Preparation of cultured cells for scanning electron microscope

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Method Article

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Abstract

Introduction

Researchers whose work focuses on self-assembled structures in the biological sciences or nanostructures in the materials sciences are interested in high-resolution imaging. Certain self-assembled structures occupy a size range where the theoretical limit of resolution by light microscopy \(LM), which is on the order of 160 nm, is not adequate to image them. The cell creates several types of protrusions. The filopodium \(plural, filopodia) is a slender, tapering extension of cytoplasm with a mean width of 50-100 nm. Filopodia are especially challenging to visualize, because they may broaden to >160 nm at the base where they integrate with the rest of the cytoplasm. Although the broader parts of the structures are often used to estimate the prevalence of filopodia on cells, the greatest number of the structures may be missing from the image viewed by LM. Thus, LM imaging gives inaccurate and misleading results. To accurately assess their prevalence, filopodia must be imaged with a high-resolution instrument or modality. The filopodia serve as only one example of a cell surface structure that is at or beyond the LM limit. Additional features, such as microvilli, coated pits, caveolae, and stereocilia, use self-assembly processes to create structures in the sub-LM range.

Reagents

3% glutaraldehyde in 0.1 M phosphate buffer at 37 degrees C 0.1 M phosphate buffer or phosphate-buffered saline \(PBS), pH 7.3 1% OsO_4 1% carbohydrazide Distilled or deionized water Graded series of ethanol or hexylene glycol for dehydration Liquid carbon dioxide for critical point dryer

Equipment

One pair of small diagonal wire cutter Critical point dryer Sputter coater

Procedure

1. Pre-warm fixative solution to 37 degrees C. 2. Remove media from plastic tissue culture dish and rapidly pour on glutaraldehyde fixative. 3. Leave 15 minutes. 4. Rinse with buffer or PBS three times over 5 minutes. 5. Fix in OsO_4, 15-30 minutes. 6. Rinse with buffer five times over 10 minutes. 7. Incubate in freshly made 1% carbohydrazide 10-30 minutes. 8. Rinse with distilled five times over 15 minutes. 9. Incubate again in 1% OsO_4, 15-30 minutes 10. Rinse three times with distilled water over 15 minutes. In the third rinse, cut dishes to fit the critical point dryer. Using small diagonal wire cutters, clip and remove edge first. Then cut bottom of dish to size. 11. Place in another dish. 12. Dehydrate through ethanol or hexylene glycol series beginning with 30% and changing to solutions of 50%, 70%, 90%, and three times 100%, over 30 minutes. 13. Dry in critical point dryer. 14. Sputter coat with 1-2 nm gold-palladium.

Timing

4-5 hours

Critical Steps

1. The major protrusions have been found to retract if the temperature of the fixative differs from that of the cells \(see Table 1). Thus, prewarmed fixative and its rapid addition to the cell culture while it is still warm are essential. 7-9. Repeating the OsO4 exposure after a carbohydrazide step adds more reduced osmium to cellular details and increases the sample conductivity \(references 1-3). Carbohydrazide is substituted for the compound used by the workers who originally defined this procedure \(reference 4). The conductivity can be increased later in the procedure by using thicker coats of noble metal, but these obscure the details of the cell edge.

Troubleshooting

1. To conserve shape conformation of mammalian cells, fixative temperature must be the same as the cells' \(37 degrees C). 10. Do not let cells dry out at any time. This exerts huge surface tension forces and collapses the structure. Samples are especially vulnerable during steps where they are cut. 12. The samples are also vulnerable to air-drying during transfer to the critical point dryer. Hexylene glycol is not volatile. This makes it better than ethanol to avoid air-drying. 14. Thick coatings \(3 nm or greater) will obscure edge details.

Anticipated Results

High resolution surface and edge detail, useful for evaluating the prevalence of filopodia and other cellular surface details.

References

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Figures

Tukey Grouping			Variable	Treatment
A			0.370	Sample C
A	B		-0.021	Sample A
	B		-0.369	Sample B

*Means with the same letter are not significantly different at the level P <0.05.

Figure 1

Supplementary Files

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