REVIEW

OPEN

Extracellular matrix: paving the way to the newest trends in atherosclerosis

Chrysostomi Gialeli[a,*], *Annelie Shami*[a,*], *and Isabel Gonçalves*[a,b]

Purpose of review
The extracellular matrix (ECM) is critical for all aspects of vascular pathobiology. In vascular disease the balance of its structural components is shifted. In atherosclerotic plaques there is in fact a dynamic battle between stabilizing and proinflammatory responses. This review explores the most recent strides that have been made to detail the active role of the ECM – and its main binding partners – in driving atherosclerotic plaque development and destabilization.

Recent findings
Proteoglycans-glycosaminoglycans (PGs-GAGs) synthesis and remodelling, as well as elastin synthesis, cross-linking, degradation and its elastokines potentially affect disease progression, providing multiple steps for potential therapeutic intervention and diagnostic targeted imaging. Of note, GAGs biosynthetic enzymes modulate the phenotype of vascular resident and infiltrating cells. In addition, while plaque collagen structure exerts very palpable effects on its immediate surroundings, a new role for collagen is also emerging on a more systemic level as a biomarker for cardiovascular disease as well as a target for selective drug-delivery.

Summary
The importance of studying the ECM in atherosclerosis is more and more acknowledged and various systems are being developed to visualize, target and mimic it.

Keywords
atherosclerosis, calcification, collagen, elastin, extracellular matrix

INTRODUCTION

The extracellular matrix (ECM) is involved in all aspects of vascular pathobiology. The major constituents of vascular ECMs are collagens, elastic fibres, proteoglycans, hyaluronan and a variety of glycoproteins, all interconnected in a complex active three-dimensional (3D) matrix network. This network regulates the biomechanical properties of blood vessels and the phenotype of the cells that reside in them, such as endothelial cells, vascular smooth muscle cells (VSMCs), adventitial fibroblasts and infiltrating immune cells from the circulation. Though many cell types mentioned can synthesize ECM macromolecules, VSMCs are considered the most prominent [1].

The initial event of atherogenesis occurs upon focal endothelial cell injury in artery branches or curvatures with nonlaminar blood flow, attracting circulating monocytes and T cells. Monocytes moving into the subendothelial intima differentiate into macrophages that secrete cytokines and exacerbate the inflammatory environment, as well as endocytose LDL particles, becoming lipid-engorged foam cells. Simultaneously, medial SMCs migrate into the intima where they proliferate and secrete collagen fibres that form a stabilizing fibrous cap. Conversely, formation of a large lipid-rich necrotic core destabilizes the lesion, potentially resulting in

[a]Department of Clinical Sciences Malmö, Lund University, Clinical Research Center, Malmö and [b]Department of Cardiology, Malmö, Skåne University Hospital, Lund University, Sweden

Correspondence to Isabel Gonçalves, PhD, MD, Department of Clinical Sciences Malmö, Clinical Research Center, Lund University, Jan Waldenströms gata 35, 214 28 Malmö, Sweden. Tel: +46 40 336852; e-mail: Isabel.Goncalves@med.lu.se

*Chrysostomi Gialeli and Annelie Shami contributed equally to this article.

Curr Opin Lipidol 2021, 32:277–285

DOI:10.1097/MOL.0000000000000775

KEY POINTS

- The atherosclerotic plaque ECM is not a simply a structural scaffold but rather plays a dynamic role in atherosclerosis.
- Elastin synthesis, cross-linking, degradation and its elastokines provide several point of potential intervention to modulate and image atherosclerosis.
- Plaque collagen affects plaque stability not only through quantity, but also fibrillar direction, and may serve as a selective target for drug delivery.
- Proteoglycans GAGs biosynthetic enzymes may be plausible target to treat atherosclerosis both in its early and advanced stages.
- Incorporating ECM structure – both in-vivo and through in-vitro simulation – will provide crucial mechanistic insights into future therapeutic interventions.

rupture or erosion, in high-risk, rupture-prone, also called vulnerable plaques, inducing thrombosis, which can lead to arterial occlusion, resulting in a myocardial infarction (MI) or stroke [2] (Fig. 1).

The vascular structure and function throughout life is defined from a balanced interplay among different ECM components. Specifically, almost half of the total normal arterial ECM consists of elastic fibre proteins, with lesser amounts of collagen, proteoglycans and glycoproteins. In atherosclerosis the balance is shifted with proteoglycans being the major ECM component in the early lesion phase, while, as the lesions progress collagens take over with decreased elastic fibres, proteoglycans and glycoproteins [3] (Fig. 1).

Taking into consideration the huge size of the field, this review summarizes the most recent strides to detail the active role of the ECM in driving atherosclerotic plaque development and destabilization and the newest trends on how to use this knowledge in future clinical applications.

PROTEOGLYCANS AND THEIR GLYCOSAMINOGLYCANS IN ATHEROSCLEROSIS

Proteoglycans (PGs) are comprised of sulphated glycosaminoglycans (GAGs) attached covalently to a protein core. Considering that a simple protein core can be modified with numerous GAGs, chondroitin sulphate (CS), dermatan sulphate (DS), keratan sulphate (KS), heparin and heparan sulphate (HS) or noncovalently bound hyaluronan, possibly of different types, it is clear that proteoglycans are extremely diverse molecules in the pathogenesis of atherosclerosis [1].

