Acta Medica Okayama

Volume 60, *Issue* 1 2006 *Article* 7

FEBRUARY 2006

In situ preparation of colloidal iron by microwave irradiation for transmission electron microscopy.

Satoru Nakatani[*] Ichiro Naito[†] Ryusuke Momota[‡]

Noriko Hinenoya[**] Kanji Horiuchi[††]

Keiichiro Nishida[‡‡] Aiji Ohtsuka[§]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]Okayama University,
[‡‡]Okayama University,
[§]Okayama University,

In situ preparation of colloidal iron by microwave irradiation for transmission electron microscopy.*

Satoru Nakatani, Ichiro Naito, Ryusuke Momota, Noriko Hinenoya, Kanji Horiuchi, Keiichiro Nishida, and Aiji Ohtsuka

Abstract

We attempted to prepare colloidal iron within tissues by means of microwave irradiation. Mouse tissue blocks were fixed with a mixture of paraformaldehyde and ferric chloride in a cacodylate buffer, immersed in a cacodylate buffered ferric chloride solution, and irradiated in a microwave processor. Colloidal iron was prepared within tissues or cells, and was observed in the form of electron dense fine granules (1-2 nm in diameter) by transmission electron microscopy. Collagen fibrils in the connective tissue showed colloidal iron deposition at regular periodical intervals. Cells in the splenic tissue showed that fine colloidal granules were deposited on the ribosomes but not on the nuclear chromatin. This finding suggests that ferric ions could not diffuse into the nucleus, which was surrounded by the nuclear envelope. The podocyte processes of the renal glomerulus were stained diffusedly. Though this microwave in situ colloidal iron preparation method has some limitations, it is convenient for use in biomedical specimen preparation in transmission electron microscopy.

KEYWORDS: colloidal iron, microwave, histochemistry, transmission electron microscopy

---

*PMID: 16508690 [PubMed - indexed for MEDLINE]

Acta Med. Okayama, 2006
Vol. 60, No. 1, pp. 59–64

Acta Medica Okayama
http://www.lib.okayama-u.ac.jp/www/acta/

Original Article

In Situ Preparation of Colloidal Iron by Microwave Irradiation for Transmission Electron Microscopy

Satoru Nakatani, Ichiro Naito*, Ryusuke Momota, Noriko Hinenoya, Kanji Horiuchi, Keiichiro Nishida, and Aiji Ohtsuka

Department of Human Morphology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700–8558, Japan

We attempted to prepare colloidal iron within tissues by means of microwave irradiation. Mouse tissue blocks were fixed with a mixture of paraformaldehyde and ferric chloride in a cacodylate buffer, immersed in a cacodylate buffered ferric chloride solution, and irradiated in a microwave processor. Colloidal iron was prepared within tissues or cells, and was observed in the form of electron dense fine granules (1–2 nm in diameter) by transmission electron microscopy. Collagen fibrils in the connective tissue showed colloidal iron deposition at regular periodical intervals. Cells in the splenic tissue showed that fine colloidal granules were deposited on the ribosomes but not on the nuclear chromatin. This finding suggests that ferric ions could not diffuse into the nucleus, which was surrounded by the nuclear envelope. The podocyte processes of the renal glomerulus were stained diffusedly. Though this microwave *in situ* colloidal iron preparation method has some limitations, it is convenient for use in biomedical specimen preparation in transmission electron microscopy.

Key words: colloidal iron, microwave, histochemistry, transmission electron microscopy

The cationic colloidal iron method, which was first developed by Hale in 1946 [1], is useful for visualizing anionic sites in tissues and cells. Preparation procedures have been modified to improve the staining ability and stability of the colloid. Sodium, ammonium or hydrazinium cacodylate buffer solution was applied in an attempt to make fine and stable colloidal iron sol [2–6]. Cacodylate buffered ferric chloride solution is boiled until its yellow tint changes to a reddish brown color. Even the finest colloidal granules cannot penetrate into the deep region of the tissue blocks, so a staining method for hydrophilic resin-embedded ultrathin sections was developed [7].

