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**EDITORIAL**

# The interdependent effects of cholesterol and substrate stiffness on vascular smooth muscle cell biomechanics

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**This editorial refers to 'Membrane cholesterol and substrate stiffness co-ordinate to induce the remodelling of the cytoskeleton and the alteration in the biomechanics of vascular smooth muscle cells', by H.J. Sanyour *et al.*, pp. 1369–1380.**

Atherosclerosis is a progressive vascular disease highlighted by an array of cellular and tissue abnormalities including a chronic inflammatory response, subendothelial lipoprotein retention, cholesterol laden foam cells, and dysfunctional vascular smooth muscle cells (VSMCs) that coalesce into an overt pathology.[1] Considerable attention has been given to the varying contributors of atherosclerosis and their role in shaping specific components of plaque progression such as fibrous cap thinning, VSMC migration, arterial stiffening, and macrophage infiltration and activity; however, significantly less attention has been given to how some of these components coordinate with one another to regulate functional and phenotypic changes. Importantly, both reduced cholesterol efflux and changes in extracellular matrix composition and stiffness of VSMCs are noted occurrences in atherosclerosis.[2–4] Currently, it is unknown how or if cholesterol and changes in ECM composition coordinate to regulate VSMC function/biomechanics and downstream pathological processes such as intimal migration and plaque progression.

Sanyour *et al.*[5] present compelling evidence that membrane cholesterol and substrate stiffness synergistically affect VSMC biomechanical properties and cytoskeletal organization. Using atomic force microscopy, they show that increased VSMC stiffness and α5β1-integrin-mediated adhesion, as a result of increasing substrate stiffness, are highly dependent upon VSMC membrane cholesterol. In addition, the authors show that substrate stiffness-regulated VSMC cytoskeletal organization is dependent upon membrane cholesterol, and that depletion of membrane cholesterol with methyl-β-cyclodextrin (MβCD) disrupts actin remodelling, whereas cholesterol enrichment in the presence of MβCD (MβCD-CHOL) increases cytoskeletal remodelling even in low substrate stiffness (3.5 kPa) conditions. *Ex vivo* analysis of rat aortic vessels treated with phenylephrine also indicated that depletion of cholesterol with MβCD-reduced contractile force, providing further support to their *in vitro* findings and overall hypothesis. Thus, Sanyour *et al.* provide important insight into how changes in substrate stiffness and cholesterol influence VSMC biomechanics, and their findings further the need to study how internal and external factors mesh to regulate cellular function. The novel findings presented in this work also raise new and interesting questions concerning the regulation of VSMC biomechanics and the potential implications for the study of atherosclerosis.

First, what is the role of cholesterol in VSMCs? Cholesterol is a well-known player in the development of atherosclerosis, due in part to its role in macrophage foam cell formation and the vascular inflammatory response, but recently, there has been an increased appreciation for its role in modulating cellular mechanics. However, there is contentious debate on this topic, as the effect of cholesterol on biomechanical properties may be cell-type dependent.[6,7] For VSMCs, recent findings indicate that human coronary atherosclerotic lesions exhibit SMCs containing excess cholesterol, and that smooth muscle cell (SMC)-derived foam cells make up a larger percentage of foam cells than previously recognized.[2] From the data presented by Sanyour *et al.*,[5] excess cholesterol in areas prone to atherosclerosis may augment age-dependent increases in cellular stiffness and α5β1-integrin-mediated adhesion, which are both implicated in atherosclerosis.[8,9] How this interplay between cholesterol and the biomechanical properties of the matrix influences other properties of VSMCs such as migration and phenotypic switching remains to be studied in healthy and diseased conditions. Given previous evidence and results presented by Sanyour *et al.*, targeted approaches to reduce VSMC cholesterol accumulation such as enhancing cholesterol efflux from VSMCs may be worthwhile.

Second, what are the mechanisms behind the coordinated signalling between cholesterol and substrates stiffness, and what role does cytoskeletal remodelling play in this response? Cholesterol is known to influence actin dynamics at the leading edge of migrating cells,[7] and depletion of membrane cholesterol impairs cardiomyocyte contraction,[10] and phenylephrine-induced aortic contraction, as presented Sanyour *et al.*[5] Likewise, mechanotransduction plays an important role in VSMC actin dynamics and integrin-mediated adhesion.[11,12] The findings presented by Sanyour *et al.* paint an interesting picture where cholesterol enrichment in low substrate stiffness conditions increases α5β1-integrin-mediated adhesion and actin stress-fibre remodelling but is unable to increase overall VSMC stiffness. It is only when VSMCs are cultured on an increasingly stiff substrate that a functional change in cellular stiffness is realized. Thus, cholesterol signalling may provide the structure through which

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increased substrate stiffness induces changes in cellular stiffness. The mechanisms behind this response remain elusive, but membrane/lipid rafts may be an integral piece to the puzzle given that their formation is dependent upon membrane cholesterol,[13] and that they have unique roles in the regulation of ECM adherence, receptor activation, and cytoskeletal organization.[14] Future studies should further explore this topic.

Lastly, as the authors point out, continued investigation using animal models of atherosclerosis is needed. The data from *in vitro* investigations provide compelling evidence for coordinated signalling between cholesterol and substrate stiffness; however, the *in vivo* environment is more complex, and it is likely that additional factors work in combination with cholesterol and ECM stiffness to regulate VSMC biomechanics, such as cell-to-cell contact and paracrine and endocrine signalling. Moreover, future studies should assess the functional consequences and possible clinical outcomes of modulating VSMC membrane cholesterol levels in plaques. This is especially relevant to statin use, as an effect of statin therapy may be the modification of VSMC biomechanics. As an extension, future studies may seek to compare patient vessel stiffness before and after statin use. It is currently unknown if the observed coordinated signalling is protective or determinantal in atherosclerosis.

In summary, the work performed by Sanyour *et al.* opens a new and intriguing line of research that has direct implications for atherosclerosis and various other cardiovascular diseases and encourages continued investigation into coordinated signalling in vascular biology.

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