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AFM imaging of fenestrated liver sinusoidal endothelial cells

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ABSTRACT

Each microscope with its dedicated sample preparation technique provides the investigator with a specific set of data giving an instrument-determined (or restricted) insight into the structure and function of a tissue, a cell or parts thereof. Stepwise improvements in existing techniques, both instrumental and preparative, can sometimes cross barriers in resolution and image quality. Of course, investigators get really excited when completely new principles of microscopy and imaging are offered in promising new instruments, such as the AFM.

The present paper summarizes a first phase of studies on the thin endothelial cells of the liver. It describes the preparation-dependent differences in AFM imaging of these cells after isolation. Special point of interest concerned the dynamics of the fenestrae, thought to filter lipid-carrying particles during their transport from the blood to the liver cells. It also describes the attempts to image the details of these cells when alive in cell cultures. It explains what physical conditions, mainly contributed to the scanning stylus, are thought to play a part in the limitations in imaging these cells.

The AFM also offers promising specifications to those interested in cell surface details, such as membrane-associated structures, receptors, coated pits, cellular junctions and molecular aggregations or domains. The AFM also offers nano-manipulation possibilities, strengths and elasticity measurements, force interactions, affinity measurements, stiffness and other physical aspects of membranes and cytoskeleton. The potential for molecular approaches is there.

New developments in cantilever construction and computer software promise to bring real time video imaging to the AFM. Home made accessories for the first generation of AFM are now commodities in commercial instruments and make the life of the AFM microscopist easier. Also, the combination of different microscopies, such as AFM and TEM, or AFM and SEM find their way to the market allowing comfortable correlative microscopy.

1. Introduction

Thanks to the improvement in chemical fixation, both transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were able to visualize and unequivocally characterize different cell types in the liver capillaries, also called liver sinusoids. After perfusion of a fixative through the vascular system of the liver, it appeared that very thin, fenestrated endothelial cells could be seen lying between the blood and the resident parenchymal cells (Wisse, 1970). These cells represent 'the kitchen' of the body and maintain a constant exchange with the ever flowing blood stream. Small open fenestrae (140 nm in rats and 100 nm in human) in these endothelial cells were found to allow, but also to restrict, the exchange of the different sized lipoproteins and other particles circulating in the blood or secreted by the liver cells (Wisse et al., 1999). The fenestrae are found to be dynamic, their numbers vary, their diameter varies and they also can completely disappear or appear in exaggerated numbers (Wisse et al., 1985). Unfortunately these fenestrae are below the resolution of a light microscope, but they indeed show up in full detail in TEM and SEM (Wisse et al., 2010). It was reasoned that the AFM could provide the necessary resolution and imaging conditions to resolve fenestrae and their dynamics in living liver sinusoidal endothelial cells (LSECs).

Studying the membrane topology of intact cells or membrane fragments has always been a topic of prime interest in biological atomic force microscopy (AFM) (Dufrêne, 2001; Frederix et al., 2009). AFM investigation of membrane-associated structures such as receptors (Fotiadis et al., 2006), channels (Philippsen et al., 2002), exo- and pinocytotic vesicles (Allison and Doktycz, 2006), coated pits (Jena et al., 2003), microdomains (Milhiet et al., 2003), cell junctions (Lal and Lin, 2001) and membrane pores (Cheng et al., 1999)—both in eukaryotic and prokaryotic cell models—have been widely studied. High-resolution multi-dimensional AFM data on isolated membrane structures are particularly successful and

