

Ultrasound-Based Precision Assessment and Intervention of Vulnerable Atherosclerotic Plaques: From Plaque Characterization to Therapeutic Modulation

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Abstract: Luminal stenosis remains central to clinical decision-making but does not fully characterize the plaque features associated with instability and embolization. This narrative review examines the progression from ultrasound-based plaque characterization to therapeutic modulation, with carotid atherosclerosis as the principal clinical focus. Contrast-enhanced ultrasound (CEUS) has the strongest human evidence among advanced methods for microvascular plaque characterization, linking intraplaque enhancement with histologic neovascularization and symptomatic disease. Superb microvascular imaging, elastography, and ultrasound localization microscopy provide complementary measures of slow flow, tissue mechanics, and microvascular architecture. Molecular ultrasound has visualized endothelial activation, platelet deposition, and angiogenic targets in animal models; studies of human endarterectomy specimens have confirmed the expression and accessibility of several candidate vascular targets *ex vivo*. Experimental nanoscale probes also target foam-cell lipids and extracellular matrix within plaque. Ultrasound-targeted delivery and sonodynamic therapy (SDT) modify macrophages, matrix, and neovessels in experimental plaques. A randomized SDT trial in femoropopliteal disease reported reduced plaque inflammation and improved walking performance; small carotid or mixed-territory studies report imaging responses. Carotid stroke-prevention benefit remains to be established. Serial ultrasound measurements during lipid-lowering treatment provide a human application of imaging-based response assessment.

Key words: Atherosclerosis; Vulnerable plaque; Molecular ultrasound; Sonodynamic therapy

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Luminal stenosis remains central to clinical decision-making but does not fully capture the biological features associated with plaque instability and embolization. Recent advances have shifted the focus toward functional plaque characterization. Con-

trast-enhanced ultrasound (CEUS) enables assessment of intraplaque neovascularization, while artificial intelligence (AI) may support the analysis and integration of plaque imaging features for risk stratification. Ultrasound is also being investigated beyond imaging, with

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targeted probes and ultrasound-mediated approaches offering potential routes to therapeutic modulation of vulnerable plaques. This narrative review traces the progression from stenosis-centric assessment to biologically informed imaging and targeted intervention, with carotid atherosclerosis as the principal clinical focus.

Beyond Luminal Stenosis

Stenosis as the clinical foundation

Duplex velocity criteria, interpreted with grayscale and color Doppler findings, underpin ultrasound grading of arterial stenosis, particularly for extracranial carotid stenosis. A systematic review of Society of Radiologists in Ultrasound thresholds found that a peak systolic velocity of 125 cm/s favored sensitivity, whereas 230 cm/s favored specificity [1]. Plaque morphology and biology provide information about embolic risk beyond the degree of narrowing.

Lesions with similar luminal narrowing can differ in surface disruption, lipid and hemorrhagic components, neovascularization, endothelial activation, and mechanical behavior. Consensus documents treat plaque morphology and composition as complementary to stenosis and symptom status [2]. Plaque-RADS organizes these findings into cross-modality categories ranging from no plaque to complicated plaque [3].

In a 2,023-patient cohort, higher Plaque-RADS categories were associated with prevalent stroke and, within the stroke subgroup, short-term recurrence [4]. The predominantly cross-sectional design, limited adjustment, and small category 4 subgroup ($n = 34$) restrict the prognostic interpretation of these findings.

Carotid management incorporates symptom laterality and recency, stenosis severity, best medical therapy, procedural risk, comorbidity, and life expectancy [5]. Advanced plaque imaging is most relevant when these factors leave uncertainty, particularly in selected asymptomatic or intermediate-risk patients. It may inform surveillance, further investigation, or trial eligibility, without delaying indicated care in recently symptomatic disease.

“Vulnerable” or “high-risk” plaques have structural, microvascular, mechanical, or molecular features plausibly related to disruption or embolization. Histologic vulnerability, association with recent symptoms, and future-event risk are distinct endpoints: tissue agreement supports biological interpretation, whereas prognosis depends on longitudinal outcomes.

