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Technical note

Development of a simple, cost-effective, semi-correlative light and electron microscopy method to allow the immunoelectron localisation of non-uniformly distributed placental proteins

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ABSTRACT

Immunoelectron microscopy is wrought with technical limitations that complicate its use. However, advances in correlative light and electron microscopy have recently lead to improvements in this field. We report the development of a semi-correlative approach to investigate the ultrastructural location of an antiphospholipid antibody within the syncytiotrophoblast. This method offers several advantages over existing methodologies, since it preserves antigenicity, shows good immunolabel penetrability and does not require specialized equipment. The use of a pre-embedding screen has also allowed us to target individual placental villi and overcome sampling limitations of the electron microscope. This simple, cost-effective method is likely to find widespread application in placental research.

1. Introduction

Immunoelectron microscopy localises antibody-labelled proteins on an ultrastructural scale. It has been used extensively to investigate the location of proteins in the placenta despite being a very challenging technique. Most reports of placental immunoelectron microscopy perform immunolabelling after resin embedding [1–4], or immunolabel ultrathin cryosections [5–10]. A few workers have immunolabelled placental tissues prior to resin embedding [11–13]. All three of these approaches are associated with cost and/or technical limitations which coupled with the sampling limitations of the electron microscope, contribute to the difficulty of this technique [14,15].

The placenta has been the backdrop to recent advances in immunoelectron microscopy, where proteins are localized using bifunctional fluorescent and electron-dense immunolabels [10,16]. These labels facilitate correlative light and electron microscopy, where additional information is gained by visualising the same tissue with both imaging modalities [17]. Dual visualisation may especially aid the investigation of proteins expressed in a patchy fashion, which is a common finding in the syncytiotrophoblast [18–20].

Here we report the development of a method that employs semi-correlative and pre-embedding approaches to address some of the difficulties associated with immunoelectron microscopy. We have used this technique to visualize the ultrastructural localisation of an antiphospholipid antibody, which is known to be rapidly internalised by the syncytiotrophoblast in culture [21].

2. Methods

Collection and use of human first trimester placentae from Epsom Day Unit, Greenlane Hospital, Auckland, was approved by the Northern X Regional Ethics Committee. All samples were obtained with written informed consent.

Villous tissue explants were excised from placentae, rinsed in phosphate buffered saline pH 7.4 (PBS) and cultured for 2 min with a murine monoclonal antiphospholipid antibody, that was generated by hybridoma culture and purified by protein G chromatography, as described previously [21,22].

Explants were fixed in 4% paraformaldehyde in 0.1 M sodium cacodylate pH 7.4 + 5% sucrose [23] overnight at room temperature (RT) before being immobilised in 4% low-gelling temperature agarose/PBS. The agarose was set on ice before the blocks (Fig. 1A) were sectioned into 70 μm slices using a vibratome.

Free-floating sections were blocked of non-specific binding overnight at 4 °C in block buffer (1% newborn goat serum in PBS + 0.5% Tween 20 (PBST)). Internalised antiphospholipid antibody was detected by incubation with biotinylated anti-mouse IgG antibody (Jackson Immunoresearch) overnight at 4 °C followed by Streptavadin-AlexaFluor® 594-FluoroNanogold™ (Nanoprobes) for 2 h RT, both diluted 1:300 in block buffer. Control sections received 1:300 anti-rabbit IgG antibody (Jackson Immunoresearch) in place of anti-mouse IgG antibody. All sections were washed in PBST, 4 × 15 min, between each incubation. Sections were then visualized using transmitted and/or fluorescent light on Zeiss LSM710 confocal and Nikon Ti microscopes.

