Investigate atherosclerosis with modified low-density lipoproteins

Fluorescently labeled oxidized LDLs and acetylated LDLs.

Alterations in lipid metabolism are known to be the root defect in atherosclerosis. Atherosclerosis is a form of arteriosclerosis in which the thickening and loss of elasticity of the artery walls is caused by the buildup of fatty plaques—composed mainly of cholesterol and other lipids in blood vessels—that inhibit or block the blood flow. The exact mechanism of atherogenesis is still subject to much debate, but high levels of low-density lipoproteins (LDLs) are considered a risk factor for atherosclerosis, and high levels of high-density lipoproteins (HDLs) appear to offer some protection.

As one of the key lipid–protein complexes in blood, LDLs are responsible for the transport and delivery of lipids, including cholesterol, triglycerides, and phospholipids, throughout the body via receptor-mediated endocytosis. Modified LDLs no longer bind to the LDL receptor and are typically cleared through "scavenger" receptors on macrophages and endothelial cells. Fluorescent conjugates of LDL (native and modified) can be employed to study these pathways using fluorescence microscopy, high-content analysis, and flow cytometry. Thermo Fisher Scientific offers unmodified LDLs and their fluorescent conjugates for studying LDL receptor–mediated endocytosis and other aspects of lipid metabolism. Here we focus on two sets of fluorescently labeled, modified LDLs—oxidized LDLs and acetylated LDLs—for investigating the scavenger receptor–mediated endocytic pathways.

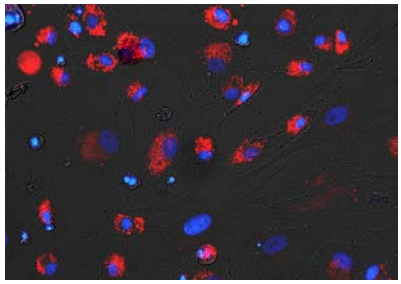

Figure 1. Endocytosis of DiI OxLDL by BPAECs. BPAECs (bovine pulmonary artery endothelial cells) were grown in poly-D-lysine–coated 96-well plates for 24 hr and then serum-starved overnight in Gibco™ FluoroBrite™ DMEM (Cat. No. A1896702) plus 0.3% BSA. On the day of the experiment, 10 µg/mL DiI OxLDL (Cat. No. L34358) was added to the medium and cells were incubated for 3 hr. After this incubation, cells were rinsed with assay buffer plus 0.3% BSA and fixed in 4% formaldehyde. Following nuclear staining with Invitrogen™ NucBlue™ Live ReadyProbes™ Reagent (Cat. No. R37605), images were acquired on the Thermo Scientific™ CellInsight™ CX5 High-Content Screening Platform.

Oxidized LDLs mimic naturally occurring processes

Oxidation of LDL is a naturally occurring process within the body, thought to be caused by the presence of free radicals. Endothelial cells and then macrophages are called into action to rid the body of oxidized LDL (OxLDL), which triggers inflammatory and immunogenic responses. Unlabeled and fluorescent OxLDL probes are therefore important tools for the study of scavenger receptor–mediated endocytosis by macrophages and endothelial cells (Figure 1), as well as the formation of macrophage-derived foam cells, a hallmark of early atherogenesis [1].

Our unlabeled OxLDL probe is generated by oxidizing the surface lipids of native or unmodified LDL using a copper sulfate incubation. During this incubation, the oxidation is continuously monitored by measuring the optical density at 234 nm [2,3]. The incubation is terminated at approximately the halfway point during the lipid peroxidation phase (Figure 2A). This oxidation level represents TBAR (thiobarbituric acid–reactive) values of approximately 25–35 nmol/mg protein. The electrophoretic mobility of this type of OxLDL preparation is found to be at least twice that of native LDL (Figure 2B). This very controlled oxidation procedure ensures that primarily lipids on the surface of the LDL are oxidized, with very limited oxidation of the surface apolipoprotein, in order to induce a physiologically relevant inflammatory response from cells.

After the proper level of oxidation is achieved and tested, fluorescently labeled OxLDL is generated by labeling with the lipid membrane intercalator DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate or $DiIC_{18}(3)$). DiI is a highly fluorescent lipophilic dye that can diffuse into the hydrophobic portion of the LDL complex without affecting the LDL-specific binding of the apolipoprotein. The degree of labeling in the DiI-OxLDL probe has been optimized to produce superior sensitivity in fluorescence imaging applications (Figure 1), as well as in flow cytometry and high-content imaging analyses. Each lot is analyzed for degree of labeling and functionally tested with bovine pulmonary artery endothelial cells (BPAECs) to help ensure that the oxidized probe

is recognized by scavenger receptors (Figures 1 and 3). This extensive quality testing makes it possible to achieve similar results from lot to lot, experiment to experiment.