From plaque characterization to therapeutic modulation

Ultrasound can characterize plaque phenotype, assess

a biological target, deliver or activate treatment, and monitor the response. B-mode and Doppler describe anatomy and flow; contrast-enhanced ultrasound (CEUS), microvascular flow imaging, elastography, and ultrasound localization microscopy (ULM) add vascular and mechanical information. Computational analysis supports quantification and integration of these measurements.

These applications are at different stages of development. Stenosis measurement is established, whereas enhancement, slow-flow signal, stiffness, and deformation are clinical research measurements. Molecular ultrasound relies mainly on animal experiments and human ex vivo plaque studies. Ultrasound-targeted delivery remains preclinical; sonodynamic therapy (SDT) has also reached early human studies, including a femoropopliteal randomized trial and carotid or mixed-territory pilots.

Existing reviews separately address carotid imaging, microbubble delivery, cardiovascular ultrasound-targeted microbubble destruction, and atherosclerosis nanomedicine [2,6-9]. This review examines the progression from plaque characterization to ultrasound-mediated therapeutic modulation, with carotid atherosclerosis as the principal clinical focus. Noncarotid and experimental studies provide evidence on molecular targets, delivery mechanisms, and treatment effects. Experimental theranostic platforms combine plaque imaging with ultrasound-activated treatment.

Plaque Biology Relevant to Ultrasound

Structural substrate and surface failure

Plaque burden, composition, surface integrity, and superimposed thrombus contribute to the embolic substrate. Ultrasound provides indirect information through acoustic backscatter, tissue motion, and blood flow. Thickness, area, and three-dimensional (3D) volume describe geometry; echogenicity and texture reflect tissue composition and imaging conditions. A surface recess or mobile element may indicate complicated plaque.

Low echogenicity can accompany lipid, hemorrhage, or loose matrix, but also changes with gain and insonation angle. A microscopic cap is often below transcutaneous resolution, while dense calcium can obscure the lumen–plaque interface and deeper tissue. Agreement between 3D ultrasound and excised plaque volume supports geometric measurement [10]; echogenicity and surface appearances are less tissue-specific.

Endarterectomy histology allows direct tissue comparison, predominantly in advanced, operable lesions.

Tissue deformation, orientation, and section matching affect image–histology correspondence. A 2025 review of 63 histology-referenced studies found wide accuracy ranges and persistent heterogeneity across ultrasound techniques [11].

Intraplaque neovascularization

Intraplaque neovascularization (IPN) is associated with hypoxia, inflammation, hemorrhage, and plaque expansion. Patent neovessels carry blood and can permit inflammatory-cell and plasma-protein entry. Ultrasound detects perfusion rather than vessel fragility or leakage; thrombosed, compressed, or extremely slow-flow channels may be histologically present but undetectable.

CEUS measures intravascular microbubble enhancement and tracer kinetics; superb microvascular imaging (SMI) detects low-velocity flow after clutter suppression; ULM reconstructs localized microbubble tracks. Human tissue studies associate CEUS and SMI signals with neovessel density [12–15]. Enhancement can correlate with vascularity without a comparable association with macrophage infiltration [13]. Multimodal plaque scores have also been associated with inflammatory markers [16].

Mechanical vulnerability and local hemodynamic loading

Plaque failure reflects interactions among tissue heterogeneity, geometry, pressure, and local flow. A soft region beside calcification can generate steep deformation or stress gradients even when mean stiffness is unremarkable; plaque shoulders experience different mechanical loading than the plaque base. Low or oscillatory luminal shear may sustain endothelial activation, whereas high shear near a stenotic throat may contribute to surface injury.

Strain imaging measures tissue deformation, while shear-wave elastography (SWE) estimates effective stiffness; deriving stress additionally requires loading and material-boundary assumptions. Ultrafast and vector-flow methods measure velocity fields used to estimate wall-shear-related indices. Human studies associate lower stiffness with symptomatic plaque and characterize regional flow patterns [17–19].