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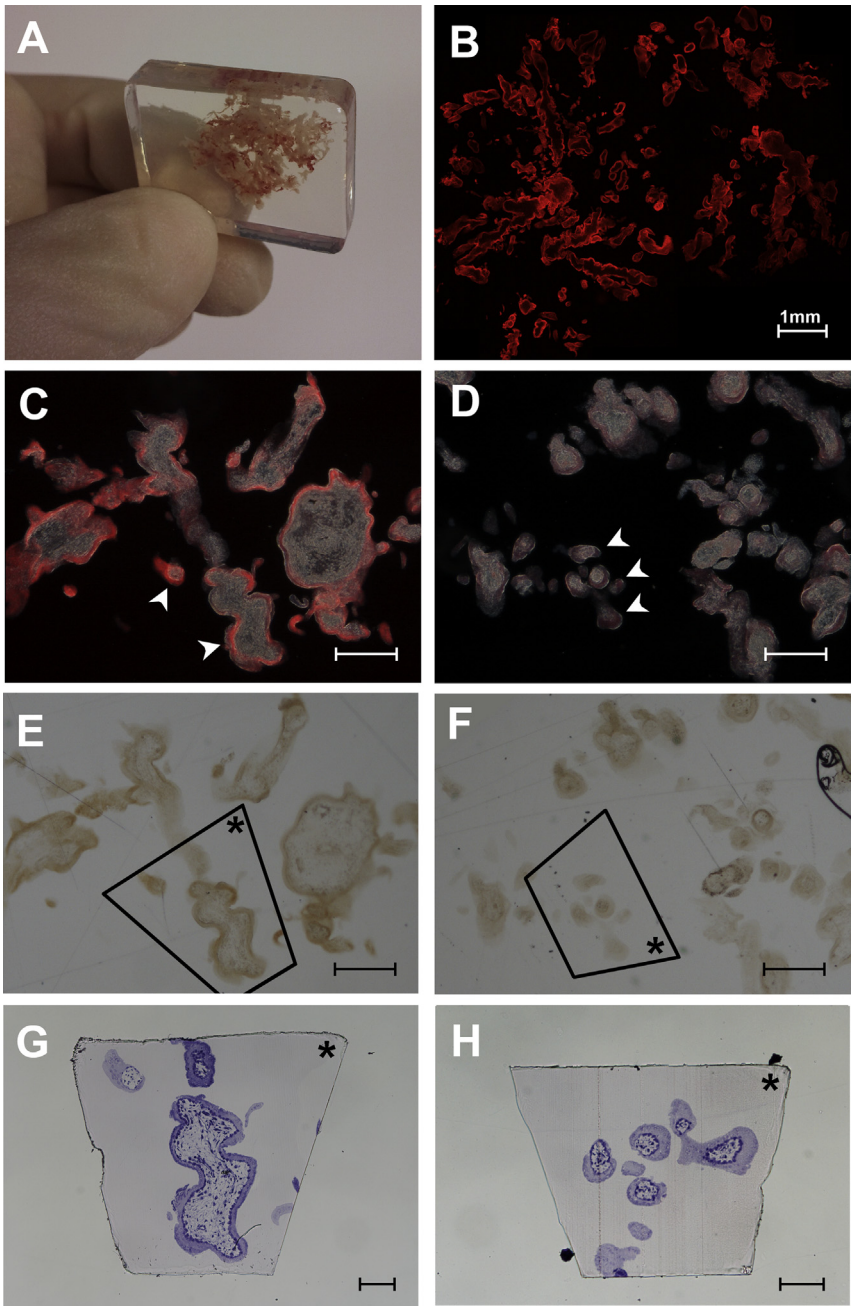

Fig. 1. Immunostaining agarose-embedded placental villous sections with FluoroNanogold allowed selective electron microscopy analysis. (A) Fixed placental explants were embedded in agarose to immobilize villi prior to vibratome sectioning. (B) To detect the internalised antiphospholipid antibody, 70 μm-thick agarose sections were probed with biotinylated anti-mouse IgG antibody and streptavidin-conjugated AlexaFluor® 594-FluoroNanogold™ and examined under fluorescent illumination. (C) At higher magnification, red-fluorescent and transmitted-light images were merged and prominently stained villi were selected (arrowheads). (D) Villi were also selected from those sections probed with anti-rabbit IgG antibody as a negative control. (E&F) After flat resin embedding, selected villi were re-located and trimmed to trapeziums (black outline). The corner of the trapezium marked with an asterisk corresponds to the asterisk-marked corner in (G&H) semi-thin toluidine blue-stained sections. All scale bars = 100 μm unless otherwise stated.

Agarose sections were rinsed in 20 mM sodium citrate pH 7.0, 3 × 5 min, followed by deionized water before enlarging FluoroNanogold with HQ Silver™ enhancement kit (Nanoprobes) for 3 min according to the manufacturer's instructions.

Sections were post-fixed in 0.5% osmium tetroxide in 0.1 M Sorenson's phosphate buffer pH 7.4 for 30 min at RT and dehydrated by washes in 70% ethanol 2 × 5 min, 85% ethanol 1 × 5 min, 95% ethanol 1 × 5 min, 100% ethanol 4 × 5 min and 100% propylene oxide 2 × 5 min. Dehydrated sections were infiltrated with Agar 100 Epoxy resin (Agar Scientific) by incubations in 2:1 propylene oxide:resin 1 h RT, 1:1 propylene oxide:resin 1 h RT, 1:2 propylene oxide:resin 1 h RT and 100% resin overnight. All dehydration and infiltration steps were performed at RT. Following infiltration, sections were laid flat on Poly-chloro-tri-fluoro-ethylene PCTFE film, or Aclar (ProSciTech), covered with a drop of fresh resin, and then a second sheet of Aclar. This Aclar sandwich was then placed between two glass slides to keep the section flat during polymerization at 60 °C for 48 h. Additionally, a BEEM capsule was filled with resin, overturned onto a separate sheet of Aclar and polymerized at 60 °C for 48 h to generate a resin block with a flat surface.

Flat resin-embedded sections were later peeled away from the two sheets of Aclar and visualised with a Nikon Eclipse E400 microscope under white light. Areas of interest were excised from the resin-embedded section and super-glued onto the flat surface of the polymerised resin block. Semi-thin sections (1 μm) were made, stained with toluidine blue and visualised on a Nikon Eclipse E400 microscope