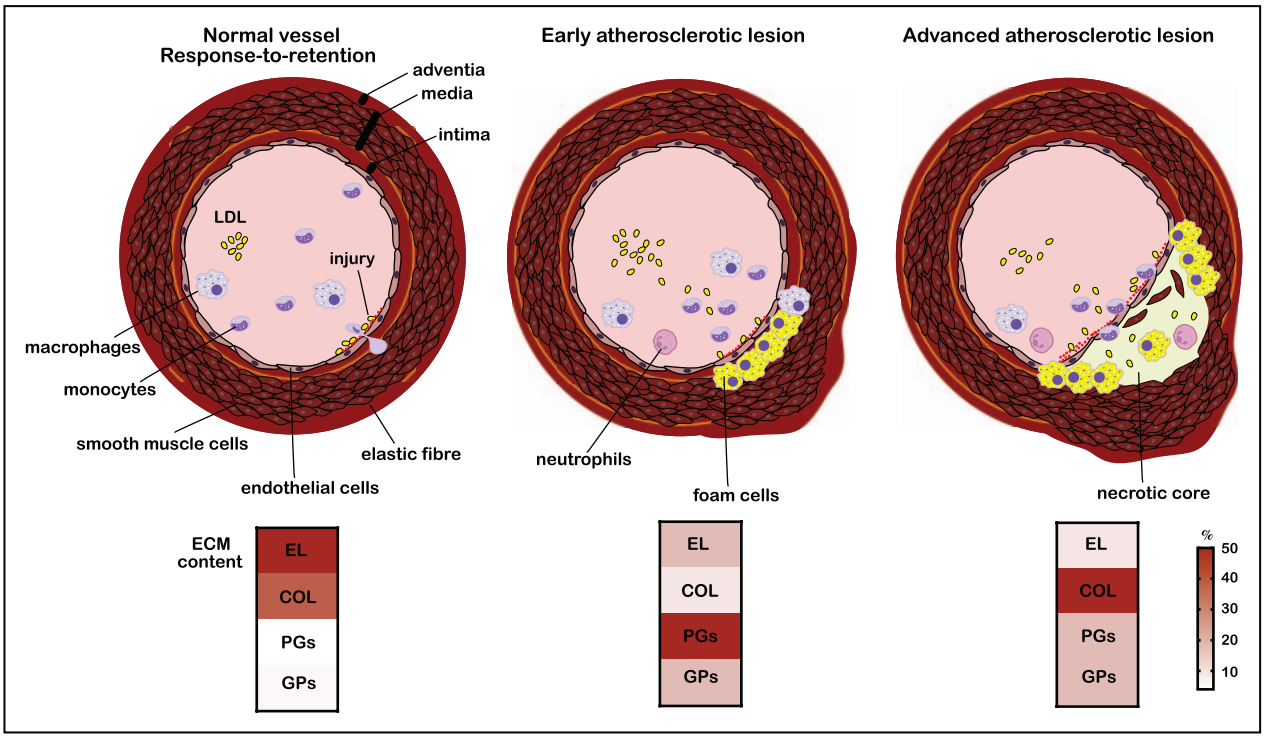

FIGURE 1. Progression of atherosclerosis and extracellular matrix components from normal vessel to early and advanced atherosclerotic lesion. COL, collagens; EL, elastin; GP, glycoprotein; PG, proteoglycan.

High-affinity complexes with lipoproteins

The current paradigm of the atherogenesis, the so-called response-to-retention hypothesis, involves the accumulation of lipoproteins in the intima driven by modified ECM molecules. Most incriminated proteoglycans in this process are versican (CSPG), biglycan (CS/DSPG), decorin and perlecan (HSPG) [3–6]. The retention ability of proteoglycans is mainly addressed in the context of SMCs and endothelial cells. However, macrophage-derived proteoglycans notably also bind lipoproteins via both their protein core and heparan sulphate GAG chains [7▪▪]. In the same study, the authors reported that the matrisome of macrophages is sensitive to macrophage polarization. A vital regulator of these interactions is GAG biosynthesis. For example, the chondroitin sulphate GAG biosynthesis gene (ChGn-2) impacts atherosclerosis by influencing the retention of lipoproteins by SMCs as well as macrophage [8▪]. Mechanistically, this occurs through a G protein-coupled receptor transactivation of TGFBR1, thus upregulating genes associated with GAG chain elongation [9]. Overall, GAG biosynthetic enzymes may be plausible targets to treat atherosclerosis both in its early and advanced stages.

Endothelial glycocalyx

The luminal surface of endothelial cells is covered by a thin negatively charged organized network of membrane-attached PGs (syndecan-1, syndecan-2, syndecan-4 and glypican-1) and secreted PGs (mimecan, perlecan and biglycan), often called the endothelial glycocalyx. It provides endothelial cells protection, while serving as molecular barrier, cell adhesion modulator and mechanosensor for blood flow. HS accounts for 50–90% of the total GAG pool, while the rest is composed of CS and hyaluronan [10]. In a recent study, endothelial cells exposed to pathological shear stress increased GAG synthesis, secretion and content, compared with those exposed to physiological shear stress [11▪]. The extent of atherosclerosis progression is associated with thinning of vascular endothelial glycocalyx, attributed to diminished expression of HSPGs [12▪].

One of the underlying mechanisms reported involves the inactivation of AMP-activated protein kinase and subsequent activation of hyaluronidase 2 that cleaves hyaluronan from the endothelial glycocalyx. This glycocalyx impairment results in increased macrophage recruitment [13]. While the presence of hyaluronan on the endothelial glycocalyx is antithrombotic and anti-inflammatory, suppressing leukocyte and platelet adhesion, hyaluronan can also be proatherosclerotic through abnormal accumulation due to hyaluronan synthase upregulation [14]. Hyaluronan, through CD44, induces VSMC migration and proliferation, which in turn leads to plaque growth and increased matrix production. Finally, macrophage activity is enhanced in plaques via their hyaluronan-Toll-like receptors, promoting macrophage accumulation and Th1 cell polarization.

Another secreted glycocalyx PG, mimecan, was recently related to a vulnerable phenotype in human plaques and increased risk of future cardiovascular death. Notably, in this study, mimecan is inversely corelated with SMCs, while *in vitro* mimecan expression is upregulated upon inflammatory stimuli in macrophages [15].

The actions of several ECM molecules also depend on the disease stage. Cartilage oligomeric matrix protein (COMP), expressed by VSMCs, protects the vasculature in early stages of plaque development by moderating disturbed flow-induced endothelial cell activation through integrin $\alpha5\beta1$/FAK inactivation [16▪▪]. In contrast, COMP expression in advanced plaques is upregulated and correlates with the vulnerable plaque phenotype by modulating the macrophage phenotype [17].