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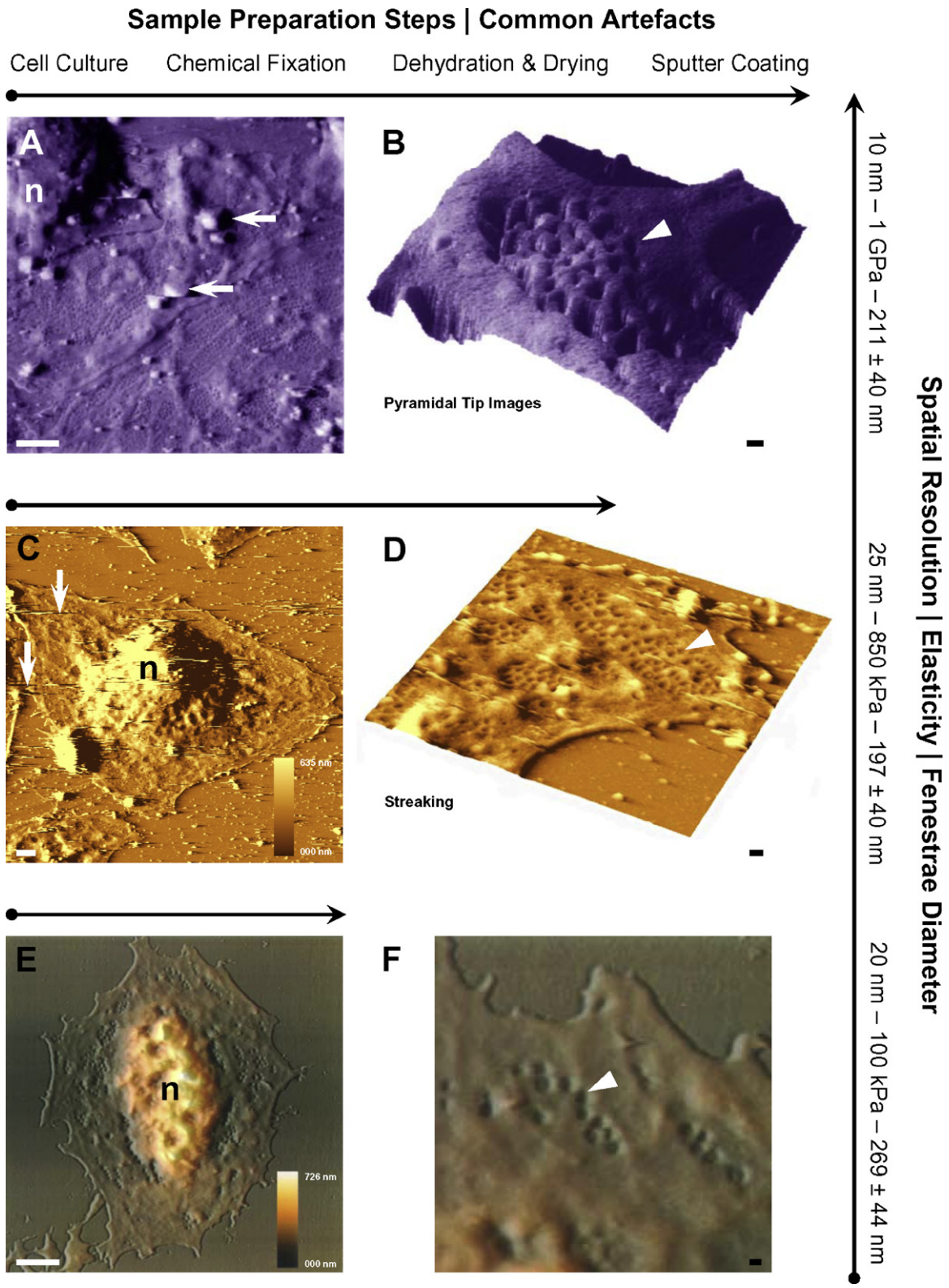

Fig. 1. From dried-fixed gold-coated (A and B), over dried-fixed uncoated (C and D) to wet-fixed (E and F) liver sinusoidal endothelial cells (LSECs) in vitro. (A) Low magnification shows a clear bulging area containing the nucleus (n) which is surrounded by flat cytoplasmic processes harbouring sieve plates with fenestrae. Pyramidal tip images (arrows) were the most dominant artefact present in those samples. (*With permission, Comparative atomic force and scanning electron microscopy; an investigation on fenestrated endothelial cells in vitro, Braet et al., J Microsc, vol. 181, pp. 10–17, ©[1996] Wiley-Liss, Inc.*). (B) Corresponding three-dimensional AFM image of a sieve plate that contain fenestrae (arrowhead). Note the granular roughness that correspondents to the gold grains of the sputter-coated layer. (C) Dried-fixed uncoated LSECs differ significantly in image quality from the ones provided with a 10 nm gold layer (see A vs. C): i.e., the presence of numerous linear streaks in the scan direction (arrows). Note that sieve plates could be depicted. Nucleus, n. (D) At a higher scan range, sieve plates and their fenestrae (arrowhead) could be resolved, but tip-induced streaking remained. *(Figure C and D with permission: Drying cells for SEM, AFM and TEM by hexamethyldisilazane: A study on hepatic endothelial cells, Braet et al., J Microsc, vol. 186, pp. 84–87, ©[1997] Wiley-Liss, Inc.*). (E) Overview image of a LSEC that underwent brief fixation with 2% glutaraldehyde and imaged under 0.1 M sodium cacodylate buffer (wet-fixed). This type of sample preparation resulted in superior AFM data of LSECs as they were largely devoid of tip-induced AFM artefacts. Some minor lateral deformation could be noticed