Precision Phenotyping Across Complementary Measurements

Structural and hemodynamic assessment

The baseline examination identifies the index plaque and assesses stenosis severity, velocity spectra, burden, surface abnormalities, and collateral flow. The relation

to the symptomatic territory and areas obscured by calcium determine the interpretation of subsequent measurements. An ipsilateral lesion is more relevant to symptom attribution than a contralateral one.

Maximum thickness samples one plane, plaque area depends on the imaging plane, and 3D volume accommodates irregular geometry. Semiautomated segmentation improves measurement efficiency [10]. Serial comparison depends on registration of the same lesion, consistent acquisition and segmentation, and known scan–rescan error. Surface recesses require confirmation of luminal communication because dropout and calcification can mimic discontinuity.

Doppler velocity describes the hemodynamic effect of a lesion, whereas surface disruption and mobile material concern a focal region. Recording plaque boundaries, insonation plane, gain normalization, and acoustic shadowing helps align these observations across visits. Grayscale thresholds are particularly sensitive to scanner processing.

Plaque-RADS organizes structural observations into a common vocabulary [3]. Representative Doppler images illustrate plaque morphology and color-flow patterns (Fig. 1). Table 1 summarizes the principal measurements and evidence for each method.

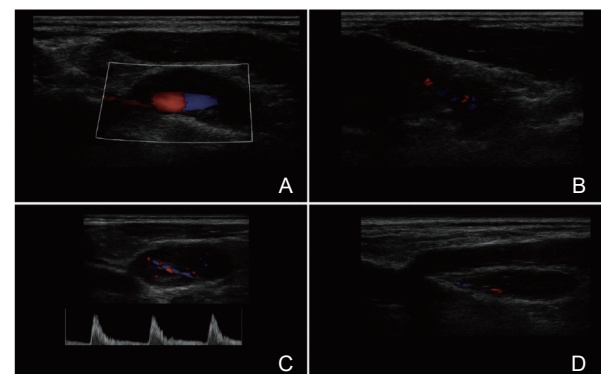


Figure 1 Representative Doppler ultrasound appearances of carotid plaque. (A) Plaque with moderate echogenicity and a smooth surface; (B) Color-flow signals within the plaque region; (C) Plaque at the carotid bifurcation with a spectral Doppler trace; (D) Long-axis view of a plaque with sparse color-flow signals. Reproduced from Wang et al. [20].

Microvascular phenotyping

Contrast-enhanced Ultrasound CEUS has the broadest human evidence among microvascular methods. Meta-analysis found greater enhancement in symptomatic plaques and better reproducibility for quantitative than visual analysis [21]. Histology-referenced diagnostic performance is moderate to high, but reference standards and thresholds vary [22]. Recent small studies examine sequential acquisition and high-frame-rate reader agreement [23–24]. Focal enhancement corresponds to CD31-positive neovessels in an endarterectomy specimen (Fig. 2) [25].

Table 1 Advanced ultrasound assessment of carotid plaque

Method	Primary quantity	Evidence	Key limitation	Role in plaque assessment
B-mode and duplex Doppler	Anatomy, burden, surface, stenosis, flow velocity	Established stenosis framework; structured morphology lexicon [1–4]	Shadowing, plane dependence, scanner settings, and limited tissue specificity	Stenosis grading and structural description
3D ultrasound and automated segmentation	Plaque volume and geometry	Histology/surgical-volume agreement and improved efficiency [10]	Segmentation failure, registration drift, and uncertain outcome meaning of volume change	Quantification of burden; serial volume change as a research endpoint
CEUS	Intravascular enhancement and perfusion related to IPN	Histology correlation, symptom association, small prospective cohorts [12–13,21–22,26–30]	Protocol and threshold heterogeneity; calcified plaques may be unevaluable; limited specificity for inflammation	Perfused neovessel assessment
SMI and related microvascular flow modes	Filtered low-velocity intraplaque flow	Agreement with CEUS and histologic neovessel density; pooled diagnostic studies [14–15,31]	Vendor-specific clutter filtering and settings; signal differs from contrast kinetics	Noncontrast vascularity assessment within a defined scanner protocol
SWE and strain elastography	Stiffness or deformation	Histology studies and symptom-association meta-analysis [17–18,36–37]	Pressure, cardiac phase, calcification, geometry, and reconstruction affect estimates	Regional mechanical characterization alongside plaque morphology
Ultrafast/vector-flow methods	Flow vectors and wall-shear-related indices	Small human surgical-validation and prospective technical cohorts [18–19]	Boundary conditions, segmentation, and fitted composites	Characterization of local flow and loading
ULM	Super-resolved microvascular tracks, density, and velocity	Two small human feasibility/discrimination studies [34–35]	Acquisition and processing dependence; outcome validation unavailable	Human feasibility mapping of microvascular architecture
AI/radiomics	Segmentation, quantification, feature integration, classification	Accurate volume measurement; high internal and emerging external discrimination [10,40–41]	Patient leakage, vendor shift, and exclusion of calcified plaques	Measurement automation and research classification of plaque phenotypes

