KALEN Biomedical, LLC

Treat Your LDL Gently

One of our clients got some aberrant results when he inadvertently added LDL aggregates to his cultures by “lightly” vortexing to “ensure” an even distribution of LDL particles. This brought forth the question of how much vortexing is necessary to cause LDL aggregation. To get a quick answer, we briefly reviewed some early pertinent publications and then performed a few simple measurements ourselves.

Review

Human low density lipoprotein (LDL) is one of the 5 major groups of lipoproteins found in plasma. It is a mixture of heterogeneous particles, varying in density and size. Small dense LDL particles are about 20 nm in diameter, while large less dense, particles approach 30 nm. These particles have a mass ranging from approximately 2000 to 3000 kDa (cf: Meyer, et. al. 1995a). Each LDL particle consists of an amorphous hydrophobic core comprised of cholesterol esters and triacylglycerols, surrounded by a thin shell of phospholipid, non-esterified cholesterol, and protein (Apo B100). Each particle contains a single molecule of ApoB100 (Laggner and Muller, 1978). This protein is the key structural element. ApoB100 is a large single chain protein, with a molecular weight of approximately 514 kDa. The variable lipid composition of the remaining particle (about 1500 to 2500 kDa) accounts for its heterogeneity. Because the LDL particle is an assembly of non-covalently bound hydrophobic and hydrophilic molecules it is susceptible to disassembly and denaturation.

Vortexing of LDL quickly leads to aggregates which can be precipitated by centrifugation at 10,000 x g for 20 minutes. Aggregated LDL is taken up by macrophage and even small amounts stimulate them to form foam cells. Stimulation from aggregated LDL can be 10 fold more than non-aggregated LDL (Khoo, et.al. 1988).The more vortexing, the more aggregates are formed and the stronger the stimulation of macrophages.

Neutron solution scattering studies and Guinier analyses performed at physiological LDL concentrations, show that the scatter from the spherical structure of LDL weakens and disappears during vortexing. The average diameter (maximum observable dimension, L) of the LDL particle quickly increased from 20 nm (approximately the size of a monomer) to about 60 nm during vortexing. The radius of gyration (R_G) and the forward scatter (extrapolated to zero concentration $I(0)$) are proportional to particle size and particle mass. Both increased with increasing vortex time. All these changes suggest the loss of native LDL and increasing amounts of aggregated LDL (Meyer, et.al. 1955b).

Electron microscopic (EM) studies generally show LDL as spheres with different diameters. In addition to the biological variability, the method of fixing and staining the LDL contribute to the diameter heterogeneity. EM studies have shown that vortexing of LDL disassociates it into precipitable aggregates, lamellar and multi-lamellar vesicles and lipid droplets (sometimes very large). Aggregates predominately contained protein (apo-B100) and esterified cholesterol. Vesicles were formed from phospholipids and non-esterified cholesterol. Lipid droplets and particles resembling intact LDL were also observed (Guyton, et.al. 1991).

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Current Study

We performed a few measurements on the effects of briefly vortexing LDL and present them here to encourage gentle treatment of LDL.

Human Low Density Lipoprotein (LDL, d=1.019 to 1.063) was prepared by serial isopynic ultracentrifugation of pooled human plasma.

To assess the physical effects on LDL by brief vortexing, we performed the following measurements on three different preparations. A solution was prepared containing 250 ug/mL (protein) of LDL in PBS EDTA . Two mL of the solution was aliquoted into each of multiple 5 mL *NUNC*tm tubes. Each tube was vortexed at room temperature, using a VWR Vortexer 2 at medium speed. Tubes were processed for 0 (control), 5, 10, 15, 20, 30, or 60 seconds. Each tube was immediately examined under a strong lamp for Tindal effect and visible aggregates, then scanned in a spectrophotometer from 220 to 800 nm. After one hour at room temperature the examinations were repeated. After 5 days at 2 – 8 °C the samples were visually examined, but not scanned.

Visual Examination

Vortex Time	Immediate					1 Hour					5 Days				
	Clear	Hazy	Turbid, Milky	Clumps, Threads	Visible Precipitate	Clear	Hazy	Turbid, Milky	Clumps, Threads	Visible Precipitate	Clear	Hazy	Turbid, Milky	Clumps, Threads	Visible Precipitate
0	+					+					+				
5	+					+					+			+/ -	
10	+			+/ -		+			+/ -		+			+	+/ -
15		+		+/ -			+		+/ -			+		+	+
20		+		+/ -			+		+			+		+	+
30			+	+				+	+				+	+	++
60			+	+				+	+	+			++	+	++

