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Molecules in focus

Platinum replica electron microscopy: Imaging the cytoskeleton globally and locally

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ABSTRACT

Structural studies reveal how smaller components of a system work together as a whole. However, combining high resolution of details with full coverage of the whole is challenging. In cell biology, light microscopy can image many cells in their entirety, but at a lower resolution, whereas electron microscopy affords very high resolution, but usually at the expense of the sample size and coverage. Structural analyses of the cytoskeleton are especially demanding, because cytoskeletal networks are unresolvable by light microscopy due to their density and intricacy, whereas their proper preservation is a challenge for electron microscopy. Platinum replica electron microscopy can uniquely bridge the gap between the "comfort zones" of light and electron microscopy by allowing high resolution imaging of the cytoskeleton throughout the entire cell and in many cells in the population. This review describes the principles and applications of platinum replica electron microscopy for studies of the cytoskeleton.

1. Introduction

Structural studies are essential for understanding functional principles in biology. They reveal how smaller components of a system work together as a whole. An ideal structural approach would combine high resolution of details with full coverage of the whole. However, it is usually difficult to satisfy both requirements. In practice, different trade-offs are needed for each biological question.

In cell biology, light microscopy (LM) and electron microscopy (EM) are main imaging techniques for structural studies. Their "comfort zones" in terms of resolution and coverage barely overlap. Conventional LM can image many cells in their entirety, but at resolution limited by diffraction to ~200 nm. EM can reach ~2 nm resolution, but is rather demanding in terms of time, skills and effort required to produce high quality samples in sufficient amounts. LM is easily compatible with live cell imaging and identification of molecules, although with little information about the structural context of labeled components. EM cannot image live cells. Moreover, many EM approaches are poorly compatible correlative light and EM (CLEM) of living cells that can link cell structure and dynamics. Incorporating immunochemistry into EM is also challenging, but can reveal labeled structures together with their surroundings.

Structure of the cytoskeleton, and especially of the actin cytoskeleton, presents particular challenges for high resolution structural analyses. The nanometer size and tight packing of cytoskeletal filaments makes them unresolvable by either diffraction-limited or subdiffraction (super-resolution) LM. This goal so far can be achieved only by EM. However, actin filaments are notoriously difficult to visualize by traditional EM. In plastic-embedded samples, organization of stable actin filament bundles can be revealed, but delicate actin filament networks, such as those in lamellipodia or at the surface of membrane organelles remain uninterpretable even in best examples (Kukulski et al., 2012; Luduena and Wessells, 1973; Tilney et al., 1980; Wu et al., 2010).

CryoEM is praised for its ability to analyze biological samples in their native hydrated state (Lucic et al., 2013). However, because of low contrast and sensitivity to irradiation of cryoEM preparations, cryoEM is mostly used to analyze samples suitable for averaging. In cells, cryoEM can image only very small regions and is best in detecting membranes, but rarely used to image the cytoskeleton (Mahamid et al., 2016; Medalia et al., 2007). The amount of cellular data that can be collected by cryoEM is limited by lengthy image acquisition, sample damage during imaging, sophisticated digital processing of each field, and availability of expensive equipment. CryoEM is also incompatible with specific identification of structural components except for educated guess (Lucic et al., 2013).

Platinum replica electron microscopy (PREM), also known as rotary shadowing EM, can uniquely bridge the gap between LM and EM, especially for structural analyses of the cytoskeleton (Svitkina, 2016; Svitkina and Borisy, 1998). PREM yields many cells per

