Supporting Information for

Profiling and imaging analysis of cholesterol and 7-dehydrocholesterol in cells via sputtered silver nanoparticle MALDI-ion mobility-MS

Libin Xu,[1,2,#] Michal Kliman,[1,2,3#] Jay Forsythe,[1,2,3] Zeljka Korade,[4] Anthony B. Hmelo,[5] Ned A. Porter,[1,2] John A. McLean[1,2,3*]

[1]Department of Chemistry, [2]Vanderbilt Institute of Chemical Biology, [3]Vanderbilt Institute for Integrative Biosystems Research and Education, [4]Department of Psychiatry and Vanderbilt Kennedy Center for Research on Human Development, [5]Vanderbilt Institute for Nanoscale Science and Engineering, Vanderbilt University, Nashville, Tennessee 37235

Abstract

Profiling and imaging of cholesterol and its precursors by mass spectrometry (MS) are important in a number of cholesterol biosynthesis disorders, such as in Smith-Lemli-Opitz syndrome (SLOS), where 7-dehydrocholesterol (7-DHC) is accumulated in affected individuals. SLOS is caused by defects in the enzyme that reduces 7-DHC to cholesterol. However, analysis of sterols is challenging because these hydrophobic olefins are difficult to ionize for MS detection. We report here sputtered silver MALDI – ion mobility – MS (IM-MS) analysis of cholesterol and 7-DHC. In comparison with liquid-based $AgNO_3$ and colloidal Ag nanoparticle (AgNP), sputtered silver NP (10 – 25 nm) provided the lowest limits-of-detection based on the silver coordinated $[cholesterol+Ag]^+$ and $[7\text{-}DHC+Ag]^+$ signals while minimizing dehydrogenation products ($[M+Ag-2H]^+$). When analyzing human fibroblasts that were directly grown on poly-L-lysine-coated ITO glass plates with this technique, *in situ*, the 7-DHC/cholesterol ratios for both control and SLOS human fibroblasts are readily obtained. The *m/z* of 491 (specific for $[7\text{-}DHC+^{107}Ag]^+$) and 495 (specific for $[cholesterol+^{109}Ag]^+$) were subsequently imaged using MALDI-IM-MS. MS images were co-registered with optical images of the cells for metabolic ratio determination. From these comparisons, ratios of 7-DHC/cholesterol for SLOS human fibroblasts are distinctly higher than in control human fibroblasts. Thus, this strategy demonstrates the utility for diagnosing/assaying the severity of cholesterol biosynthesis disorders *in vitro*.

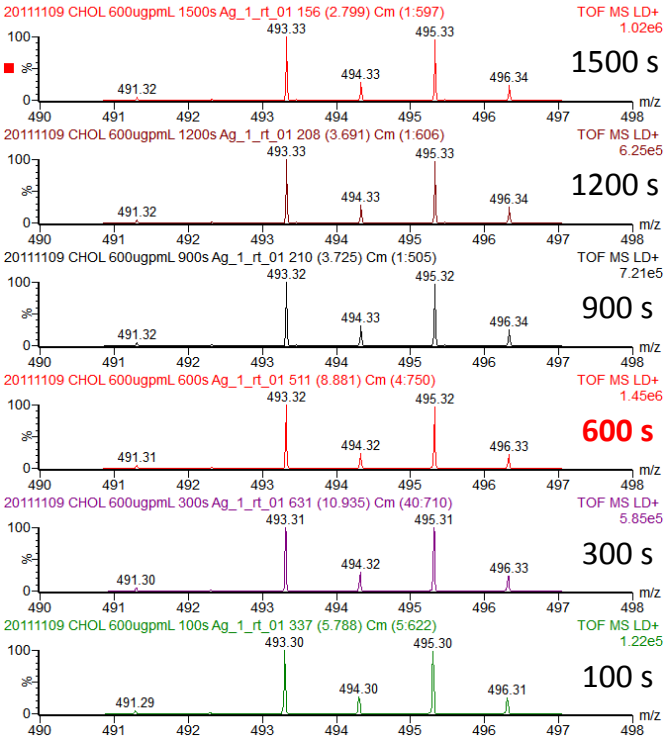

Figure S1. Optimization of sputtering time for cholesterol standard. Triplicate experiments were performed for each sputtering time condition for 0.78 nmol cholesterol standard.