Microwave irradiation has been applied to the histological processing of biomedical specimens for fixation, immunohistochemistry, staining and embedment [8–12]. However, it has not been used to make colloidal iron sol or any other staining solution within the tissue blocks. In the present study, we attempted to prepare colloidal iron sol within the tissue using microwave irradiation to stain the cellular structures readily in transmission electron microscopy.

Materials and Methods

Preliminary microwave irradiation. To determine the time and temperature control for pre-

Received August 26, 2005; accepted October 6, 2005.
*Corresponding author. Phone: +81–86–235–7091; Fax: +81–86–235–7095
E-mail: naito16@md.okayama-u.ac.jp (I. Naito)

paring colloidal iron by microwave irradiation, 10 ml of 0.1 M sodium cacodylate-HCl buffer (pH 7.3) containing 0.01 M ferric chloride in each glass sample bottle was irradiated using a microwave processor (H2500: Bio-Rad) at upper temperature limits of 45, 55, 65, 75, 85, 95 and 100 °C (Table 1). When ferric ions were converted to colloidal iron, the color of the solution changed from light yellow to dark reddish brown. In each case, the timing of the beginning of the color change and its completion were recorded.

Animals. Adult female Balb/c mice (12–16 weeks) (Charles River Japan, Yokohama, Japan) were used. They were maintained at a constant temperature (22 °C) and light cycle (light 6:00–18:00) with free access to water and food (MF, Oriental Yeast, Tokyo, Japan). All of the experimental procedures were reviewed and approved by the Guidelines for Animal Experimentation of Okayama University Medical School.

In situ colloidal iron preparation. Mice were anesthetized with diethyl ether inhalation and perfused with physiological saline through the left ventricle followed by 4% paraformaldehyde and/or 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) containing 0.01 M ferric chloride. The abdominal cavity was opened, and the spleen and kidney were excised and cut into $2 \times 2 \times 2$ mm blocks with razor blades. The tissue blocks were immersed in the same fixative containing ferric chloride for 6 h or overnight, and washed twice with 0.01 M ferric chloride in 0.1 M cacodylate buffer for 2 h or more. The specimens in the buffered ferric chloride solution were irradiated with microwaves for 5 min at 75 °C until the color of the solution changed to dark reddish brown.

Electron microscopy. The irradiated samples were immediately cooled in ice, washed in 0.1 M cacodylate buffer, and then rinsed with physiological saline. The samples were dehydrated through an ethanol series, embedded in epoxy resin, and cut into ultrathin sections. Sections were mounted on copper grids and observed without any metal staining in a transmission electron microscope (H-700 or H-7100: Hitachi (Tokyo, Japan); Equipped in the Central Research Laboratory, Okayama University Medical School) at an accelerating voltage of 75 kV.

Table 1 Duration of microwave irradiation until the color change of colloidal iron sol at different upper limits of temperature

Upper limit of temperature (°C)	Time of color change from yellow to reddish brown	
	Beginning (sec)	Completion (sec)
45	no change	no change
55	135	360 <
65	47	185
75	30	110
85	30	80
95	30	55
100	30	50

Note, when the upper limit of the temperature was 75 °C or higher, the color began to change at 30 sec, and this change was completed within 120 sec.

Results

To determine the conditions for the microwave irradiation, ferric chloride solution buffered with sodium cacodylate was irradiated with microwaves at 45–100 °C. The results are summarized in Table 1. As shown in Table 1, when the upper limit temperature was 75 °C or higher, the color began to change at 30 sec, and the change was completed at 110 sec (75 °C) to 50 sec (100 °C). When the upper limit of the temperature was 65 °C or less, it took 3 min or more to accomplish the colloidal conversion. The irradiation time and temperature should be sufficient to produce the colloidal iron within the tissues, but the same conditions should not cause damage to the ultrastructures. Thus, the irradiation condition was determined to be 5 min at a temperature upper limit of 75 °C in a microwave processor.