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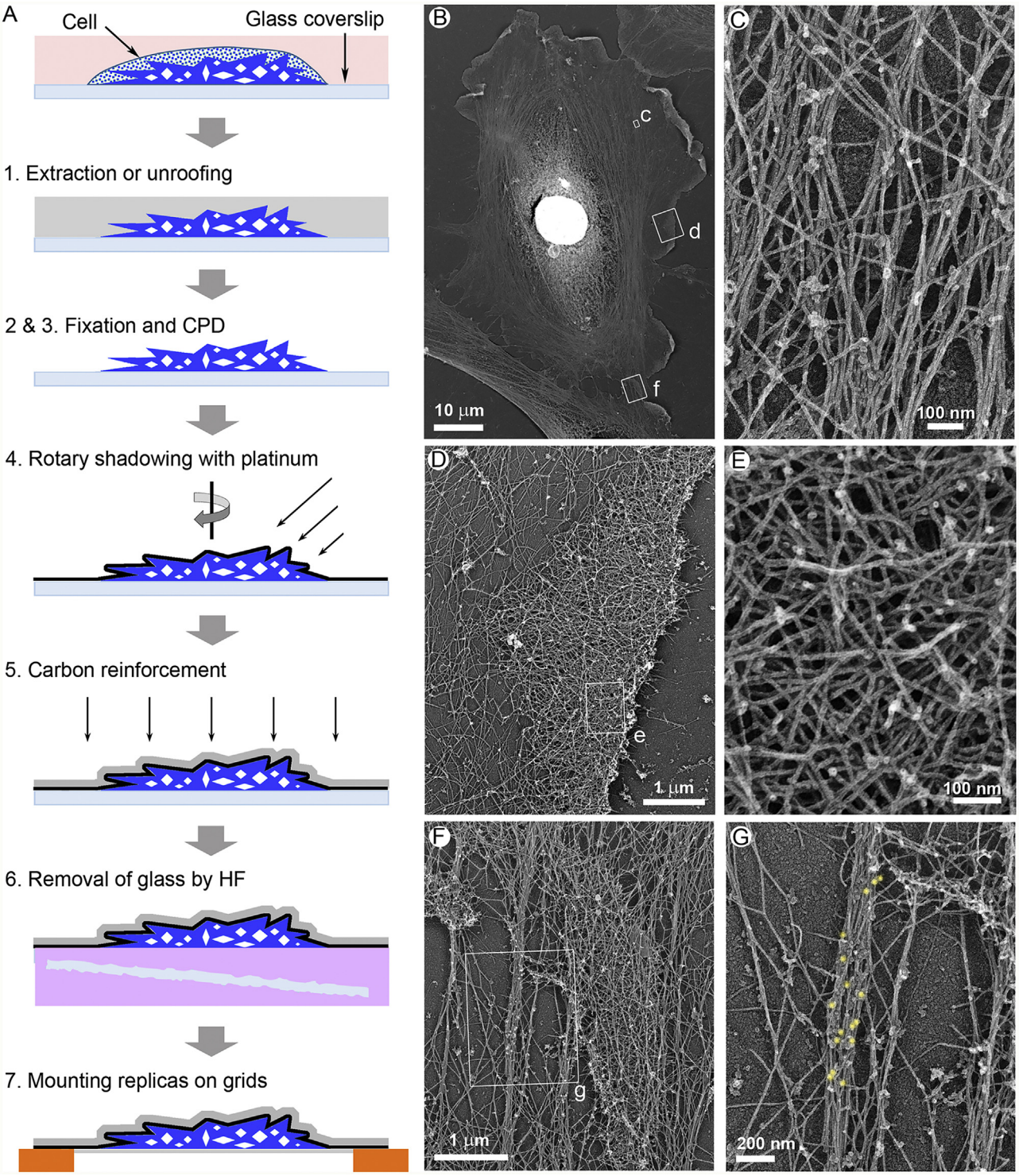

Fig. 1. PREM of a human umbilical vein endothelial cell stained with anti-VE-cadherin immunogold. (A) Workflow of PREM of cultured cells. (B) Overview of the cell with labeled regions enlarged in panels C–G. (C, D) Actin filament networks in the lamellum (C) and the lamellipodium (D). (E) Enlarged region of the branched actin network from the box in D. (F) Junction between two cells. (G) Enlarged focal adherens junction from the box in F; gold particles labeling VE-cadherin are pseudocolored in yellow.

experiment and affords high resolution typical for EM. Various cytoskeletal structures including dynamic actin networks can be well preserved and analyzed by PREM at a broad range of scales up to single filaments, even when they are densely packed (Fig. 1). PREM data are suitable for direct visual evaluation, thus allowing immediate screening of many pleiomorphic cells to sort out their general and variable structural features. Sample preparation for PREM, including immunolabeling, is fast and efficient compared with other EM techniques. PREM is compatible with CLEM and requires inexpensive equipment and short training. As any other technique, PREM is not universal. It is limited to relatively thin samples, requires samples attached to glass surfaces and is not ideal for studies of membrane structures. However, the combination of its strengths and weaknesses makes PREM a highly productive and informative approach to study cytoskeleton organization in different cells at various physiological conditions.