Immunomodulatory properties of proteoglycans and their glycosaminoglycans

After the last decades of atherosclerosis research, proteoglycans and subsequently, their GAGs have been mainly considered in the context of SMC and endothelium biology [18,19▪]. However, the last years' research offers fresh insight on how immune cells with PGs and their GAGs can orchestrate atherosclerosis progression. Esko *et al.* [20] reviewed the leading role of neutrophils, endothelium and macrophage HSPGs in atherogenesis, describing direct effects on for example macrophage polarization, also corroborated by Swan *et al.* For example, monocyte syndecan-1 expression modulated type I interferon signalling, promoting nonclassical macrophage activation, while lack of heparan sulphate sulphation promotes classical inflammatory activation. On the other hand, macrophage syndecan-4 acts as scavenger receptor, promoting foam cell formation. Of note, the syndecan-4/L-selectin axis is reported to modulate neutrophil infiltration. In turn, neutrophils extracellular traps are formed inducing inflammatory signalling by macrophages. In support of these observations, it was reported that HS biosynthesis is fine-tuned during macrophage polarisation, depicting their resolving or proinflammatory macrophage phenotype during atherosclerosis-related inflammation [21]. Recently, it was reported that M1 macrophages produced PGs with a higher level of HS chain sulphation compared with M2 macrophages [7▪▪]. These observations encourage further studies on the

significance of HS in the immune signature of the plaques that may lead to future improved treatment against plaque destabilization.

Proteoglycans and their glycosaminoglycans: therapeutic potential

Advances on the molecular mechanisms underlying the actions of PGs and their GAGs in atherosclerosis, have lately emerged through different targeting approaches. The rationale to develop peptidomimetics to mimic the protective role or block detrimental actions of ECM molecules for the plaque was described by Lv *et al.* [16▪▪] for COMP. In addition, several studies have employed CD44-hyaluronan interactions for targeting atherosclerosis, designing hyaluronan-coated polymeric micelles targeting CD44 overexpressing macrophages [22,23]. Further research is needed to characterise proteoglycan remodelling by degrading enzymes for example proteoglycanases, heparinase, at different stages of the atherosclerosis, introducing novel targets or biomarkers.

ELASTIN IN ATHEROSCLEROSIS

Elastin is an essential protein to provide elasticity and resilience to arteries, maintaining constant adaptation to haemodynamic changes during the cardiac cycle. This has been elegantly reinforced in mice models by Stoka *et al.* [24]. With a half-life of several decades, elastic fibres are extremely stable over time and very resistant to chemical physical attacks such as pH changes, heat and stretching [25]. After synthesis, elastin cross-linking by lysyl oxidase family members (LOX and LOXL-1) is also a process that can affect several stages of atherosclerosis, as brilliantly reviewed by Martinez-Gonzalez *et al.* [26]. Taken together, the elastic fibres are more than inert physical scaffolds, involved in dynamic signalling between cells and their environment.

Elastin in mineralization induction

The calcium-binding capacity of elastin is due to the presence of mineralization nucleation sites, in particular sulfydryl and carboxyl groups even in the absence of cells. Boraldi *et al.* [27▪] have recently shown that mineral deposition is favoured on hydrolysed fibrillar structures. Exposure of multiple charged sites increase the adsorption of Ca^{2+}, attracting phosphate and increasing the local ion concentration past the point of supersaturation. Even small damages on the surface of elastin fibrillar structures are sufficient to increase the mineralization process and act in synergy with peptides released from the whole elastin molecule, as inducers of an osteogenic response in VSMCs, actively modulating gene expression.

Ectopic calcification through targeted gene analysis was recently studied by Dayekh and Mequanint [28▪]. They showed that partially soluble elastin upregulated VSMC osteogenic genes accompanied by downregulation of smooth muscle myosin heavy chain (Myh11), a late-stage SMC differentiation marker, suggesting a skewing towards a synthetic phenotype: two important features of vascular calcification. Another recently described mediator – specifically in stiffness-dependent VSMC calcification – is a positive feedback loop between the collagen receptor discoidin domain receptor-1 activity and actomyosin contractility [29▪].

The proatherogenic effects of elastokines

Peptides obtained by degradation of elastin are known as elastokines, elastin-derived or elastin-related peptides, many of which are listed in a recent review by Heinz [30]. The elastokine-binding receptor – elastin receptor complex (ERC), first described in the1980s – has three components: first, the splice variant of β-galactosidase; second, cathepsin A; and third, neuraminidase (Neu1). Binding of elastokines to ERC activates Neu1, which then modulates the CD36 sialylation, in turn regulating oxLDL uptake [31,32]. This newly discovered interaction between CD36 and the Neu1 component of ERC further contributes to the proatherogenic effects of the elastokines. Furthermore, it emphasizes the elastokine effects as modulators of macrophage functions during atherogenesis, namely phagocytosis, cytokine release, migration and reactive oxygen species production. They promote angiogenesis, induce proteases expression, modulate the vascular tone affecting NO production and relaxation of endothelial cells; contractility and proliferation of vascular SMC as well as chemotaxis and cytokine release from immune cells. Significantly, Gayral *et al.* [33] already in 2014 showed that elastokines injected in mice led to an increase in atherosclerotic plaque size through the immune Neu1-PI3Kgamma pathway. Moreover, Neu1 is involved in the mediation of elastokine effects in insulin resistance and thrombosis [34,35]. Actually, elastokines through lactosylceramide/p-ERK1–2 signalling pathway are also involved in adipocyte dedifferentiation, which can be relevant in the metabolic syndrome subject who often suffer from atherosclerosis too [31].

A very recent study by Bocquet *et al.* [36▪] focused on the inhibition of neuraminidases such as Neu1 to modulate atherosclerosis, by testing oseltamivir phosphate (a widely used anti influenza sialidase inhibitor) in $Ldl^{-/-}$ mice on high fat diet.

They showed a decrease in plasma LDL and aortic elastin fragmentation, but unfortunately without effect on plaque size, and in concert with increased liver inflammation and fibrosis.