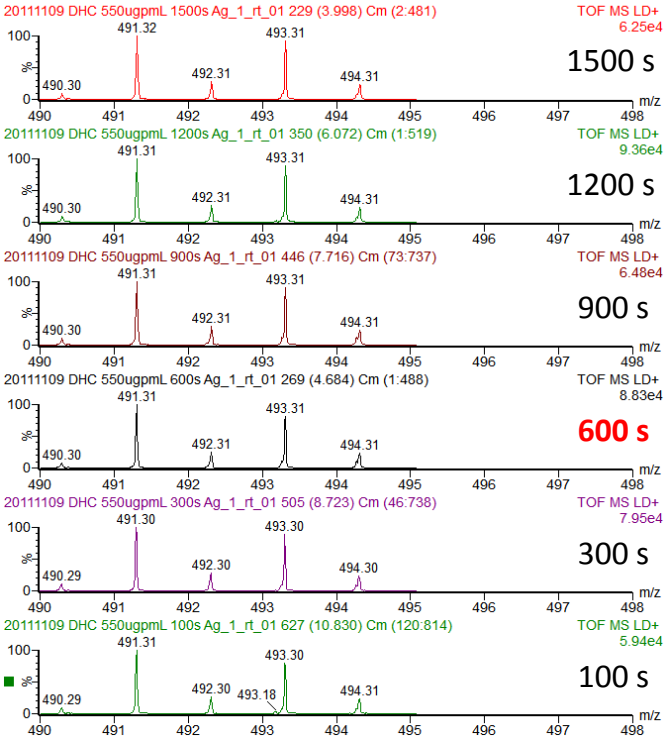

Figure S2. Optimization of sputtering time for 7-dehydrocholesterol (7-DHC) standard. Triplicate experiments were performed for each sputtering time condition for 0.72 nmol 7-DHC standard.

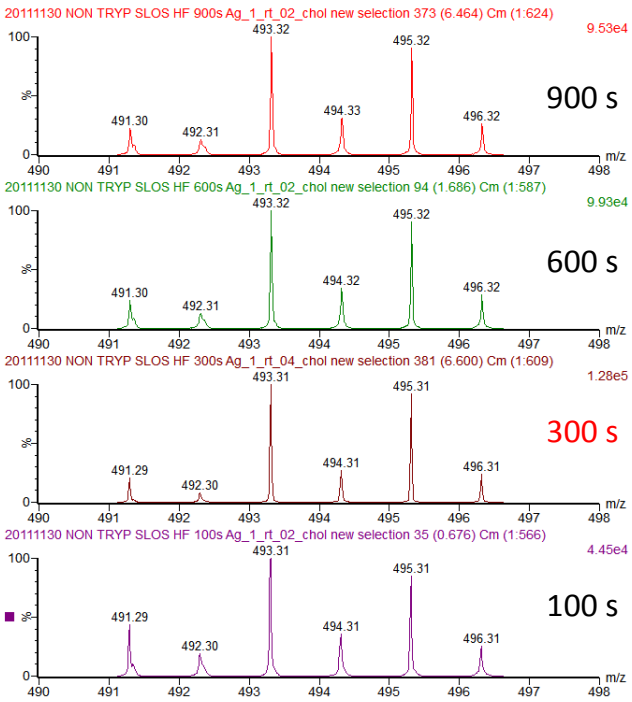

Figure S3. Optimization of sputtering time on cells using non-trypsinized SLOS human fibroblasts that were grown on poly-L-lysine and ITO-coated glass MALDI plates. Triplicate experiments were carried out for each condition. 300 s of sputtering was found to give the highest signal intensity, but 100 s was found to give the highest 7-DHC/Cholesterol ratio, suggesting less dehydrogenation peaks were formed from $[7\text{-DHC+Ag}]^+$.

