doi:10.1017/S143192761100571X
Microsc. Microanal. 17 (Suppl 2), 2011

Whole-Cell Analysis of the Effect of Cholesterol on LDL-Gold Nanoparticles Uptake in Macrophages by STEM Tomography and 3D STEM.

J.P Baudoin*, M.J Dukes* **, W.G Jerome,*** and N. de Jonge*

*Molecular Physiology and Biophysics, Vanderbilt University Medical Center, 2215 Garland Ave, Nashville TN 37232-0615
**Department of Chemistry, Vanderbilt University, 7330 Stevenson Center, Nashville, TN 37235
***Department of Pathology, Vanderbilt University Medical School, 1211 Medical Center Drive, Nashville, TN 37232

Macrophage cholesterol accumulation is considered a critical process in the development of atherosclerotic plaques, the cause of most heart attacks and strokes. LDL, the main carrier of blood cholesterol, enters blood vessels where monocyte-derived macrophages take it up, converting macrophages into so-called foam cells [1]. Most of the studies about cholesterol accumulation by macrophages use LDL-gold nanoparticles uptake to monitor the amount of LDL taken up by the cells. Semi-thin sections provide an elegant way to look by conventional transmission electron microscopy (TEM) at the localization of LDL-gold along the endocytic pathway and to quantify them [2]. But such quantifications are time consuming and an accurate quantification of LDL-gold uptake at the whole cell level is still missing. Tilt-series TEM is the traditional methodology to resolve unique parts of the three-dimensional (3D) cellular ultra-structure [3]. A cubic volume is reconstructed from projections obtained by mechanically tilting the sample stage. The resolution of the reconstruction is in the range of 1–20 nm [4]. A new development in three-dimensional (3D) electron microscopy is the use of scanning transmission electron microscopy (STEM), which can detect high atomic number elements inside an embedding medium of a low atomic number [5-8].
In this study we used an approach combining the advantages of tilt-series STEM and focal-series STEM to image gold-nanoparticles within the 3D content of whole cells [9]. We applied this new methodology to quantify the effect of cholesterol on LDL-gold nanoparticles uptake by macrophages. THP1 monocytes differentiated as macrophages were grown directly on silicon microchips supporting thin silicon nitride (SiN) windows. We applied different concentrations of cholesterol for different time periods, and then loaded the cells with LDL-gold and/or BSA-gold [10] nanoparticles. Cells were fixed and processed for electron microscopy with an optimized protocol [9]. The samples were first inspected by TEM at 120 KeV (CM12, FEI) to characterize at the whole-cell level the nanoparticles internalization pattern (Fig.1A,B). To obtain 3D information while optimizing for reduced damage we recorded a tilt-series with minimal acquisitions/angle range with a 200 KeV STEM (CM200, FEI) (Fig.2A,B). The tilt-series reconstruction was processed with the Etomo/3Dmod softwares [11]. As a second 3D approach we recorded focal series using aberration corrected STEM at 200 KeV (JEOL 2200 FS), and applied deconvolution. We compared and combined the results obtained with both 3D approaches. The obtained data describes the localization and quantification of gold-nanoparticles inside whole-cells with a nanometer resolution. [12]

References

[1] W.G. Jerome and P.G. Yancey, *Microsc. Microanal.* 9 (2003) 54.
[2] W.G. Jerome et al., *Microsc Microanal.* 14 (2008) 138.
[3] H. Stahlberg and T. Walz, *ACS Chem. Biol.* 3 (2008) 268.
[4] S.W. Hell, *Science* 316 (2007) 1153.
[5] N. de Jonge et al., *Microsc. Microanal.* 16 (2010) 54.
[6] M.F. Hohmann-Marriot et al., *Nat Methods* 6 (2009) 729.
[7] K. Aoyama et al., *Ultramicroscopy* 109 (2008) 70.
[8] A.E. Yakushevska et al., *J. Struct. Biol.* 159 (2007) 381.
[9] M. Dukes et al., Submitted (2011).
[10] W. Möbius et al., *Traffic* 4 (2003) 222.
[11] J.R. Kremer et al., *J Struct. Biol*. 116 (1996) 1116:71.