In transmission electron microscopy, the tissue and cellular elements, including cell organelles, were well preserved after microwave irradiation for 5 min at 75 °C. In the splenic tissues, 1–2 nm electron dense particles of colloidal iron were deposited on the cellular surface and cytoplasmic structures, including ribosomes (Fig. 1). The nucleus, however, was not stained with colloidal iron. Mitochondria were also stained with colloidal iron in the space between the inner and outer membranes and the space in the cristae.

The renal glomeruli in the kidney samples, which were treated using the microwave *in situ* colloidal

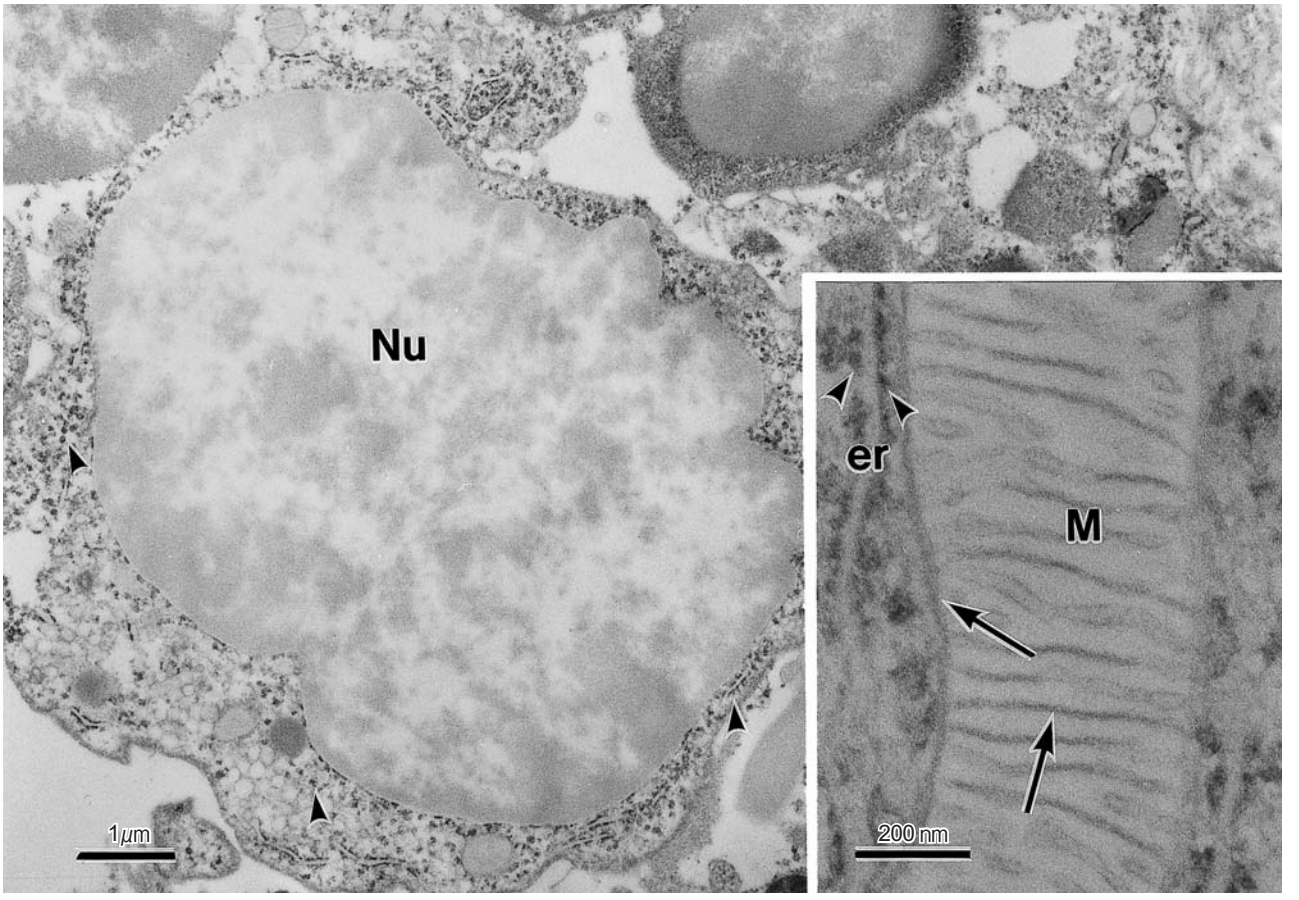

Fig. 1 Transmission electron micrographs of the splenic tissue, which was immersed in ferric chloride solution and irradiated with microwaves (*in situ* colloidal iron preparation method). Electron dense colloidal particles are deposited on intracellular structures such as ribosomes (arrowheads) in the splenic cell. The nucleus (Nu) is not stained by this method. Inset: A high magnification micrograph of a mitochondrion (M) shows the space between the inner and outer membranes and the space in the cristae were stained by the colloidal iron (arrows). er, endoplasmic reticulum. Scale bars: 1 μm; inset, 200 nm.

iron method, showed good contrast with the *in situ* prepared colloidal iron with appropriate staining, though the nuclei of the podocytes and mesangial cells were not stained in the present specimens (Fig. 2A). The podocyte foot processes or pedicles were well stained with *in situ*-prepared colloidal iron and showed a diffuse staining pattern (Fig. 2B).

In the connective tissue, collagen fiber bundles were well stained. The colloidal granules were deposited periodically at approximate intervals of 42–44 nm along the collagen fibrils (Fig. 3). In the transverse section of the collagen fibrils, the colloidal particles were observed around the fibrils.

Discussion

The present paper describes a new method for preparing fine granular colloidal iron. This method is characterized by the use of microwave irradiation to make colloidal iron within the tissue blocks, which have been immersed in cacodylate-buffered ferric chloride solution. In the previous colloidal iron staining methods, the colloidal sol was prepared by a combination of dialyzed iron and acetic acid [1], by glycerin and ammonia [13], or by pouring ferric chloride into boiling distilled water [14, 15]. These methods have been further improved in order to prepare fine colloidal iron particles using sodium [2], ammonium [3] or hydrazinium [4] cacodylate buffer. Though these colloidal particles are as small as 0.5–