Elastin as an imaging target

Another interesting use of the ECM composition is the possibility to target molecules for diagnostic purposes, particularly if the targeted molecules are specific for characteristics of the rupture-prone plaques so that patients at high risk can be timely identified before symptoms occur. Dysfunctional elastogenesis occurs during atheroma formation, leading to the formation of tropoelastin in plaques which fail to cross-link into functional elastic fibres [37]. This has been implemented in imaging attempts from several groups on the last decade, culminating with the in-vivo detection and quantification of different MRI markers in a single scan now proven feasible using simultaneous assessment of plaque-burden and inflammatory activity by dual-probe molecular MRI of progressive atherosclerosis [38,39]. A 3T MRI scanner with the novel superparamagnetic iron oxide nanoparticle contrast agent and an elastin-targeted gadolinium agent enabled the visualisation of plaque burden in mice. No significant difference between the uptake of either contrast agent between stable versus vulnerable plaques was described, though it should be noted that the concept of vulnerable plaque in mice, is in itself highly controversial [40▪].

Recent advances of atomic force microscopy have shown, with unprecedented resolution, a new view on the stiffness process and elasticity impairment at the molecular level, in mice. Such precise quantification of fibre elasticity may enable tracking of fibre remodelling with improved stringency to predict plaque rupture in the future [41▪].

COLLAGEN STRUCTURE AND PLAQUE INTEGRITY

The common feature of collagens – the most abundant proteins in mammals – is the rigid triple helical structure (Gly-X-Y repeats) intermingled with more flexible, nontriple helical regions. The resulting aggregates determine the biomechanical properties of tissues, such as the tensile strength of arteries. Of the 28 types described so far, collagen type I and III are the main forms expressed in atherosclerotic plaques [42].

The artery wall depends on the various mechanical parameters of each layer for proper function and contractility. Recently, the visualisation of such structures have become increasingly feasible, exemplified by Johnston *et al.* [43▪▪] who – using the small angle light scattering technique – found that collagen fibre alignment in the circumferential direction in the plaque cap plays the most dominant role for determining plaque structural stability. These plaques exhibited superior load bearing (i.e. higher stresses and lower strains) before failure, while plaques with caps with predominately axial fibres displayed the opposite trend, suggesting a possible therapeutic utilisation to identify rupture-prone plaques. Significantly, the study also indicates that collagen content alone does not correlate with ultimate tensile stress and strain [43▪▪]. In a murine model ($Apoe^{-/-}$), similar changes in aortic adventitial collagen organization was associated with aging, with a skewing towards longitudinal – as opposed to circumferential – orientation in mice given normal chow, curiously enough with the opposite effect in mice on a high-fat diet [44▪].

Collagen as a cardiovascular disease marker

While plaque collagen structure exerts very palpable effects on its immediate surroundings, a new role for collagen or its fragments is also emerging as an overall cardiovascular disease (CVD) indicator on a systemic level. Circulating collagen type VI alpha 2 chain (COL6A) was recently put forth by Liu *et al.* [45▪] as an independent predictor – and thus potential noninvasive marker – of plaque erosion in patients undergoing optical coherence tomography during ST-segment elevated MI. Increased baseline levels of plasma endostatin – a type XVIII collagen cleavage product – has been associated with increased risk of mortality and severe disability following ischemic stroke in a large Chinese population. Moreover, adding plasma endostatin to a basic model constructed with conventional factors significantly improved risk stratification in this population [46▪]. The collagen advanced glycation end product glucuronidine/LW-1 found in skin, rather than in the circulation, was also associated with established coronary heart disease and calcified plaque volume in diabetic patients [47▪].

Collagen: therapeutic potential

Adding yet another layer to the role of collagen in CVD, is its selective targeting as an exciting strategy for drug-delivery. To this end, Kassam *et al.* has developed a collagen-targeted peptide amphiphile-based nanofibre for the prevention of neointimal hyperplasia after arterial injury. Of benefit are differing pharmacokinetics, including rapid elimination clearance, compared with traditional small molecules, which may potentially achieve a

therapeutic effect with comparatively lower plasma concentrations [48▪]. Peptide probes have also been devised for selective detection of denatured collagen by incorporating nonimino acids at the X and Y positions of the characteristic helical $(Gly\text{-}X\text{-}Y)_n$ amino acid collagen sequence pattern [49▪▪]. Moreover, nanoparticles recognizing collagen type IV, developed by Molinaro *et al.* [50▪▪] for selective drug delivery to regions of endothelial cell sloughing and collagen type IV-rich basement membrane exposure was confirmed to successfully target *in vivo* experimentally eroded murine endothelium.

Plaque collagen is a widely used indicator of atherosclerotic plaque stability, and effects inferred by a multitude of elements have been described recently. One especially interesting family of such factors is the microRNAs (miRNA/miR), manipulations of which in murine models demonstrate a clear role in atherogenesis with promising therapeutic potential. Promoting or inhibiting a variety of miRs has been reported to infer plaque stabilization (miR-9, miR-335-5p, miR-520a-3p, 14q32 miR-494 [51▪,52▪,53▪▪,54▪]), attenuated plaque growth (miR-9, miR-495–3p, miR-520c-3p, miR-21, miR-520a-3p [51▪,54▪–57▪]) and destabilization (miR-208w [58▪]).

CONCLUSION

The importance of studying the ECM is increasingly acknowledged and various systems are under development both to track – such as the utilization of ECM-derived biomarkers – and to mimic it. An example of the latter, a simple, pumpless, close-loop, easy-to-replicate and miniaturized flow device introduced by Hosseini *et al.* [59▪▪], concurrently exposes 3D engineered vascular smooth muscle tissues to high-velocity pulsatile versus low-velocity disturbed flow conditions, manipulating elastin and collagen type I assembly. They showed that gene expression of matrix metalloproteinase and their inhibitors, rather than that of collagens and elastin, seemed to be flow-dependent and were also able to halt the effect of low-velocity flow on the ECM remodelling through treatment with doxycycline, an MMP inhibitor. Elastin-like polymers produced