Acetylated LDLs for specific scavenger receptors

The scavenger pathways in macrophages and endothelial cells, and the formation of foam cells, have been traditionally studied using acetylated LDL (AcLDL). Unlike the oxidation of LDL, which primarily involves modification of the surface lipids, the acetylation of LDL alters the single copy of apolipoprotein B100 on the surface of the LDL. When AcLDL complexes accumulate within macrophages and endothelial cells, the cells assume an appearance similar to that of foam cells found in atherosclerotic plaques. However, Wang and coworkers have reported that native LDL, OxLDL, and AcLDL are each trafficked to different endosomes and accumulate in distinct lysosomal compartments, indicating different endocytic pathways [4].

Thermo Fisher Scientific offers several different fluorescent AcLDL derivatives that can be used on a variety of fluorescence-based platforms. DiI AcLDL is routinely used to identify endothelial and microglial cells in primary cell cultures (Figure 4). The Invitrogen™ Alexa Fluor™ 488, Alexa Fluor™ 594, and BODIPY™ FL AcLDL derivatives may be preferred in some applications because the dyes are covalently bound to the modified apolipoprotein portion of the LDL complex and are therefore not extracted during subsequent manipulations of the cells. Given the rich publication history for both OxLDL and AcLDL, researchers can choose the appropriate modification for their particular studies and experimental setup.

Lipid droplets in foam cells

The study of foam cells plays an important role in dissecting the mechanism of atherosclerosis and developing the next generation of drugs that reduce the risk of coronary heart disease. The lipid droplets that accumulate in these foam cells are typically detected using neutral lipid stains. With an extremely high affinity for neutral lipid droplets, the Invitrogen™ HCS LipidTOX™ neutral lipid stains were developed to detect intracellular lipid droplets or globules and characterize the effects of drugs and other compounds on lipid metabolism in mammalian cell lines. These reagents are added after cell fixation (Figure 5), →

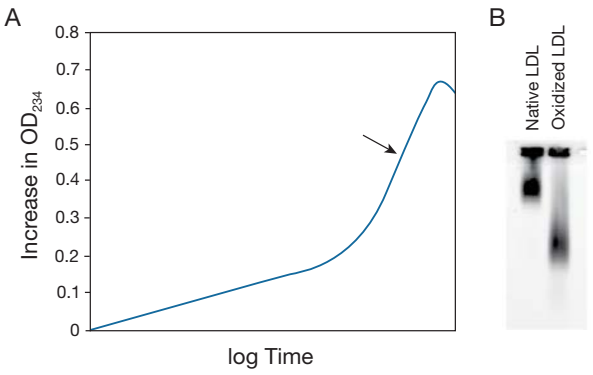

Figure 2. Real-time monitoring of the formation of OxLDL. (A) The generation of OxLDL is continuously monitored by measuring the optical density at 234 nm (OD_{234}) with a Thermo Scientific™ NanoDrop™ Spectrophotometer during the incubation of native LDL with copper sulfate. The incubation is terminated during the lipid peroxidation phase (indicated by the arrow), and the OxLDL lot is collected for further treatment. **(B)** Electrophoretic mobility of oxidized LDL (OxLDL) is at least two times greater than the mobility of native LDL.

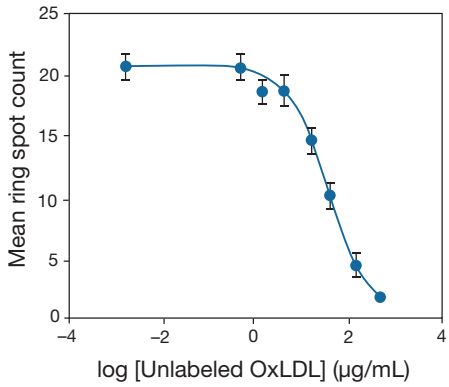

Figure 3. Specificity of DiI OxLDL for the OxLDL receptor, as determined by competitive inhibition. Bovine pulmonary artery endothelial cells (BPAECs) were plated in poly-D-lysine–coated 96-well plates for 24 hr and then serum-starved overnight in Gibco™ FluoroBrite™ DMEM (Cat. No. A1896702) plus 0.3% BSA. On the day of the experiment, cells were pretreated with a dilution series of unlabeled OxLDL (from 0 to 500 µg/mL) for 30 min at 37°C, labeled with 10 µg/mL DiI OxLDL (Cat. No. L34358) for 3 hr in a cell culture incubator, rinsed with assay buffer plus 0.3% BSA, and fixed in 4% formaldehyde. Following nuclear staining with Hoechst™ dye, images were acquired and enumerated on the Thermo Scientific™ CellInsight™ CX5 High-Content Screening Platform. Data analysis was performed with the internalization and spot count module in the Thermo Scientific™ HCS Studio™ Cell Analysis Software to quantify the number of label-positive spots per cell. Five hundred cells were sampled per well, with n = 3 wells per data point.

making them compatible with immunocytochemistry protocols, and they do not require subsequent wash steps after incubation with the sample. In addition, they are more sensitive and photostable than traditional neutral lipid stains such as Invitrogen™ BODIPY™ 493/503 dye or Nile Red, and are available with green, red, and deep red fluorescence emission.