CEUS, contrast-enhanced ultrasound; IPN, intraplaque neovascularization; SMI, superb microvascular imaging; SWE, shear-wave elastography; ULM, ultrasound localization microscopy

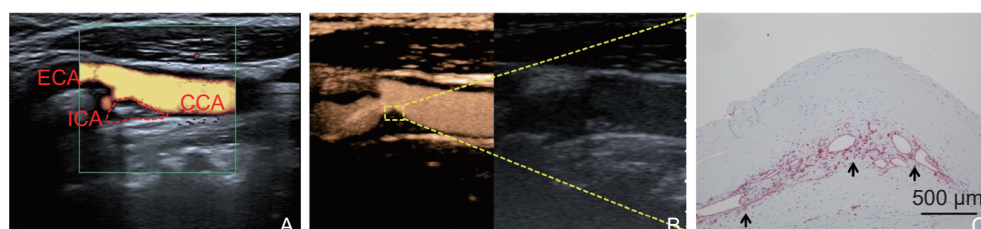


Figure 2 Contrast-enhanced ultrasound (CEUS) and histologic neovascularization in human carotid plaque. (A) Color Doppler ultrasound at the carotid bifurcation; the red dashed contour marks the plaque; (B) CEUS of the same region, with focal intraplaque enhancement outlined by the yellow box; dashed lines connect this region to the corresponding histology in panel C; (C) Anti-CD31 immunohistochemistry from the corresponding plaque region; arrows identify CD31-positive neovessels, and the scale bar is 500 μm . Reproduced from Schmidt et al. [25].

Evidence relating CEUS to future events is mixed. Small prospective cohorts associated high-grade IPN with later ischemic or cardiovascular events, with limited event counts and wide confidence intervals [26–27]. A coronary-disease cohort found no independent association [28], while a larger diabetes cohort assessed a systemic cardiovascular composite rather than ipsilateral stroke [29]. A 2025 meta-analysis predominantly classified prevalent stroke and showed modest discrimination [30].

Superb Microvascular Imaging Prospective SMI comparisons show agreement with CEUS and histologic neovessel density [14–15]. Meta-analysis found high sensitivity but lower specificity across heterogeneous implementations and reference standards [31]. A 2026

internally split nomogram combined SMI vascularity with clinical and structural variables to classify concurrent acute stroke [32]. Low- and high-grade transverse and longitudinal patterns are illustrated in figure 3 [33]. Wall filter, persistence, gain, scale, vendor processing, and motion affect the displayed signals.

Ultrasound Localization Microscopy A 26-patient ULM histology-feasibility study reconstructed maps at approximately 60 μm resolution, with tracked-bubble rates distinguishing plaques with and without histologic neovascularization [34]. Paired density and velocity maps describe the microvascular distribution (Fig. 4). A subsequent 65-patient study found more tracks in symptomatic than asymptomatic plaques using standard low-frame-rate CEUS acquisitions [35].