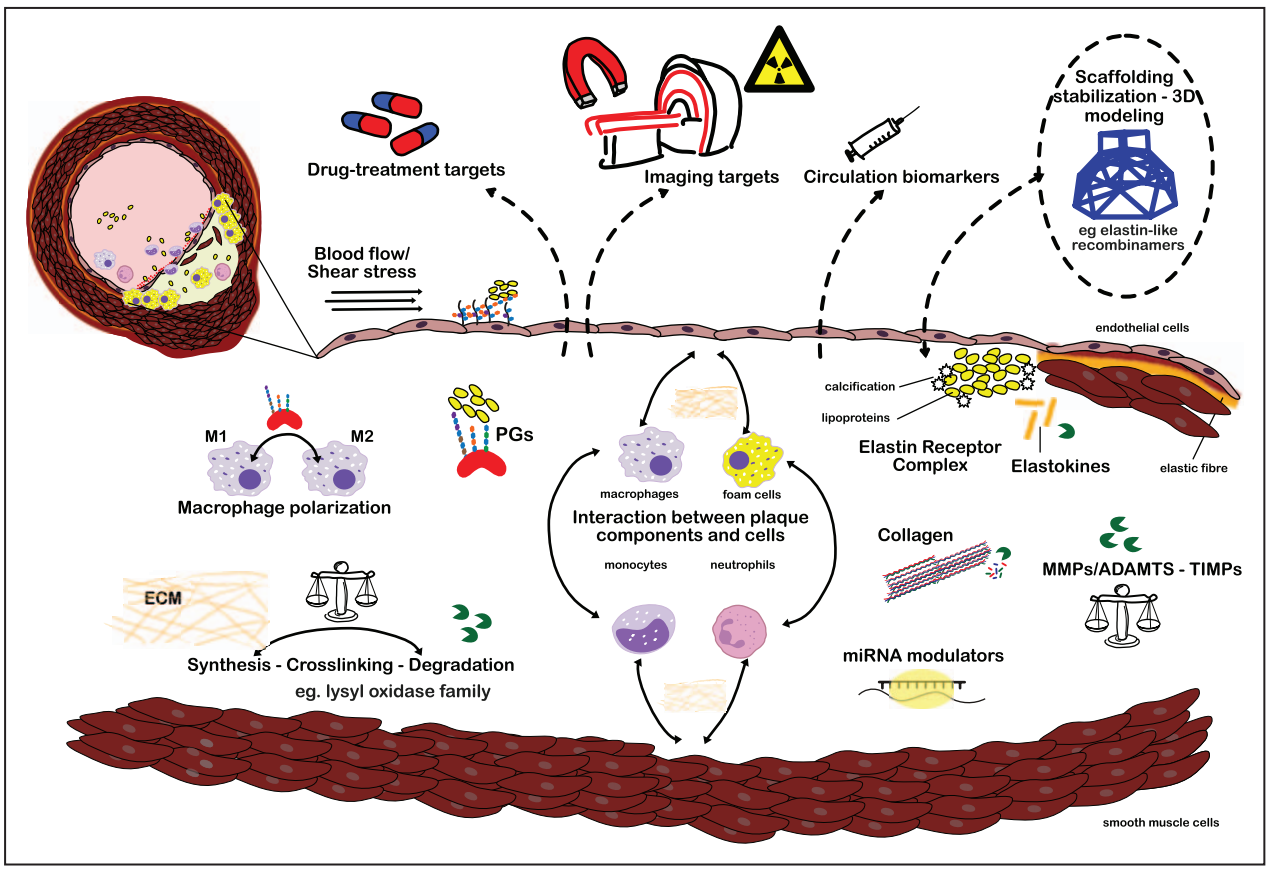

FIGURE 2. Overview of futures approaches against atherosclerosis using the current and most recent knowledge on extracellular matrix. ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; MMPs, metalloproteinases; TIMPs, Tissue inhibitors of metalloproteinases.

by recombinant techniques, also called elastin-like recombinamers seem particularly promising to manufacture an elastic fibrous scaffold, that can receive different cell adhesion sequences and be a potential tool in regenerative medicine [60]. Therefore, these types of platforms can be exploited for drug efficacy studies by providing crucial mechanistic insights into therapeutic interventions with effects on the ECM: an approach we are likely to see included more and more often as we look towards future intervention studies in the field of vascular pathobiology.

Overall, future investigations will need to address how tissue specific and temporal differences in ECM impact atherosclerosis. Of importance in the line with personalized treatment is the notion that individual unique changes to enzymes that affect GAGs sulphation and elongation may reflect differences in susceptibility to atherogenesis or may pinpoint a vulnerable plaque (Fig. 2).

Acknowledgements

None.

Financial support and sponsorship

This work was supported by funding from the Swedish Research Council, Swedish Heart and Lung Foundation, ALF Grants Region Skåne, SUS foundations and funds, Stroke foundation, the Royal Physiographic Society in Lund, The Swedish Heart and Lung Association, The Swedish Stroke Association and Swedish Foundation for Strategic Research (Dnr IRC15-0067).

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Karamanos NK, Theocharis AD, Piperigkou Z, *et al.* A guide to the composition and functions of the extracellular matrix. FEBS J 2021. https://doi.org/10.1111/febs.15776. [Online ahead of print]
2. Durham AL, Speer MY, Scatena M, *et al.* Role of smooth muscle cells in vascular calcification: implications in atherosclerosis and arterial stiffness. Cardiovasc Res 2018; 114:590–600.
3. Wight TN. A role for proteoglycans in vascular disease. Matrix Biol 2018; 71–72:396–420.
4. Wight TN. Provisional matrix: a role for versican and hyaluronan. Matrix Biol 2017; 60–61:38–56.
5. Gubbiotti MA, Neill T, Iozzo RV. A current view of perlecan in physiology and pathology: a mosaic of functions. Matrix Biol 2017; 57–58:285–298.
6. Scuruchi M, Poti F, Rodriguez-Carrio J, *et al.* Biglycan and atherosclerosis: lessons from high cardiovascular risk conditions. Biochim Biophys Acta Mol Cell Biol Lipids 2020; 1865:158545.
7. ■■ Ng CY, Whitelock JM, Williams H, *et al.* Macrophages bind LDL using heparan sulfate and the perlecan protein core. J Biol Chem 2021; 296:100520.

This study revealed that HS composition was sensitive to macrophage cell type and polarization, whereas CS profile was not affected. In addition, M1 macrophages-derived HS was more sulphated than M2. This article highlights the importance of GAGs biosynthesis and subsequent expression of PGs on macrophages phenotype. In addition, the authors identified perlecan as HSPG responsible for the LDL retention.

