Some Notes on Re-Embedding EM Samples

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Most of us have been here more than once: a critical specimen, often the only one of its kind, does not embed properly. Usually the problem is incompletely polymerized resin. After the panic subsides, a limited number of options are left, and if the specimen is irreplaceable, there are *no* options, other than trying to salvage it the best way you can. I've had this problem before with low viscosity resins, but the following suggestions should work for other resins as well.

First of all, realize that reprocessed specimens are rarely as good as they would be if things went fine the first time around. But when only a few sections are needed from a critical block, I would suggest trying the following, in this order:

1) Crank up the heat in the polymerizing oven, and really "cook" the specimen for an additional 12 to 24 hours. If the resin polymerizes around 70° C, try repolymerizing the block at 90° to 100° C. This will frequently harden a marginal block enough to get at least a few sections from it.

2) If repolymerization doesn't work, try using propylene oxide to remove most of the gooey stuff. Use the binocular scope and the trimming block on the ultramicrotome to remove most of the resin from around the specimen. Use lots of razor blades, changing them frequently. I like to use the thinner, double-edge blades for this. Then cut under the specimen to remove it from the block. Be careful at this step. If too much force is used, the valued specimen will go flying across the room, never to be seen again.

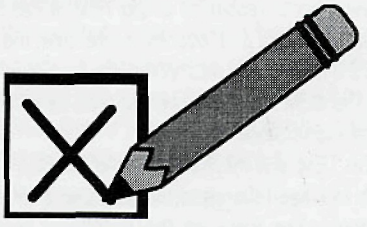
It depends on how much crosslinking has taken place in the soft parts, but it should be possible to remove at least some of the soft resin with propylene oxide.

Place the unblocked specimen in a vial with a large excess of propylene oxide, and place the vial on a rotator or an agitator. Make frequent changes of propylene oxide over a period of 24 to 48 hours. From this point, it should be possible to infiltrate as usual. Try an ascending series of resin concentrations that are cut with propylene oxide, then pure resin overnight. Apply vacuum during the infiltration with pure resin if possible. Using a vacuum oven for polymerization is also a good idea.

The objective here is to adequately infiltrate and polymerize good, hard resin into the specimen as far as possible. The center of the specimen will probably still be soft, so when sectioning the re-embedded specimen, be sure to concentrate on collecting sections from the first few micrometers of the surface.

An interesting alternative (which I have not personally tried) might be to use sodium ethoxide instead of propylene oxide to remove resin from the specimen. Sodium ethoxide is *extremely* corrosive. A one minute incubation on a drop of sodium ethoxide will completely digest the resin from an 80 nm thin section, so it should work great to remove soft resin from a specimen. Sodium ethoxide is sodium hydroxide in ethanol, so dehydration with ethanol is needed following the treatment, then go into propylene oxide then infiltrate in the usual fashion. If anyone has tried this I would like to hear the results.

Maybe there's a grad student out there who is looking for a little experiment to do this weekend? ■



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