Learn more about our LDL probes

Thermo Fisher Scientific offers the most complete portfolio of unlabeled and fluorescently labeled native LDL, OxLDL, and AcLDL for the study of lipid metabolism by fluorescence imaging, high-content analysis, and flow cytometry. All of our LDL products are derived from human LDLs isolated from human plasma, which is sourced from a blood bank and tested for HIV, hepatitis B and C, syphilis, and other infectious diseases. Visit **thermofisher.com/cellanalysisbp76** to see a summary of our cell analysis products by application, as well as our diverse collection of instrument platforms, including the Invitrogen™ EVOS™ Imaging Systems, the Thermo Scientific™ CellInsight™ High-Content Analysis Platforms, and the Invitrogen™ Attune™ NxT Flow Cytometer. ■

References

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2. Parthasarathy S, Augé N, Santanam N (1998) *Free Radic Res* 28:583–591.
3. Ray K, Fahrmann J, Mitchell B et al. (2015) *Pain* 156:528–539.
4. Wang MD, Kiss RS, Franklin V et al. (2007) *J Lipid Res* 48:633–645.

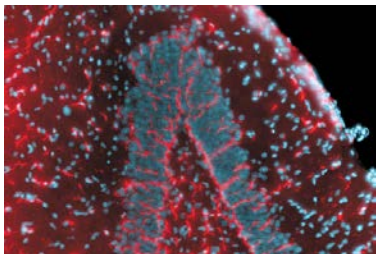

Figure 4. Microglial cells in a rat hippocampus cryosection labeled with DiI AcLDL. Microglial cells in a rat hippocampus cryosection were labeled with red-orange–fluorescent DiI AcLDL (Cat. No. L3484) and counterstained using blue-fluorescent DAPI nucleic acid stain (Cat. No. D1306).

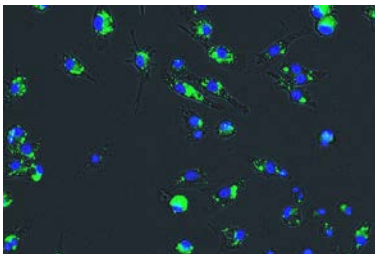

Figure 5. Accumulation of lipid droplets in RAW cells treated with OxLDL. RAW cells grown on MatTek dishes were serum-starved overnight and treated with 50 µg/mL unlabeled OxLDL (Cat. No. L34357) for 24 hr. Serum medium was then added back to the cells overnight, and cells were fixed in 4% formaldehyde and stored at 4°C. On the day of the experiment, cells were rinsed in PBS, stained with Invitrogen™ NucBlue™ Live ReadyProbes™ Reagent (Cat. No. R37605) and Invitrogen™ HCS LipidTox™ Green Neutral Lipid Stain (Cat. No. H34475) for 30 min, and rinsed in PBS again. Images were collected on an Invitrogen™ EVOS™ FL Auto 2 Imaging System at 10x magnification.

Product	Ex/Em*	Quantity	Cat. No.
Unmodified low-density lipoproteins			
Low-Density Lipoprotein from Human Plasma (LDL)	NA	200 µL	L3486
Low-Density Lipoprotein from Human Plasma, BODIPY™ FL complex (BODIPY™ FL LDL)	515/520	200 µL	L3483
Low-Density Lipoprotein from Human Plasma, DiI complex (DiI LDL)	545/571	200 µL	L3482
Low Density Lipoprotein from Human Plasma, pHrodo™ Green conjugate (pHrodo™ Green LDL)	509/533	200 µL	L34355
Low Density Lipoprotein from Human Plasma, pHrodo™ Red conjugate (pHrodo™ Red LDL)	560/585	200 µL	L34356
Oxidized low-density lipoproteins			
Low Density Lipoprotein from Human Plasma, Oxidized (OxLDL)	NA	200 µL	L34357
Low Density Lipoprotein from Human Plasma, Oxidized, DiI complex (DiI-OxLDL)	545/571	200 µL	L34358
Acetylated low-density lipoproteins			
Low-Density Lipoprotein from Human Plasma, Acetylated (AcLDL)	NA	200 µL	L35354
Low-Density Lipoprotein from Human Plasma, Acetylated, Alexa Fluor™ 488 conjugate (Alexa Fluor™ 488 AcLDL)	495/519	200 µL	L23380
Low-Density Lipoprotein from Human Plasma, Acetylated, Alexa Fluor™ 594 conjugate (Alexa Fluor™ 594 AcLDL)	590/617	200 µL	L35353
Low-Density Lipoprotein from Human Plasma, Acetylated, DiI complex (DiI AcLDL)	545/571	200 µL	L3484
Neutral lipid stains			
HCS LipidTOX™ Green Neutral Lipid Stain, for cellular imaging	495/505	1 each	H34475
HCS LipidTOX™ Red Neutral Lipid Stain, for cellular imaging	577/609	1 each	H34476
HCS LipidTOX™ Deep Red Neutral Lipid Stain, for cellular imaging	637/655	1 each	H34477

*Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm. NA = not applicable.