Technical methods

3-Aminopropyltriethoxysilane (APES): a new advance in section adhesion

P H MADDOX, D JENKINS *From the Department of Pathology, Whittington Hospital, London*

Various adhesives have been used to increase the adherence of tissue sections to glass slides and to prevent separation during staining. Traditionally glycerin albumin¹ and gelatin² solutions have been widely used in routine histology. More recently poly-L-lysine has been used to improve section adhesion, particularly in immunocytochemistry.³ This method has been of great value but it is time consuming and slides cannot be stored.

For the simultaneous immunostaining of T6 and S100 in tissue sections, we have found it necessary to use frozen sections of tissue fixed in formol calcium.⁴ With poly-L-lysine used as the adhesive, there was a high loss of sections after initial protease digestion. An alternative method for increasing adhesion was therefore sought.

For over 20 years alkoxysilanes have been used in an industrial context as coupling agents in coating and dyeing a variety of materials.⁵ Weetall *et al*^{6,7} described their use as coupling agents for insolubilising enzymes on inert surfaces, emphasising that the strong covalent bonding effect between the aminoalkyl groups and aldehyde or ketone functions of a reactive surface would withstand repeated washings with a variety of inorganic solvents. More recently aminoalkylsilane has been used as an adhesive for enhancing chromosome spreading on glass slides,⁸ and for in situ hybridisation of frozen sections.⁹

In this paper we describe a new modification to the coating method for hybridisation originally introduced by Rentrop *et al*.⁹ The modified method is simple, cheap, and can be used in the routine laboratory, whenever improved adhesion of sections is necessary.

Method

3-AMINOPROPYLETRIETHOXYLSILANE (APES)

COATING OF GLASS SLIDES

1 Soak slides in 10% Decon at room temperature overnight.

2 Wash in running tap water at 60°C for at least one hour.

3 Rinse in distilled water.

4 Dry overnight at 60°C.

(At this stage the slides should be clean and sparkling, as any stain or foreign material remaining on the slides will affect the coating).

5 Immerse rack of slides for at least five seconds in a 2% solution of 3-aminopropyltriethoxysilane (Sigma Chemical Co, Poole, Dorset, England, No A-3648), in 99% industrial methylated spirits imms. The 2% solution should be freshly prepared.

6 Wash in 99% industrial methylated spirits.

7 Wash in distilled water.

8 Dry overnight at 37°C.

A 2% solution of APES (200 m) in 99% industrial methylated spirits can be used for 200-300 slides. No modifications to the usual laboratory methods of section drying is necessary.

Result

APES as an adhesive produces improved section bonding over other commonly used adhesives such as poly-L-lysine, glycerin albumin, and gelatin and withstands protease digestion. There is no background staining and it does not seem to interfere with the routine histological or immunostaining methods used in our laboratory. A large supply of coated slides can be stored in boxes, at room temperature until required. The bonding effect can be instantaneous, so that positioning of the section on the coated slide can be very difficult.

Discussion

An alcoholic solution of APES fulfils the requirements for an adhesive for the immunostaining of prefixed frozen sections. It also provides a useful method of stabilising histological and cytological preparations on glass slides for other purposes. It is a sufficiently simple, low cost technique (@ 50 pence for 200 slides), making its use in routine histological practice feasible.

The APES concentrate is a toxic chemical that is moisture sensitive and requires storage at 0-5°C; it is not carcinogenic but is an irritant and can cause burns. Normal laboratory precautions, including the wearing of gloves and the use of a fume cupboard, should be taken when handling the concentrate solution. The method offers considerable benefit in saving laboratory time as it becomes possible to use routinely available spare sections for techniques such as immunocytochemistry and in situ hybridisation or

other lengthy rigorous cytochemical techniques.

Our thanks to Mr RJ Yabsley (Senior Chief MLSO), Mr D Lake (Chief MLSO), and all members of the departments of histopathology and cytology at the Whittington Hospital for their support and cooperation in the routine use and assessment of the coated slides.

