Four microscopy methods unveil liver fenestrations: correlative SEM, SIM, STED, AFM targeting single cell type

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Liver sinusoidal endothelial cells (LSECs) are an excellent research target for developing high-resolution imaging methods due to their unique morphology [1]. LSEC membrane is perforated with multiple transcellular pores called fenestrations. Being in the range of 50-350 nm [2], fenestrations remain largely beyond the resolution limits of conventional optical microscopy, limited by light diffraction. LSECs play a crucial role in realizing the bidirectional transport of substances, especially lipoproteins, between blood and hepatocytes in the liver. Reduced LSEC porosity, called defenestration is often associated with ageing and chronic liver diseases such as hepatitis, steatosis, and cirrhosis. In vitro studies have shown that LSEC porosity can be altered pharmacologically [2,3]. These findings promise the development of a proper treatment allowing to reopen once closed fenestrations or to tune their diameters to achieve proper filtration properties within the liver [4]. Due to the great importance of fenestrations diameters, acting as a functional sieve for particles of a certain size into and out of the liver, the determination of the "true" fenestration size is extremely desirable.

Over the years various nanoscopy techniques have been challenged to disclose fenestrations in LSEC [5]. Obtained values of their diameters often significantly varied between the used techniques. The only way to understand these discrepancies was to visualise the exact same fenestrated areas using several techniques in a correlative manner. Each microscopy technique has its benefits and limitations originating e.g. from image acquisition, resolution, and level of sample preparation. We applied four different microscopy techniques, namely scanning electron microscopy (SEM), structured illumination microscopy (SIM), stimulated emission-depletion microscopy (STED), and atomic force microscopy (AFM), all to address the reported differences in fenestration size distribution.

A quantitative and qualitative description of the differences was made in a correlative manner [1]. Scanning electron microscopy (SEM) was combined with optical nanoscopy methods (STED, SIM). Additionally, atomic force microscopy (AFM) was used in combination with SEM (Figure) and STED, all in order to better understand the differences between literature reports on fenestration dimensions. The latter employed the prototype of a device in which AFM and STED were combined in one device, providing ease in directly overlaying obtained images. Finally, we showed the potential of correlative and comparative 4-dimensional imaging (3D + time) of living cells using AFM with fluorescence microscopy (conducted at the final stage of the experiment).

We concluded that dehydration needed in SEM causes fenestration enlargement, esspecially at the edges of so called sieve plates (Figure, arrowheads). Additionally, some fenestrations closed in hydrated samples, appear as opened after dehydration in SEM. An enlargement of fenestrations is observed when probing sample with an AFM tip if a sample is mildly fixed using formaldehyde. The effect vanishes after fixation using glutaraldehyde. The point of spread function (PSF) should be considered when calculating fenestrations from images collected using optical nanoscopy. In our hands, smallest fenestrations, below 50 nm in diameter could not be disclosed with STED nor with SIM. Thanks to correlative studies and

analysis of fenestrations in a one-to-one manner, we proposed the mathematical formula allowing for recalculation of fenestration size in order to compare data from LSEC imaged using different nanoscopy techniques. It will allow researchers to compare the results collected using different nanoscopy techniques. Finally, our results show promise of a better understanding of biological processes at the nanoscale, by combining the benefits of each method when used in a correlative way.

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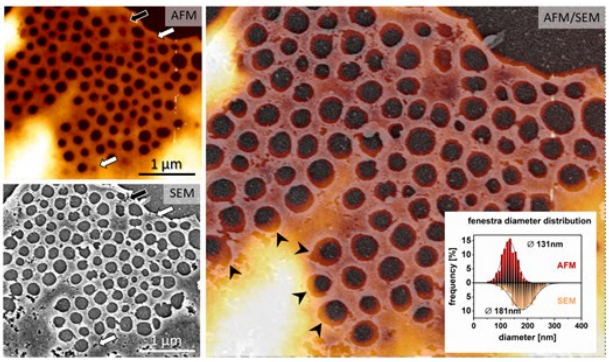
Graphic:



Keywords:

fenestration, liver, correlative microscopy, AFM

Reference:

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