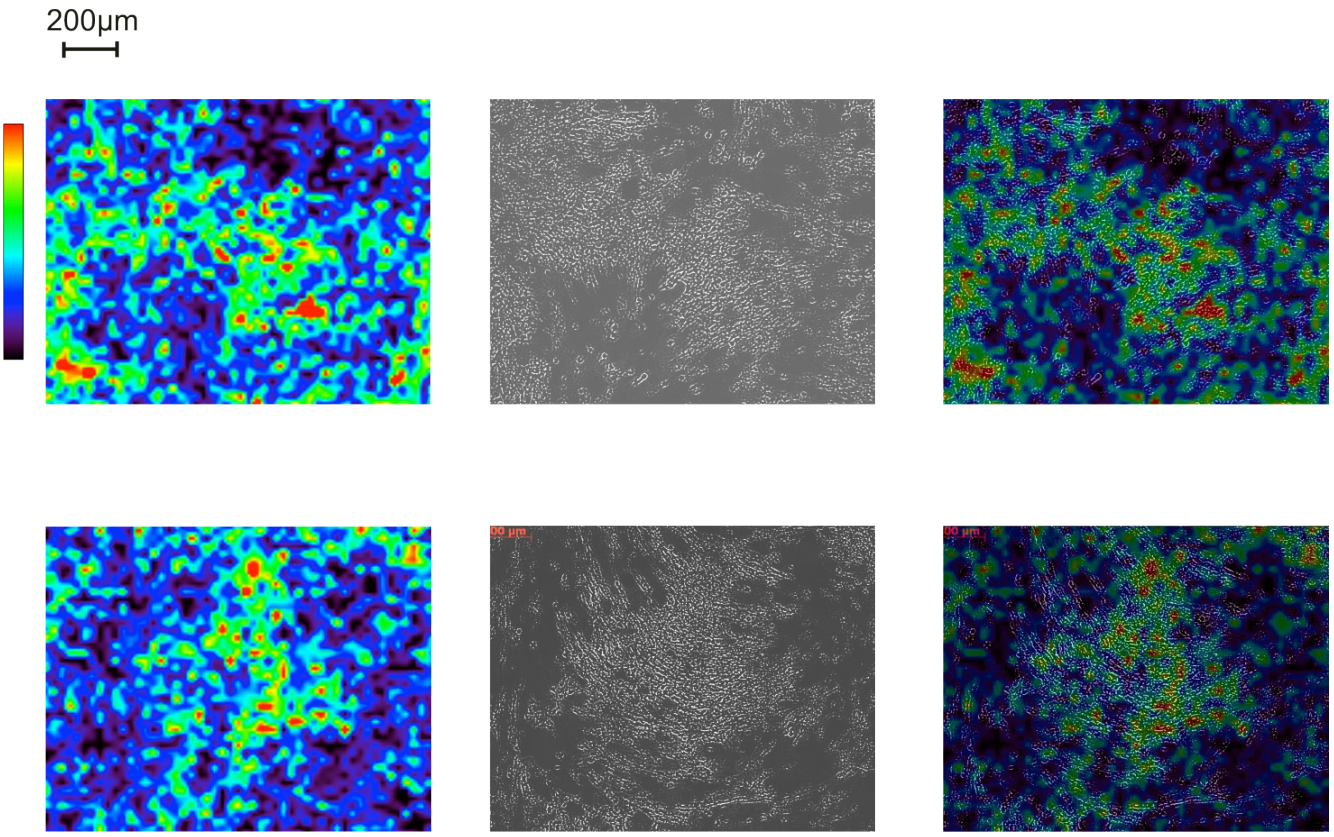

Figure S4. Overlay (right) of MS images (left, *m/z* 495) and optical images (center) of the control fibroblast cells in the same regions of ITO-coated glass plates where cells were grown.

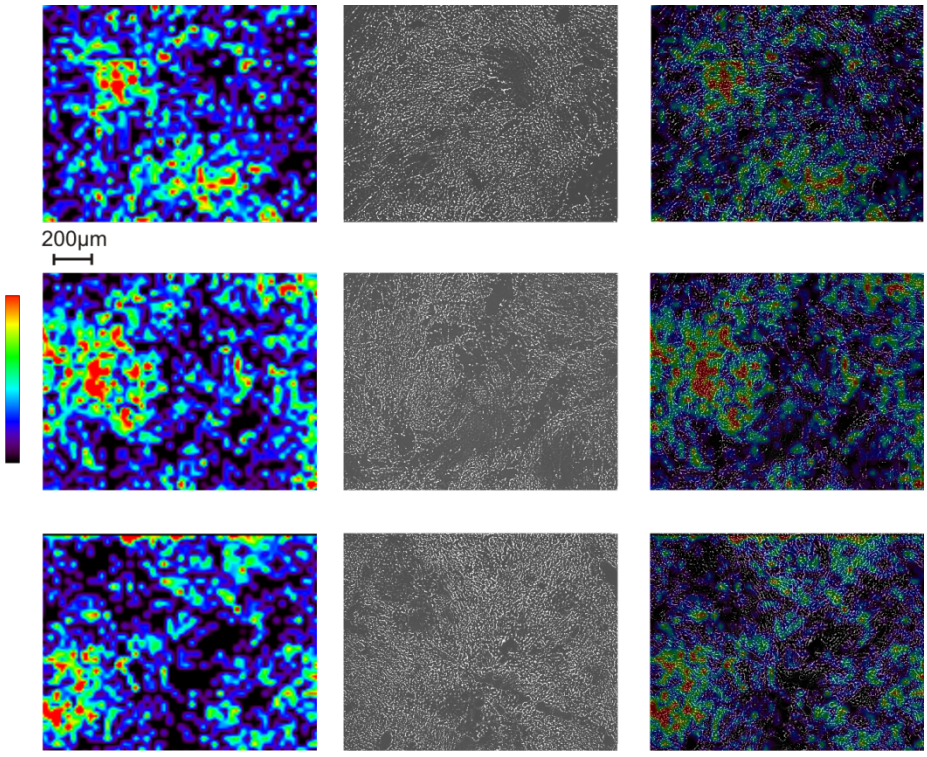

Figure S5. Overlay (right) of MS images (left, *m/z* 495) and optical images (center) of the SLOS fibroblast cells in the same regions of ITO-coated glass plates where cells were grown.