8. ■ Adhikara IM, Yagi K, Mayasari DS, *et al.* Chondroitin sulfate *N*-acetylgalactosaminyltransferase-2 impacts foam cell formation and atherosclerosis by altering macrophage glycosaminoglycan chain. Arterioscler Thromb Vasc Biol 2021; 41:1076–1091.

In this study, the authors present the CS elongation enzyme, ChGn-2, as an attractive therapeutic target. ChGn-2 deletion reduces macrophage foam cell formation and atherosclerosis developed in mice. Mechanistically, ChGn-2 mediates oxidized LDL binding on the macrophage surface.

9. Zhou Y, Little PJ, Cao Y, *et al.* Lysophosphatidic acid receptor 5 transactivation of TGFBR1 stimulates the mRNA expression of proteoglycan synthesizing genes XYLT1 and CHST3. Biochim Biophys Acta Mol Cell Res 2020; 1867:118848.
10. Yilmaz O, Afsar B, Ortiz A, Kanbay M. The role of endothelial glycocalyx in health and disease. Clin Kidney J 2019; 12:611–619.
11. ■ Russo TA, Banuth AMM, Nader HB, Dreyfuss JL. Altered shear stress on endothelial cells leads to remodeling of extracellular matrix and induction of angiogenesis. PLoS One 2020; 15:e0241040.

In this study, endothelial cells exposed to shear stress induced morphological changes to the endothelium, as well as altered functional properties. Overall changes reported included increased expression and secretion of HSPGs, as well as collagen and growth factors.

12. ■ Kang H, Sun A, Wu Q, *et al.* Atherogenic diet-diminished endothelial glycocalyx contributes to impaired vasomotor properties in rat. Am J Physiol Heart Circ Physiol 2020; 319:H814–H823.

The authors reported that high-cholesterol diet reduces *in vivo* the thickness of the endothelial glycocalyx. This was not a result of an enhanced shedding, rather attributed to a transcriptional reprogramming of endothelial cells, downregulating HSPG-related genes.

13. Zhang J, Kong X, Wang Z, *et al.* AMP-activated protein kinase regulates glycocalyx impairment and macrophage recruitment in response to low shear stress. FASEB J 2019; 33:7202–7212.
14. Homann S, Grandoch M, Kiene LS, *et al.* Hyaluronan synthase 3 promotes plaque inflammation and atheroprogression. Matrix Biol 2018; 66:67–80.
15. Tengryd C, Nielsen SH, Cavalera M, *et al.* The proteoglycan mimecan is associated with carotid plaque vulnerability and increased risk of future cardiovascular death. Atherosclerosis 2020; 313:88–95.
16. ■■ Lv H, Wang H, Quan M, *et al.* Cartilage oligomeric matrix protein fine-tunes disturbed flow-induced endothelial activation and atherogenesis. Matrix Biol 2021; 95:32–51.

This study characterized in detail the protective properties of COMP on the ECs upon shear stress. The authors attributed this effect to the ability of COMP to bind directly to integrin $\alpha 5$ via its C-terminus part, thus inactivating the integrin $\alpha 5\beta 1$ mediated signal transduction. To this end, the authors developed peptidomimetic for this interaction and reported its efficiency to alleviate atherosclerosis *in vitro* and *in vivo*.

17. Hultman K, Edsfeldt A, Bjorkbacka H, *et al.* Cartilage oligomeric matrix protein associates with a vulnerable plaque phenotype in human atherosclerotic plaques. Stroke 2019; 50:3289–3292.
18. Allahverdian S, Ortega C, Francis GA. Smooth Muscle Cell-Proteoglycan-Lipoprotein Interactions as Drivers of Atherosclerosis. In: Handbook of Experimental Pharmacology. Springer, Berlin, Heidelberg. 2020:1–24. https://doi.org/10.1007/164_2020_364.
19. ■ Holm Nielsen S, Jonasson L, Kalogeropoulos K, *et al.* Exploring the role of extracellular matrix proteins to develop biomarkers of plaque vulnerability and outcome. J Intern Med 2020; 287:493–513.

This study showed that in humans COMP is associated with a vulnerable carotid plaque phenotype. In addition, there are indications that COMP modulates macrophage phenotype.

20. Gordts P, Esko JD. The heparan sulfate proteoglycan grip on hyperlipidemia and atherosclerosis. Matrix Biol 2018; 71–72:262–282.
21. Swart M, Troeberg L. Effect of polarization and chronic inflammation on macrophage expression of heparan sulfate proteoglycans and biosynthesis enzymes. J Histochem Cytochem 2019; 67:9–27.
22. Mu D, Li J, Qi Y, *et al.* Hyaluronic acid-coated polymeric micelles with hydrogen peroxide scavenging to encapsulate statins for alleviating atherosclerosis. J Nanobiotechnology 2020; 18:179.
23. Hou X, Lin H, Zhou X, *et al.* Novel dual ROS-sensitive and CD44 receptor targeting nanomicelles based on oligomeric hyaluronic acid for the efficient therapy of atherosclerosis. Carbohydr Polym 2020; 232:115787.
24. Stoka KV, Maedeker JA, Bennett L, *et al.* Effects of increased arterial stiffness on atherosclerotic plaque amounts. J Biomech Eng 2018; 140:051007.
25. Fhayli W, Boete Q, Harki O, *et al.* Rise and fall of elastic fibers from development to aging. Consequences on arterial structure-function and therapeutical perspectives. Matrix Biol 2019; 84:41–56.
26. Martinez-Gonzalez J, Varona S, Canes L, *et al.* Emerging roles of lysyl oxidases in the cardiovascular system: new concepts and therapeutic challenges. Biomolecules 2019; 9:610.