[12] We thank C.E Romer, B. Cox, M.L Dawes, and B.K Choi for discussions and help. A portion of this research was conducted at the SHaRE User Facility, which is sponsored by the Division of Scientific User Facilities, Office of Basic Energy Sciences, U.S. Department of Energy. Support by NIH grant R01-GM081801.

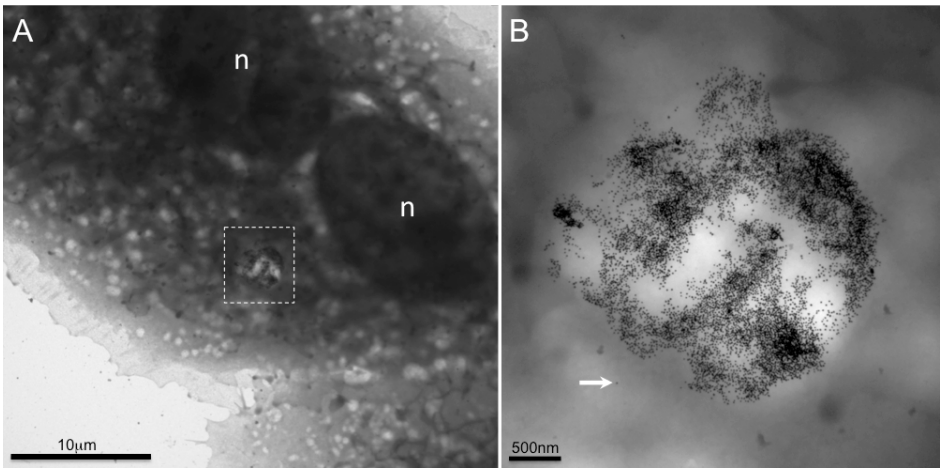

FIG. 1 A. TEM view of a whole macrophage containing LDL-gold nanoparticles. n: nucleus.
B. Magnification of the dashed box from (A) depicting nanoparticles (arrow), probably in a vesicle.

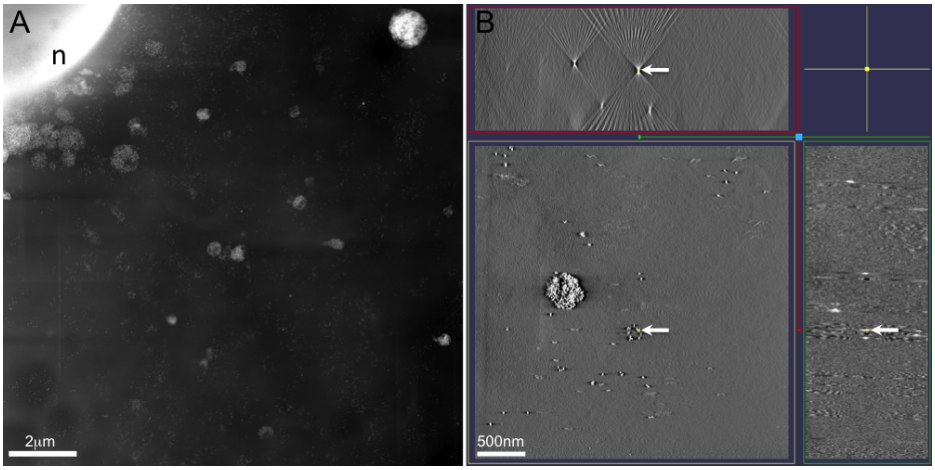

FIG. 2 A. STEM view of LDL-gold nanoparticles in the peri-nuclear region of a macrophage.
B. XYZ view of a 3D tomogram depicting single gold-nanoparticles (arrow). n: nucleus.

https://doi.org/10.1017/S143192761100571X Published online by Cambridge University Press