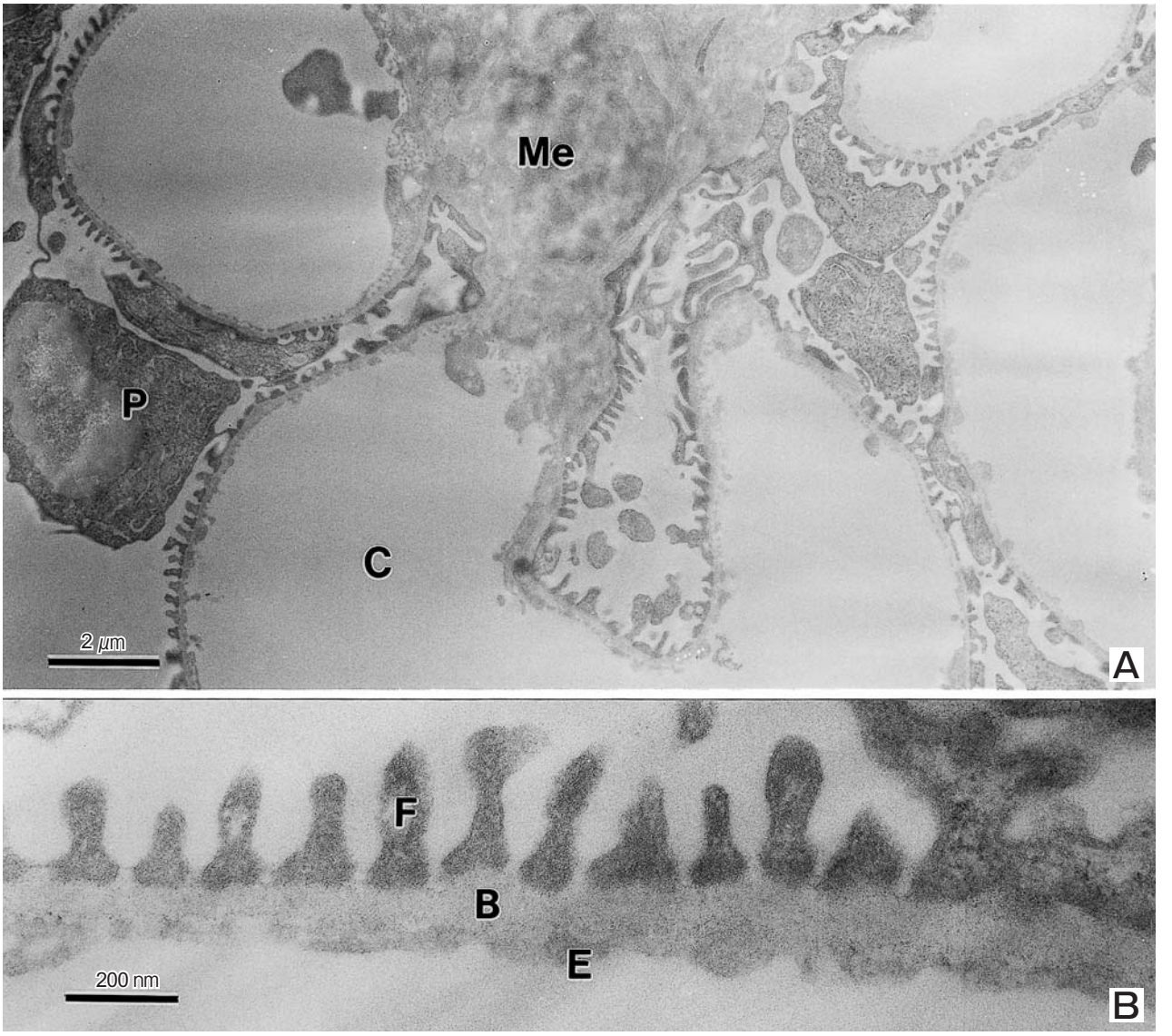

Fig. 2 Transmission electron micrographs of the renal tissue, which were treated with the *in situ* colloidal iron preparation method. **A**: Cell bodies and foot processes of podocytes (P) in the renal glomerulus are stained well by the colloidal iron. **B**: High power view of the glomerular filtration membrane. Podocyte foot processes (F) are diffusely stained with the colloid. B, basal lamina; C, capillary lumen of the renal glomerulus; E, endothelial cell; Me, mesangial cell. Scale bars: **A**, 2 μm; **B**, 200 nm.

1.0 nm in diameter [4], they do not readily diffuse into the deep regions of the tissue blocks. Our present microwave *in situ* colloidal iron preparation method can stain deeper regions. Furthermore, in the present method, the colloidal sol preparation and staining are performed at the same time. As far as we know, such a staining principle is novel in histochemical or histological procedures.

Microwave irradiation has been applied in tissue fixation [8, 9, 11], staining [12, 16], polymerizing embedding polymers [17] and antigen unmasking [10–12, 18]. These effects are brought about by rapid heating by means of irradiation. In the present method, ferric ions in sodium cacodylate buffer, which are materials of colloidal iron, move into deep regions of the tissues by diffusion, and are then transformed into the colloid by irradiated microwave energy *in situ*.