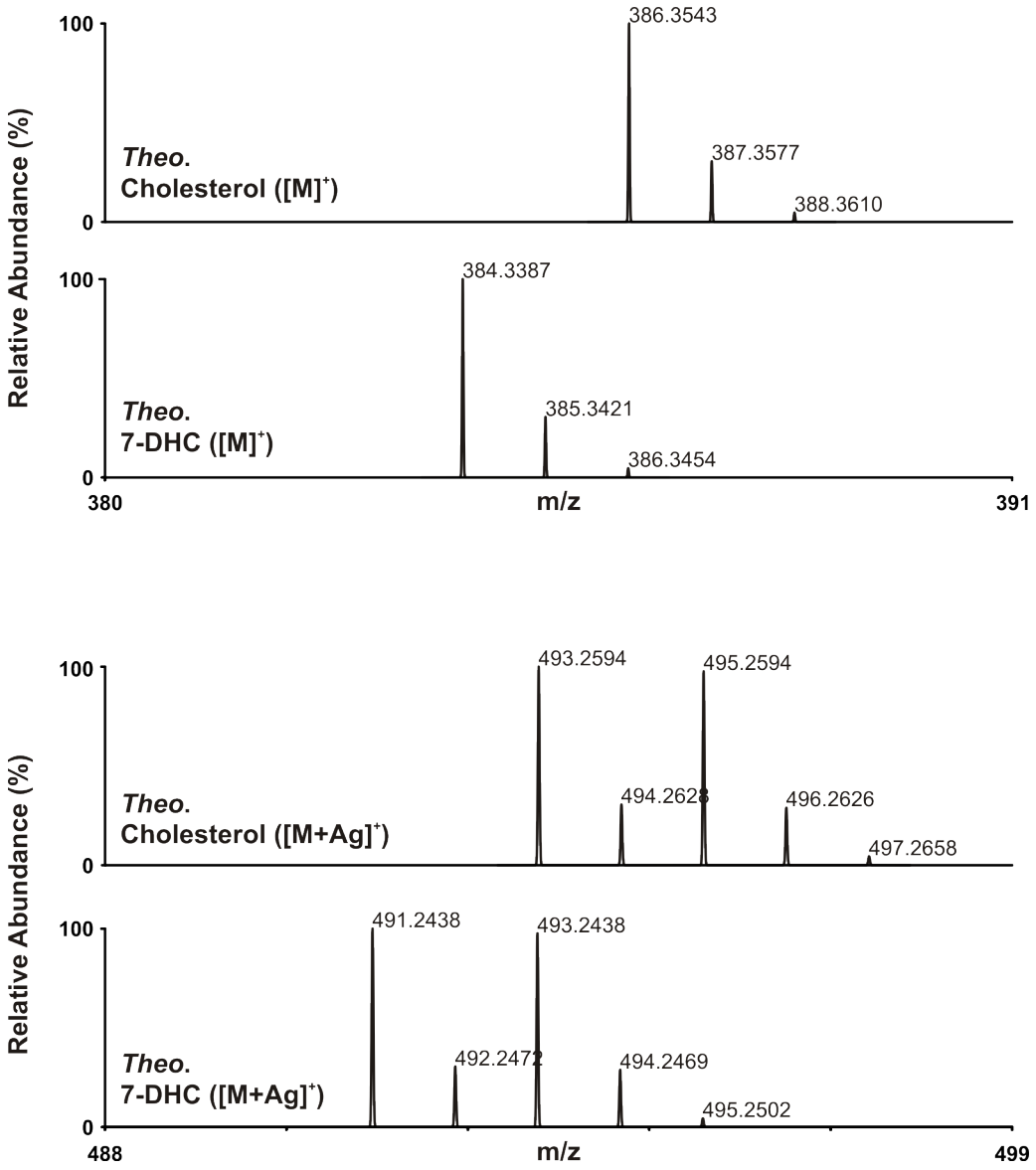

Figure S6. Theoretical isotope distributions for cholesterol and 7-DHC (top) and their respective silver-coordinated species (bottom).