A New Universal Acrylic Embedding Resin for Both Light and Electron Microcopy

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Successful immunolabeling in electron microscopy of animal and plant tissues requires a combination of excellent antigen preservation while maintaining the original structure of the tissue. One important element is tissue embedding which accomplishes two goals for the immunohistochemist, the preservation of tissue specimen structure and maintenance of biological antigenicity. Tissue embedding in plastic resins is a common method in which several important elements[1] must be considered.

1. Fine tissue structure must not be damaged by the polymerization.
2. The plastic must be stable to the electron beam.
3. Light scattering properties of the plastic should be minimal.
4. The plastic should cut easily.
5. The plastic must be of sufficiently low viscosity to infiltrate the tissue.

And, most importantly for immunolabeling, the antigenicity must be retained.

There are two basic chemical categories of plastic resins in common use, the epoxy crosslinked (hydrophobic) resins and the acrylic crosslinked (hydrophilic) resins. The epoxy resins preserve tissue structures well, but absorb water poorly, allowing limited access by immunolabels. Successful labeling on hydrophobic resins usually requires a treatment to increase accessibility by aqueous reagents through the oxidization of hydrophobic chemical side chains (i.e. with hydrogen peroxide or periodic acid) and/or the breaking (etching) of resin cross-links with alkoxides[2].

The acrylic resins are hydrophilic in nature allowing good accessibility by immunolabels while still allowing reasonable preservation. Another large advantage with the acrylics are the low temperature polymerization (curing) procedures employing ultraviolet light which provide excellent antigen conservation. The aliphatic cross-linked series (LOWICRYL products, Chemisch Werke Lowi. Germany)) and the aromatic cross-linked series (London Resin products, Hants, UK)) are excellent resins and have experienced common use in immunohistochemistry for several decades[2].

A new hydrophilic resin, described herein, is comprised of a mixture of four methacrylate precursors of similar molecular weights which combines the characteristics of even penetrability with excellent polymerization properties. This product call BioAcryl[3] or UNICRYL™ provides excellent tissue preservation and staining qualities[4]. At the light microscopy level, the use of a variety of histological stains gave excellent results comparable to those seen when staining paraffin sections[4,5].

UNICRYL (British BioCell International, Cardiff, UK) preserves tissue structure with minimal chemical interaction such that proteins, nucleic acids and other macromolecules are revealed at the section surface upon cutting. The excellent polymerization qualities are thought to be due to the monomer precursors beint of similar molecular weights assuring even penetration into the tissue. The following eight points highlight the characteristics of the new embedding medium.

1. Single Solution: The unique characteristics of UNICRYL provide other advantages. It is available as a single solution, assuring less user exposure and convenient handling, while retaining a rather long shelf life when stored cold (-20° C). UNICRYL is miscible with alcohols and retains low viscosity characteristics even at -50° C.

2. Polymerized by heat or . . . UNICRYL may be polymerized or cured by high temperature when tissue antigens are not temperature sensitive. Users should remember the exothermic nature of the polymerization process and consider that one of several cooling methods may be employed to maintain low temperature. The table below provides typical heat polymerization times for UNICRYL.

Temperature	Polymerization Times
50° C	2 - 3 days
60° C	1 - 2 days
70° C	1 day (brittle)

3. Polymerized by ultraviolet light: Ultraviolet light may be used to polymerize between temperatures ranges of -10° to 20° C for approximately two days. UV polymerization at 4° C is most common but, if higher temperatures are used, extra care should be taken to account for heat of the exothermic polymerization reaction. Once the tissue is dehydrated and infiltrated with UNICRYL at low temperature, it may be possible to polymerize at higher temperatures (i.e. 30-37° C), but once chosen, the operation should be continuous and temperature unchanged. Partial polymerization can be reversed, however, by solubilization in unreacted monomer. The table below provides typical heat polymerization times for UNICRYL.

Temperature	Direct Illumination - (2/8 watt lamps)				
	1 cm	5 cm	10 cm	15 cm	20 cm
20° C	brittle	brittle	brittle	1-2 days	2-3 day
4° C	brittle	britle	1-2 days	2-3 days	3-4 days
-10° C	1-2 days	2 days	2-3 days	3-4 days	4-5 days

4. Abbreviated protocol for polymerization: Process the tissue for heat or UV polymerization as follows:
a. fix tissue appropriately for EM or LM applications.
b. wash
c. dehydrate through alcohols or acetone (3X10 min. for each solvent).
d. infiltrate with resin (2X1 hr) with gentle agitation. (Use a ratio of 100X tissue volume). Fresh tissue should be infiltrated for at least 8 hours.
e. place vials in oven or UV chamber.

5. Recommended resin volumes: Small pieces of tissue (0.5 mm^3) may be processed in single, capped 1 ml eppendorf vials. A smaller resin volume will ensure a more even polymerization (better light penetration!) and permit better temperature control of the exothermic reaction. the volume should be sufficient to achieve good infiltration and to provide an adequate portion to handle and section.

6. Flat embedding: Either indirect or direct light may be used and the direction of illumination will vary with the specimen and types of containers. For flat embedding, lighting from above of uncovered molds is usually acceptable. When heat curing at high temperatures (55-60° C) the molds should be covered to prevent evaporation. Polypropylene molds are more suitable than softer plastic or silicone rubber molds.

7. Capsule embedding: The use of either BEEM capsules, gelatin capsules, and eppendorf capsules is acceptable and most polypropylene capsules are impermeable to UNICRYL. It is not necessary to exclude oxygen from the resin surface during the process, however, during thermal polymerization, the capsules should be sealed to limit exposure to fumes and prevent evaporation.

8. Unique cutting and staining properties: UNICRYL embedded sections display cutting properties which allow the instrument to follow the contours of the tissue surface exposing surface antigens well. This allows easy access of immunostains and other histological stains as has been reported[4,6]. ■

1. Dawes, CJ (1984) Biological Techniques for Transmission & Scanning Electron Microscopy. Ladd Research Industries, Burlington, VT
2. Causton, BE (1984) *The choice of resins for electron immunocytochemistry*, IN (Polak, Varndell, eds.) Immunolabeling for Electron Microscopy. Elvsevier Science Publishers, New York, pp 29-36.
3. Product discovered and developed by Dr. C.S. Scala, University of Bologna
4. Scala, C.S. et al (1992) J Histochem Cytochem 40, 1799-1804
5. Scala, CS. et al (1993) Histochem J 25, 670-7
6. Manara, GC et al (1993) Eur J Dermatol 3, 235-8.

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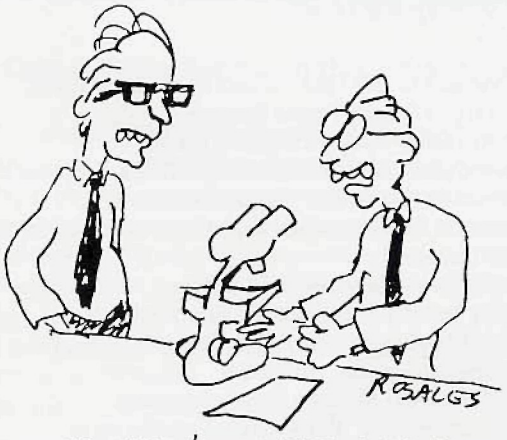

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