27. Boraldi F, Moscarelli P, Lofaro FD, *et al.* The mineralization process of
■ insoluble elastin fibrillar structures: ionic environment vs degradation. Int J Biol Macromol 2020; 149:693–706.
This study sheds light on the capacity of elastin fibres to calcify and the effects of the ionic environment on this process.
28. Dayekh K, Mequanint K. The effects of progenitor and differentiated cells on
■ ectopic calcification of engineered vascular tissues. Acta Biomater 2020; 115:288–298.
This study reports that partially soluble elastin promotes a pro-osteogenic phenotype in SMCs.
29. Ngai D, Lino M, Rothenberg KE, *et al.* DDR1 (discoidin domain receptor-1)-
■ RhoA (Ras homolog family member A) axis senses matrix stiffness to promote vascular calcification. Arterioscler Thromb Vasc Biol 2020; 40:1763–1776.
This in-vitro study describes a positive feedback loop between DDR1 activity and actomyosin contractility in mediating stiffness-dependent vascular SMC (VSMC) calcification.
30. Heinz A. Elastases and elastokines: elastin degradation and its significance in health and disease. Crit Rev Biochem Mol Biol 2020; 55:252–273.
31. Hocine T, Blaise S, Hachet C, *et al.* Lactosylceramide induced by elastin-derived peptides decreases adipocyte differentiation. J Physiol Biochem 2020; 76:457–467.
32. Kawecki C, Bocquet O, Schmelzer CEH, *et al.* Identification of CD36 as a new interaction partner of membrane NEU1: potential implication in the pro-atherogenic effects of the elastin receptor complex. Cell Mol Life Sci 2019; 76:791–807.
33. Gayral S, Garnotel R, Castaing-Berthou A, *et al.* Elastin-derived peptides potentiate atherosclerosis through the immune Neu1-PI3Kgamma pathway. Cardiovasc Res 2014; 102:118–127.
34. Kawecki C, Hezard N, Bocquet O, *et al.* Elastin-derived peptides are new regulators of thrombosis. Arterioscler Thromb Vasc Biol 2014; 34:2570–2578.
35. Blaise S, Romier B, Kawecki C, *et al.* Elastin-derived peptides are new regulators of insulin resistance development in mice. Diabetes 2013; 62:3807–3816.
36. Bocquet O, Wahart A, Sarazin T, *et al.* Adverse effects of oseltamivir phos-
■ phate therapy on the liver of LDLR$^{-/-}$ mice without any benefit on atherosclerosis and thrombosis. J Cardiovasc Pharmacol 2021; 77:660–672.
This study assessed the effects of oseltamivir phosphate, an antiinfluenza sialidase inhibitor, on atherosclerosis. Decreased levels of plasma LDL cholesterol and elastin fragmentation are reported in the aorta: effects that, however, did not translate to reduced plaque size or thrombosis.
37. Phinikaridou A, Lacerda S, Lavin B, *et al.* Tropoelastin: a novel marker for plaque progression and instability. Circ Cardiovasc Imaging 2018; 11:e007303.
38. Reimann C, Brangsch J, Kaufmann JO, *et al.* Dual-probe molecular MRI for the in vivo characterization of atherosclerosis in a mouse model: simultaneous assessment of plaque inflammation and extracellular-matrix remodeling. Sci Rep 2019; 9:13827.
39. Chen W, Cormode DP, Vengrenyuk Y, *et al.* Collagen-specific peptide conjugated HDL nanoparticles as MRI contrast agent to evaluate compositional changes in atherosclerotic plaque regression. JACC Cardiovasc Imaging 2013; 6:373–384.
40. Evans RJ, Lavin B, Phinikaridou A, *et al.* Targeted molecular iron oxide contrast
■ agents for imaging atherosclerotic plaque. Nanotheranostics 2020; 4:184–194.
In this study, novel MRI contrast agents were developed based on superparamagnetic iron oxide nanoparticles and later confirmed to accumulate in vascular regions in a manner comparable with gadolinium-based contrast agent (Gd-ESMA) targeted to elastin, known to accumulate in plaque.
41. Berquand A, Wahart A, Henry A, *et al.* Revealing the elasticity of an individual
■ aortic fiber during ageing at nanoscale by in situ atomic force microscopy. Nanoscale 2021; 13:1124–1133.
Utilizing the latest developments in atomic force microscopy and the related nanomechanical modes, this study explores the morphology and elasticity of murine aorta cross sections. It is shown that – beyond elastin and collagen content changes – with ageing, vascular elasticity is impaired at the molecular level: novel insight into the arterial stiffness process.
42. Xu J, Shi GP. Vascular wall extracellular matrix proteins and vascular diseases. Biochim Biophys Acta 2014; 1842:2106–2119.
43. Johnston RD, Gaul RT, Lally C. An investigation into the critical role of fibre
■■ orientation in the ultimate tensile strength and stiffness of human carotid plaque caps. Acta Biomater 2021; 124:291–300.
This study shows for the first time how collagen fibre architecture governs the strength and stiffness of the fibrous cap in human carotid plaques. It is further demonstrated that caps with collagen fibres aligned in a principally circumferential direction exhibit superior load bearing compared with caps with mainly axial fibres. Potential therapeutic use in the identification of plaques with a high risk of rupture is suggested.
44. Watson SR, Cooper KM, Liu P, *et al.* Diet alters age-related remodeling of
■ aortic collagen in mice susceptible to atherosclerosis. Am J Physiol Heart Circ Physiol 2021; 320:H52–H65.
This study reports a shift in aortic adventitial collagen fibres to a longitudinal rather than circumferential direction with ageing in *Apoe*$^{-/-}$ mice on chow diet while the opposite trend was seen with a high-fat diet.
45. Liu J, Wang S, Hou J, *et al.* Proteomics profiling reveals insulin-like growth
■ factor 1, collagen type VI alpha-2 chain, and fermitin family homolog 3 as potential biomarkers of plaque erosion in ST-segment elevated myocardial infarction. Circ J 2020; 84:985–993.
This study demonstrates an association between circulating COL6A and plaque erosion in ST-segment elevated myocardial infarction, suggesting its use as a potential biomarker.
46. Zhang C, Qian S, Zhang R, *et al.* Endostatin as a novel prognostic biomarker in
■ acute ischemic stroke. Atherosclerosis 2020; 293:42–48.
