedded tissues exhibit much rougher surfaces, are less crosslinked and are more hydrophilic than epoxy embedded tissues. Thus, immunostaining fluids penetrate acrylic sections with relative ease. Furthermore, strong oxidizing agents worsen both the known instability of acrylic sections in the electron beam and the structural preservation of tissue during exothermic polymerization.

Method For Pretreatment With Sodium m-Periodate

We have found hydrogen peroxide too deleterious to our tissues, and sodium methoxide varies unpredictably in apparent strength and therefore is difficult to standardize. The sodium m-periodate procedure will vary according to embedments and materials employed and must be empirically determined. To insure optimal interaction between the antigen and antibody, we use an embedment of low crosslinkage (Araldite 502, 15 ml, Eponate 12, 25 ml, DDSA, 55 ml, dibutyl phthalate, 1%, DMP-30, 1.5%) which allows aqueous immunostaining liquids to penetrate the surface of these embedded tissues better than other, more highly crosslinked formulations[3].

Tissues embedded as described above require only about 10 - 15 minutes in sodium m-periodate while heavily crosslinked formulations such as Spurr's may demand 60 minutes in contact with pretreatment solutions. To determine optimum sodium m-periodate exposure times for a specific embedding media, trial thin sections on nickel grids may be exposed to saturated sodium m-periodate for 5, 10, 15, 30, and 60 minutes, and are then compared for immunoreactivity and beam stability by TEM before a large number of grids is committed to the below procedure:

1. Prepare a saturated solution of sodium m-periodate (Sigma, Cat#S-1878) using 1 gm of sodium m-periodate in 5 ml of distilled water. Pass through a 0.22 micrometer pore filter.
2. Float grids on distilled water 2 X 5 min. to hydrate.
3. Float sections on 50 microliter drops of the sodium m-periodate solu tion.
4. Rinse well. Pass grids through 6 changes of distilled water, 2 min. each.

The grids are now ready for immunostaining. The grids must not be allowed to dry. After the last rinse in water the grids should be soaked in a buffer appropriate to the immunostaining protocol. If pretreatment of sections with oxidizing agents is undesirable, it may be possible to use a procedure which eliminates osmium, instead preserving the tissue with tannic acid, uranyl acetate, platinum chloride, and p-phenylindiamine[4]. ■

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3. Causton, B., Does the Embedding Chemistry Interact with Tissue? Science of Biological Specimen Preparation for Microscopy and Microanalysis, in Scanning Electron Microscopy, Inc. Illinois, 1985, p.209.

4. Phend, K., Rustloni, A. and Weinber, R., An Osmium-free Method of Epon Embedment That Preserves both Ultrastructure and Antigenicity for Post-embedding Immunocytochemistry. J. Histochem and Cytochem,. Vol 43, No. 3, p.283, 1995.

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SEM History

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Manfred von Ardenne was an EM pioneer and the first to publish a complete concept of a scanning electron microscope. A German overseas newscast reported the death of Manfred von Ardenne on 28 May. During the 1930s he was involved in cathode ray work and later lived In East Germany and did significant cancer research. Not mentioned in the newscast was his conceptual development of the SEM in the late 1930s.

He outlined the underlying principles of SEM operation: an electron probe scanning a small region of the specimen, the emitted electrons are captured, amplified and time-sequentially displayed on a cathode ray tube. Magnification is the ratio between the areas scanned and displayed. His was the fantastic notion of a microscope without a magnifying lens.

There are a two earlier SEM related papers by Knoll, but they were short and specific to secondary electrons only and not in the context of an SEM. Incidentally, Knoll was Ernst Ruska's supervisor. Ruska with Knoll built the first EM (TEM) in 1931.

Von Ardenne's article entitled "Des Elektronen-Raster-Mikroskop" was published in the "Zeitschrift Technischen Physik" 19, (1938) 407-416. (In English the title reads "The Scanning Electron Microscope".) It is astonishing that this was published some 27 years before the first commercial SEM was produced.

It is interesting that the now more complex TEM was developed from the practical TEM in 1933 (the first with a specimen port) and was a high performer in the early 1950s. Only in about 1960 was the Cambridge group, under Oatley. able to build the first SEM and the first commercia! SEM was produced by Cambridge Instrument Co. in 1965.

A lot of technology (especially TV/CRT and the secondary detector) had to mature before SEM was possible and the Cambridge group deserves great credit. But the development of several unique concepts of a then quite futuristic instrument is to von Ardenne's enduring credit.

After posting a note on the microscopy server, the writer received an email from Thomas Everhart, the co-inventor of the secondary detector.

"Your note about von Ardenne was forwarded to me. While I agree with what you write, you might note that Stinzing received a German patent on the concept of the SEM in about 1929, if memory serves me right. So far as I know, he did nothing to reduce the concept to practice. Knoll also used the concepts in the mid thirties, slightly before von Ardenne got involved. However, von Ardenne was the first to get serious about resolution in the SEM and he did have many ingenious Ideas." ■