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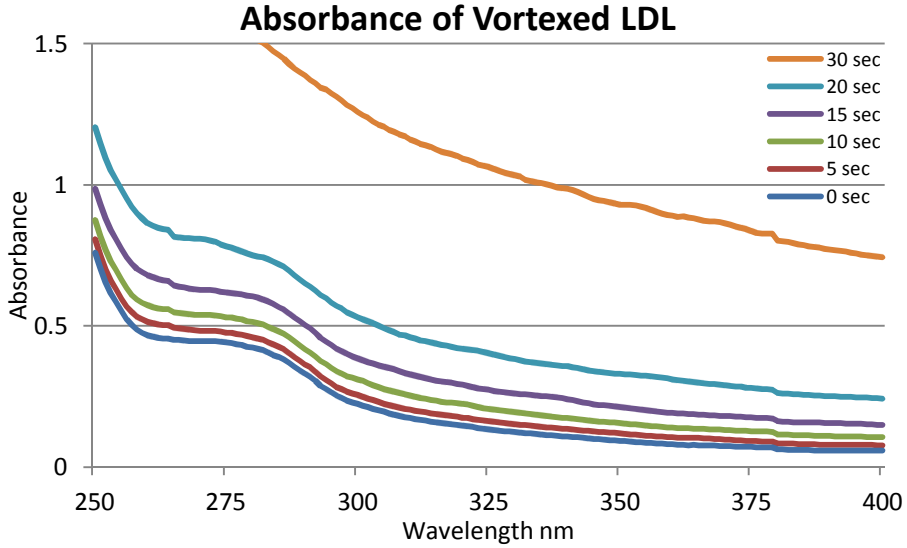
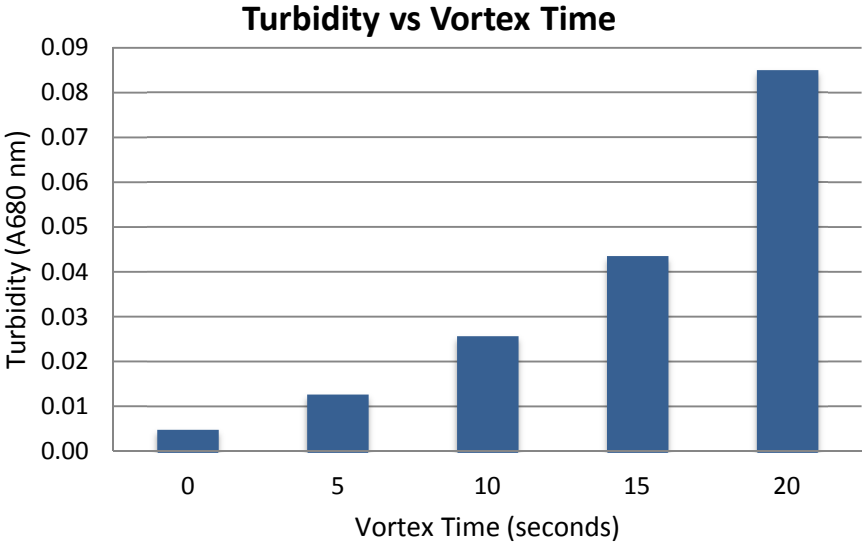
Spectral Scan



Because of the very large number of ester bonds in the lipid moiety of LDL, a typical protein spectrum with a distinct peak in the vicinity of 280 nm is not observed with LDL. Only the spectrum from 250 nm to 400 nm is shown above.

Each increase in the vortexing time resulted in a larger increase in optical density.

Turbidity from Aggregates



Aggregation was estimated by turbidity, measured by absorbance at 680 nm. The 30 second time point is off scale and not shown.

This data clearly shows the increase in LDL aggregation, even at the shortest vortexing times. Each increase in the vortexing time resulted in a larger increase in turbidity.

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Observations

Importantly, after only 5 seconds of vortexing, even though there were no visible signs of aggregation or clumping, the spectral scan and the turbidity measurement did show signs of aggregation. After 5 days at 5 degrees C, the solution was still clear, but a few white, thread-like clumps had formed.

After 10 seconds, the solution remained clear but a few thread like clumps were present. There was no apparent visual change at 1 hour. The spectral scan and turbidity measurement showed signs of aggregation. After storage, the number of clumps had increased and a slight precipitate had formed.

After 15 seconds, the solution was slightly hazy, with clumps. There was no apparent visual change at 1 hour. The spectral scan and turbidity measurement showed signs of aggregation. After storage, the number of clumps had increased and a precipitate had formed.

After 20 seconds, the solution was hazy, with clumps. The number of clumps had increased at 1 hour. The spectral scan and turbidity measurement showed increased signs of aggregation. After storage there were fewer, but bigger clumps with some sticking to the sides of the tube. A precipitate had formed.

After 30 seconds, the solution was obviously turbid, cloudy and had many clumps. After one hour, it had become more turbid and somewhat milky. The spectral scan and turbidity measurement both showed a marked increase from the 20 second data. The turbidity value is off scale on the bar chart and is not shown. After storage a heavy precipitate had formed.

After 60 seconds, the solution was turbid and milky, precluding spectral measurements. After 1 hour a precipitate had formed. After storage it was very milky and a heavy precipitate had formed.

Each increase in the vortexing time resulted in a larger, accelerating increase in aggregation.

Conclusions

Even relatively mild vortexing of LDL solutions causes structural changes and aggregation.

Do not vortex, mix vigorously, nor forcefully pipette LDL solutions unless you want aggregates. Aggregated LDL is a good stimulant of macrophage (and perhaps other cell types). Even small amounts of aggregate contamination in your cultures can cause aberrant results.

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References

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