An association is found between endostatin and increased risk of mortality and severe disability after ischaemic stroke and, furthermore, it is achieved improved risk stratification by including endostatin in a basic model constructed with conventional risk factors.
47. Holte KB, Svanteson M, Hanssen KF, *et al.* Collagen methionine sulfoxide and
■ glucuronidine/LW-1 are markers of coronary artery disease in long-term survivors with type 1 diabetes. The Dialong study. PLoS One 2020; 15:e0233174.
This study identifies an association between the collagen advanced glycation end-product glucuronide/LW-1 and established coronary heart disease in type 1 diabetes.
48. Kassam HA, Bahnson EM, Cartaya A, *et al.* Pharmacokinetics and biodis-
■ tribution of a collagen-targeted peptide amphiphile for cardiovascular applications. Pharmacol Res Perspect 2020; 8:e00672.
This study describes the differing pharmacokinetics of a collagen-targeted peptide amphiphile compared with those of small molecules.
49. Wei W, Li D, Cai X, *et al.* Peptide probes with aromatic residues Tyr and Phe
■■ at the X position show high specificity for targeting denatured collagen in tissues. ACS Omega 2020; 5:33075–33082.
In this study, novel peptide probes against denatured collagen were constructed. Successful detection of denatured collagen is shown in both mouse connective tissues and human pathological tissues. Utilization of these probes may potentially be of diagnostic value in disease characterized by collagen-damage.
50. Molinaro R, Yu M, Sausen G, *et al.* Targeted delivery of protein arginine
■■ deiminase-4 inhibitors to limit arterial intimal NETosis and preserve endothelial integrity. Cardiovasc Res 2021; 142:A15700.
In this study, development of nanoparticles targeting collagen type IV is shown to successfully reach sites of experimentally eroded endothelial regions in a mouse model. The study further succeeds in using a protein arginine deiminase (PAD)-4 inhibitor to reduce neutrophil extracellular trap (NET) formation at such sites, both confirming the role of PAD-4 and NETs in plaque erosion and suggesting future therapeutic use for selective drug delivery to sites of intimal injury.
51. Zhang R, Song B, Hong X, *et al.* microRNA-9 inhibits vulnerable plaque
■ formation and vascular remodeling via suppression of the SDC2-dependent FAK/ERK signaling pathway in mice with atherosclerosis. Front Physiol 2020; 11:804.
This study reports that upregulation of miR-9 has stabilizing and growth-reducing effects on murine atherosclerotic lesions.
52. Sun D, Ma T, Zhang Y, *et al.* Overexpressed miR-335-5p reduces athero-
■ sclerotic vulnerable plaque formation in acute coronary syndrome. J Clin Lab Anal 2021; 35:e23608.
This study found overexpression of miR-335-5p to reduce atherosclerotic vulnerable plaque formation through targeting JAG1.
53. van Ingen E, Foks AC, Kroner MJ, *et al.* Antisense oligonucleotide inhibition of
■■ MicroRNA-494 halts atherosclerotic plaque progression and promotes plaque stabilization. Mol Ther Nucleic Acids 2019; 18:638–649.
This study observed halted atherosclerosis progression accompanied by stabilization of advanced lesions through treatment with third-generation antisense inhibition of 14q32 miRNA-494 in an atherosclerotic mouse model. In particular, the reported effect on existing lesions makes this a relevant potential therapeutic strategy from a translational perspective.
54. Qi JR, Zhao DR, Zhao L, *et al.* MiR-520a-3p inhibited macrophage polarization
■ and promoted the development of atherosclerosis via targeting UVRAG in apolipoprotein E knockout mice. Front Mol Biosci 2020; 7:621324.
In this study, silencing of miR-520a-3p reduced murine atherosclerosis progression, in part by skewing macrophage polarization towards nonclassical activation.
55. Fu DN, Wang Y, Yu LJ, *et al.* Silenced long noncoding RNA activated by DNA
■ damage elevates microRNA-495-3p to suppress atherosclerotic plaque formation via reducing Kruppel-like factor 5. Exp Cell Res 2021; 401:112519.
This study found elevated expression of miR-495-3p to suppress murine atherogenesis through the reduction of Kruppel-like factor 5.
56. Wang J, Hu X, Hu X, *et al.* MicroRNA-520c-3p targeting of RelA/p65
■ suppresses atherosclerotic plaque formation. Int J Biochem Cell Biol 2021; 131:105873.
This study describes how miR-520c-3p inhibits PDGF-BB-mediated the proliferation and migration of human aortic VSMCs.
57. Gao L, Zeng H, Zhang T, *et al.* MicroRNA-21 deficiency attenuated ather-
■ ogenesis and decreased macrophage infiltration by targeting Dusp-8. Atherosclerosis 2019; 291:78–86.
This study reports miR-21 deficiency in mice to result in murine plaques of reduced size but of a less stable phenotype.
58. Chen R, Zhang Y, Zhao C. CHOP increases TRIB3-dependent miR-208
■ expression to potentiate vascular smooth muscle cell proliferation and migration by downregulating TIMP3 in atherosclerosis. Cardiovasc Drugs Ther 2021, https://doi.org/10.1007/s10557-021-07154-6.

This study describes reduced atherosclerosis progression through effects on the TRIB3/miR-208/TIMP3 axis caused by inhibition of C/EBP homologous protein (CHOP).

59. Hosseini V, Mallone A, Mirkhani N, *et al.* A pulsatile flow system to engineer ■■ aneurysm and atherosclerosis mimetic extracellular matrix. Adv Sci (Weinh) 2020; 7:2000173.

This study describes a pumpless, close-loop and miniaturized flow device which make it possible to gain crucial mechanistic insight into tissue growth ECM assembly, that may be used as a novel way to evaluate therapeutic interventions. The functionality of the method is presented by comparing ECM synthesis in 3D engineered vascular smooth muscle tissues exposed to high-velocity pulsatile flow versus low-velocity disturbed flow, where the former was observed to promote elastin while impairing collagen type I assembly, and the latter impairing elastin while promoting collagen type I assembly.

60. Putzu M, Causa F, Nele V, *et al.* Elastin-like-recombinamers multilayered nanofibrous scaffolds for cardiovascular applications. Biofabrication 2016; 8:045009.

