Oxidized cholesterol makes stronger and stiffer cells, promotes blood vessel formation

Atherosclerotic plaques that clog major arteries lead to heart attack and stroke because of the loss of the local blood supply to the heart or to brain tissues. It is also increasingly recognized today that formation and growth of atherosclerotic plaque requires blood supply into the plaque, similarly to how a malignant tumor requires blood supply to grow. Neovascularization of the plaque is also a major compounding factor in plaque formation associated with plaque rupture resulting in thrombosis. The process of neovascularization starts with endothelial cells that normally constitute the inner lining of blood vessels, growing out and forming new capillary tubes extending the blood supply into the avascular core of the plaque. In this study, we explore how endothelial biomechanical properties, resistance to deformation and the ability to exert force, contribute to their ability to form functional blood vessels.

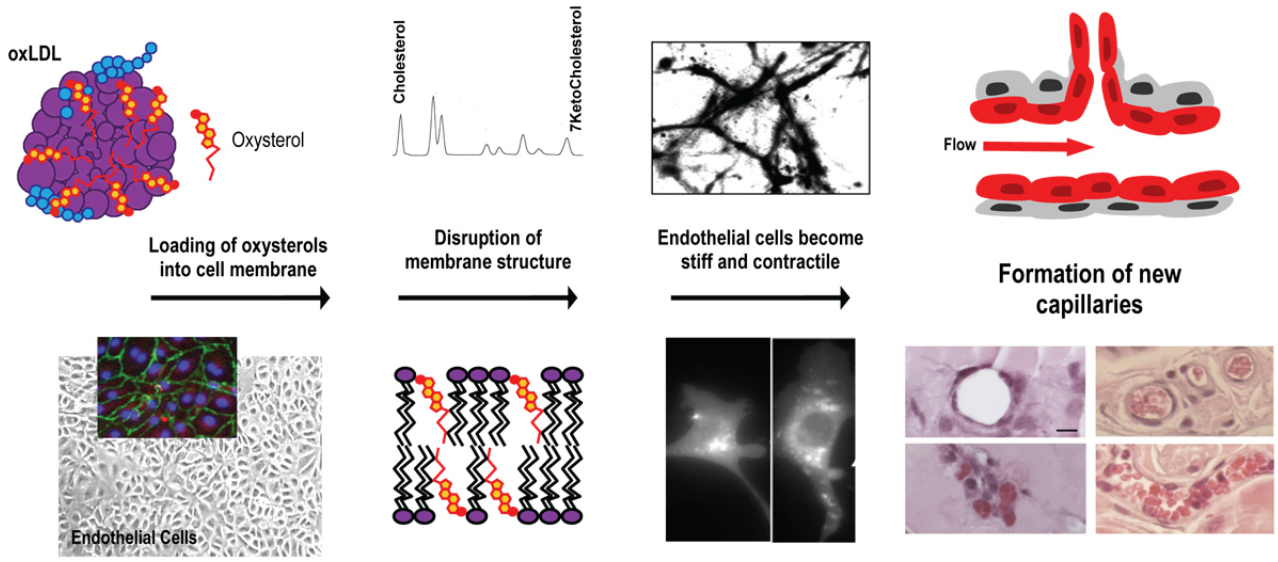

Fig. 1. Oxidized cholesterol: from disruption of membrane structure to endothelial stiffening to formation of new capillaries
From left to right: Top: oxLDL particle, Bottom: confluent endothelial cells in culture (inset shows endothelial-specific marker, PECAM1, and a nuclear stain); Top: Gas Chromatography profile of cholesterol/oxysterol profile of oxLDL exposed endothelial cells (from Shentu et al 2012); Bottom: schematic representation of disruption of membrane structure (from Ayee et al, 2016); Top: Enhanced formation of endothelial enclosures in 3-dimentional cell culture (Shentu et al 2010); Bottom: Increased stiffness/decreased deformability in oxLDL-treated endothelial cells (Byfield et al 2006, Oh et al, 2016); Top: Schematic representation of angiogenesis (formation of new capillaries); Bottom: Histological sections of new blood vessels formed in a matrigel plug exposed to oxLDL and implanted into a mouse (Oh et al 2016).

First, we investigated the impact of cholesterol and its oxidized modifications, oxysterols, on endothelial stiffness. What we found is that contrary to the general belief, cholesterol and oxysterols have opposite effects on endothelial biomechanics. Specifically, using Atomic Force Microscopy, we found that while cells exposed to oxidized low-density lipoproteins (oxLDL), the major carrier of oxidized lipids in blood, become stiffer and stronger, enriching cells with cholesterol reversed this effect resulting in more pliable and softer cells. Furthermore, pre-exposing the cells to cholesterol prevented the stiffening effect of oxLDL or oxysterols. The main novelty of these findings is the dichotomy of cholesterol and oxLDL or oxysterols. This is in contrast to the current general belief that exposure to oxLDL actually loads cells with cholesterol. We show that this is not really the case. Instead, oxLDL loads the cells not with cholesterol but with oxysterol. Furthermore, cholesterol can prevent the cells from taking up oxysterols. This distinction is critical because we also show that cholesterol and oxysterols have opposite effects on the fundamental properties of endothelial cells.

Next, we asked – so which one, cholesterol or oxysterols, are more detrimental in terms of plaque vascularization. To address this question we pre-exposed the cells to cholesterol or to oxLDL/oxysterols and then seeded them into a small artificial gel plugs inserted into a mouse abdomen. This procedure allows evaluating the efficiency of new blood vessels forming and growing into the gel in a simulation of the vessels growing into a plaque. Using this approach, we found that stiffer and stronger endothelial cells are more efficient in forming functional blood vessels than softer cells. In other words, cells exposed to oxLDL/oxysterols that make cells stiffer are better in providing the plaque with the blood supply and facilitating its growth. Furthermore, consistent with our findings described above about the dichotomy between cholesterol and oxysterols, pre-exposing cells to cholesterol inhibits neovascularization. We also show that this process is mediated by the cellular contractile apparatus.

Finally, what are the implications of these findings to the role of cholesterol in plaque formation? The implications are that it is not cholesterol but its oxidative modifications that facilitate plaque formation via strengthening vascular endothelial cells and enhancing their ability to form new blood vessels. In contrast, normal non-modified cholesterol may even have a protective effect. These unexpected findings may pave the way to new dietary recommendations or therapeutical interventions.

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Publication

Oxidized LDL signals through Rho-GTPase to induce endothelial cell stiffening and promote capillary formation.

Oh MJ, Zhang C, LeMaster E, Adamos C, Berdyshev E, Bogachkov Y, Kohler EE, Baruah J, Fang Y, Schraufnagel DE, Wary KK, Levitan I
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