The cationic colloidal iron has been used to detect anionic sites in tissues such as hyaluronic acid or

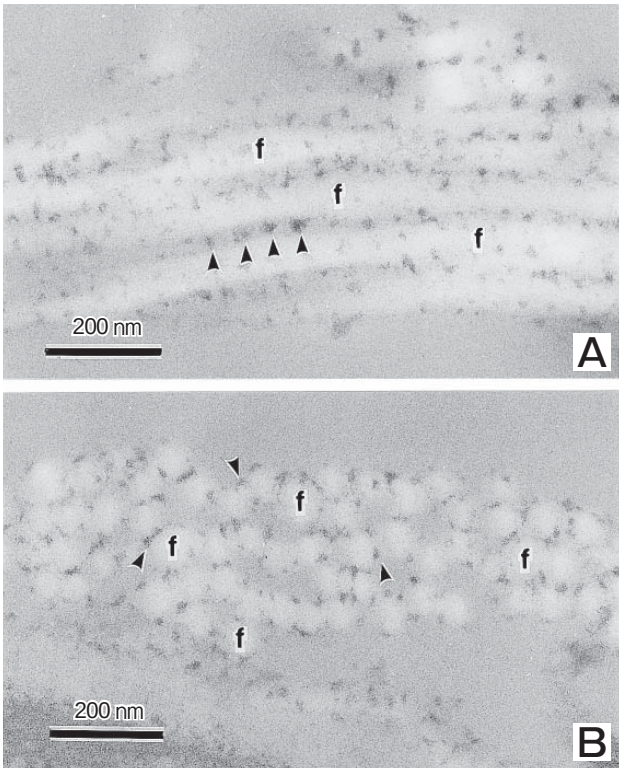

Fig. 3 Collagen fibrils in the mouse renal connective tissue stained using the *in situ* colloidal iron preparation method. **A**: Longitudinal section of the fibrillar band shows that colloidal iron particles (arrowheads) are deposited along the fibrils (f) at constant intervals. **B**: Transverse section of the fibrillar bands shows that each fibril (f) is surrounded by a deposition of colloidal particles (arrowheads). Scale bars: **A**, 200 nm; **B**, 200 nm.

other sulfated proteoglycans [1]. When these previous colloidal iron sols are used, the specimen for transmission electron microscopy is prepared by means of bulk staining [4] or by the staining of hydrophilic-resin embedded sections [7]. In bulk staining, specimens are immersed in the staining solution for a long period of time.

The present *in situ* microwave irradiation colloidal iron preparation method successfully stained intracellular ultrastructures, such as ribosomes. The staining pattern of the intracellular structures is similar to that of the ultrathin sections stained with the cationic colloidal iron, which is deposited on the ribosomes at pH 4.0 or 7.0 [7]. The ribosomes contain much ribonucleic acid whose phosphate groups were ionized at these pH values. Though we have little definitive evidence, this similarity in staining patterns suggests that the present colloidal iron particles prepared by microwaves may have positive charges. Moreover, we could not observe non-specific deposition with the present colloidal iron, though such deposition may suggest that excessive colloid remained within the cell.

The podocyte foot processes of the renal glomerulus were diffusely stained by this method. This staining pattern is quite similar to those seen at pH 7.0 using previous colloidal iron methods [3, 4]. At the neutral pH range, almost all of the anionic groups are ionized, so that specifically negatively charged sites may be forbidden.

It is noteworthy that the collagen fibrils in the connective tissue spaces were well stained with the present method, and that they showed periodic deposits of colloidal iron. Fibrillar procollagen molecules such as type I collagen have 2 possible glycosylation motifs [19]. These deposits of the colloidal iron particles suggest the presence of acid glycosaminoglycans bound to collagen fibrils.

Acknowledgements. The authors thank Mr. H. Urata (Central Research Laboratory, Okayama University School of Medicine), for his assistance in electron microscopy, and, also thank Mr. H. Mizoguchi, Mr. M. Narasaki, Mr. H. Osugi, and Mr. T. Komiyama (Department of Human Morphology) for their technical helps.