References

- 1 Mayer P. Einfache Methode zum Aufkleben mikroskopischer Schnitte. *Mitt Zool Stat Neapel* 1883;2:521-2.
- 2 Koninski K. Eine neue Methode, Paraffinschnitte auf dem Objektträger zu fixieren. *Z Wiss Mikrosk Mikrosk Tech* 1898;15:161-3.
- 3 Huang WM, Gibson P, Qu J, Polsk JM. Improved section adhesion for immunocytochemistry using high molecular weight polymers of L-lysine as a slide coating. *Histochemistry* 1983;77:275-9.
- 4 Maddox PH, Tay SK, Jenkins D. A new fixed cryosection technique for the simultaneous demonstration of S100 and T6

- 5 Langerhans' cells in normal human cervix. *Histochemical J* 1987;19:35-8.
- 6 Plueddeman EP. Adhesion through silane coupling agents. *Journal of Adhesion* 1970;2:184-201.
- 7 Weetall HH. Trypsin and papain, covalently coupling to porous glass. *Science* 1969;166:615-7.
- 8 Weetall HH, Hersh LS. Preparation and characterisation of glucose oxidase covalently linked to nickel oxide. *Biochem Biophys Acta* 1970;206:54-60.
- 9 Van Prooijen-Knegt AC, Reap AK, Van Der Burg MJM, Vrolijk J, Van Der Ploeg M. Spreading and staining of human metaphase chromosomes on aminoalkylsilane treated glass slides. *Histochemical J* 1982;14:333-44.
- 9 Rentrop M., Knapp B, Winter H, Schweizer J. Aminoalkylsilane-treated glass slides as support for in situ-hybridisation of keratin and c-DNAs to frozen sections under varying fixation and pretreatment conditions. *Histochemical J* 1986;18:271-6.

Requests for reprints to: Dr PH Maddox, Department of Pathology, Whittington Hospital, London N19, England.

Rapid method for stained renal biopsy specimens embedded in epoxy resin

J BRIERLEY, W LAWLER, G WILLIAMS *From the Department of Pathology, Manchester University*

Embedding small biopsy specimens in epoxy resin (by preparing 1.5 µm sections and staining them with a variety of techniques) enhances optical resolution and accuracy of histopathological diagnosis.

For many years, all our renal biopsy specimens, routinely embedded in paraffin wax, were stained with haematoxylin and eosin, periodic acid Schiff, Martius scarlet-blue and periodic acid-methenamine silver methods,¹ which we were anxious to retain after switching to plastic embedded specimens. Epoxy resin was found to be preferable to acrylic resin, mainly because it could be partially removed to allow for good staining. Some technical modifications were necessary as thin plastic sections require more intense staining. We found that some stains benefitted from being heated while others required their times extending; and phloxine replaced eosin as it gave similar but stronger results.²

Since 1979, all routine, non-urgent renal biopsy specimens in this department have been embedded in epoxy resin. Until now, this procedure has taken at least four days, because specimens had to be hand

processed and the resin required polymerisation overnight at 60°C. The drying (overnight at 35°C) and staining of sections were also time consuming, making the process unsuitable for urgent cases (patients with rapidly deteriorating renal function and most transplant biopsy specimens).

During the summer of 1986, an automatic "Lynx electron microscopy Tissue Processor" was purchased for all the various users of the electron microscopy suite of our department, the following method was devised to combine the benefits of plastic embedding with the availability of stained sections within 24 hours of biopsy.

Methods

After collection the specimen is processed overnight on the tissue processor with a sufficient delay in 10% buffered formalin to permit adequate fixation. For needle cores, the programme takes five hours and is as follows:

- 1 35% alcohol for 30 minutes
- 2 75% alcohol for 30 minutes
- 3 95% alcohol for 30 minutes
- 4 100% alcohol I for 30 minutes
- 5 100% alcohol II for 30 minutes
- 6 Propylene oxide I for 15 minutes
- 7 Propylene oxide II for 15 minutes
- 8 50:50 resin:propylene oxide for 60 minutes at 40°C
- 9 Neat resin for 60 minutes at 40°C.

When larger pieces of tissue are submitted (such as small open wedge biopsy specimens), processing times