References

1. Hale CW: Histochemical demonstration of acid polysaccharides in animal tissues. Nature (1946) 157: 802.
2. Tanaka A: Phagocyte properties of lung alveolar wall cells. Acta Med Okayama (1974) 28: 69–79.
3. Seno S, Ono T and Tsujii T: Macromolecular charge and cellular surface charge in adhesion, ingestion, and blood vessel leakage. Ann N Y Acad Sci (1983) 416: 410–425.
4. Murakami T, Taguchi T, Ohtsuka A, Sano K, Kaneshige T, Owen RL and Jones AL: A modified method of fine-granular cationic iron colloid preparation: its use in light and electron microscopic detection of anionic sites in the rat kidney glomerulus and certain other tissues. Arch Histol Jpn (1986) 49: 13–23.
5. Ohtsuka A and Murakami T: Fine anionic iron colloid and its use in light and electron microscopic detection of cationic sites in the rat kidney. Arch Histol Jpn (1986) 49: 543–552.
6. Endo R, Murakami S, Masuda Y, Taguchi T, Ohtsuka A, Nishizaki K and Murakami T: The perineuronal proteoglycan surface coat in the adult rat brain, with special reference to its reactions to Gömöri's ammoniacal silver. Acta Med Okayama (2000) 54: 111–118.
7. Ohtsuka A, Kikuta A, Taguchi T and Murakami T: A hydrophilic resin-embedding method for light and electron microscopic detection of tissue anionic sites with cationic colloidal iron: as applied to mouse Paneth cells. Arch Histol Cytol (1993) 56: 423–430.
8. Mayers CP: Histological fixation by microwave heating. J Clin

Pathol (1970) 23: 273–275.
9. Mizuhira V, Notoya M and Hasegawa H: New tissue fixation method for cytochemistry using microwave irradiation. I. General remarks. Acta Histochem Cytochem (1990) 23: 501–523.
10. Login GR, Schnitt SJ and Dvorak AM: Rapid microwave fixation of human tissues for light microscopic immunoperoxidase identification of diagnostically useful antigens. Lab Invest (1987) 57: 585–591.
11. Hopwood D, Coghill G, Ramsay J, Milne G and Kerr M: Microwave fixation: its potential for routine techniques, histochemistry, immunocytochemistry and electron microscopy. Histochem J (1984) 16: 1171–1191.
12. Login GR and Dvorak AM: A review of rapid microwave fixation technology: its expanding niche in morphologic studies. Scanning (1993) 15: 58–66.
13. Rinehart JF and Abul-Haj SK: An improved method for histologic demonstration of acid mucopolysaccharides in tissues. AMA Arch Pathol (1951) 52: 189–194.
14. Müller G: Über eine Verfachung der Reaktion nach Hale (1946). Acta Histochem (1955) 2: 68–70.
15. Mowry RW: Improved procedure for the staining of acidic polysaccharides by Muller's colloidal (hydrous) herric oxide and its combination with the Feulgen and periodic acid Schiff reaction. Lab Invest (1958) 7: 566–576.
16. Hanker J and Giammara B: Mirowave-accelerated cytochemical stains for the image analysis and the electron microscopic examination of light microscopy diagnostic slides. Scanning (1993) 15: 67–80.
17. Giammara BL: Microwave embedment for light and electron microscopy using epoxy resins, LR White, and other polymers. Scanning (1993) 15: 82–87.
18. Cattoretti G, Pileri S, Parravicini C, Becker MH, Poggi S, Bifulco C, Key G, D'Amato L, Sabattini E, Feudale E, Reynolds F, Gerdes J and Rilke F: Antigen unmasking on formalin-fixed, paraffin-embedded tissue sections. J Pathol (1993) 171: 83–98.
19. Olsen BR and Ninomiya Y: Fibrillar collagens; in Extracellular Matrix, Anchor, and Adhesion Proteins., Kreis T and Vale R eds, 2 nd Ed, Oxford University Press, New York (1999) pp 383–387.