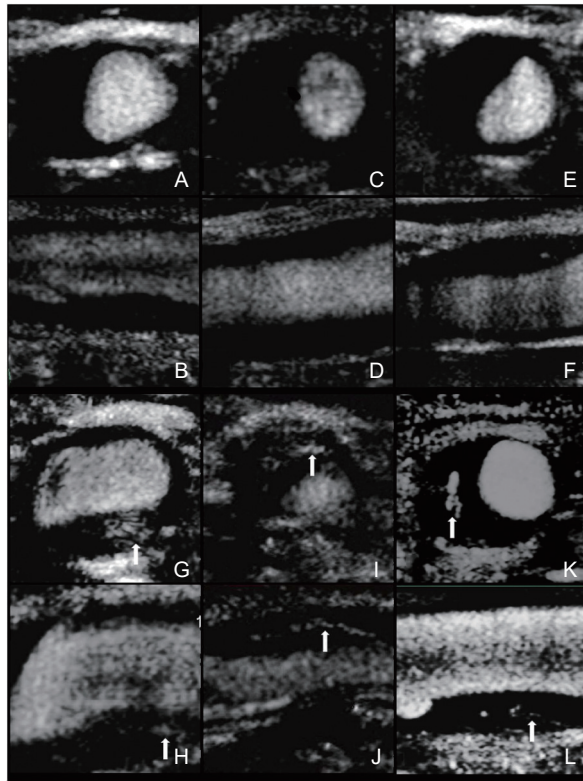


Figure 3 Standard carotid ultrasound and superb microvascular imaging (SMI) in community participants at high stroke risk. (A-F) show transverse and longitudinal views with limited intraplaque vascular signal (SMI grade 1); (G-L) show multiple short linear or strip-like signals in transverse and longitudinal views (SMI grade 2), marked by arrows in the source. Reproduced from Wang et al. [33].

ULM density and velocity maps depend on bubble concentration, recording duration, motion correction, localization and tracking rules, and the tissue area used for normalization. Arterial-wall motion, out-of-plane displacement, luminal signal contamination, and acoustic shadowing can affect the reconstructed microvasculature.

Mechanical and hemodynamic phenotyping

A meta-analysis of 14 studies and 1,781 plaques found lower mean stiffness in symptomatic plaques, with substantial methodological heterogeneity [17]. Surgical studies associate CEUS vascularity and lower stiffness with high-risk tissue features [36-37]. However, mean stiffness and wall-shear measures inconsistently separated vulnerable histology; a fitted composite performed better within a small development dataset [18]. Regional analysis may therefore be more informative than a single whole-plaque stiffness threshold.

In one endarterectomy case, a low-stiffness, hypoechoic, low-density region corresponded to gross hemorrhage, while a stiff, hyperechoic, high-density region corresponded to fibrous calcification (Fig. 5) [38]. Viscoelastic measurements also differ between plaque shoulders and echogenic subregions [39]. Whole-plaque

means can obscure these focal differences.

Spatial registration and consistent cardiac phase and probe angle allow stiffness and vascularity to be compared within the same tissue compartment. Co-registered maps can compare soft, highly perfused regions, stiff calcific regions, and heterogeneous plaque shoulders, alongside whole-plaque summaries.

Ultrafast and vector-flow methods characterize luminal loading. In asymptomatic 30%–70% stenosis, velocity-vector reconstruction distinguished straight and bending jets; bending jets were associated with more complex vortices and different wall-shear-related indices [19]. These indices describe regional flow, with uncertainty from velocity estimation, wall segmentation, pressure, and unmeasured three-dimensional geometry.

Multiparametric integration and artificial intelligence

Artificial intelligence (AI) supports segmentation, registration, and quantitative analysis. Semiautomated analysis improves 3D plaque-volume measurement efficiency [10], while motion correction and longitudinal matching can reduce acquisition-related variability. Radiomics, combined B-mode/CEUS, and longitudinal multimodal models have reported high classification performance [40-43]. Their labels include prior symptoms, vulnerability categories, and short-term treatment response, which represent different clinical questions.

Bilateral plaques and serial scans from one patient can leak information between training and test sets. Scanner-dependent beamforming, texture, and contrast processing affect transfer between vendors. Excluding calcified plaques, failed segmentations, or missing modalities narrows the population in which reported performance applies.

Multiparametric studies suggest that vascular and mechanical measurements capture related but nonidentical features. A retrospective short-term cohort found an IPN–stiffness association in mild asymptomatic stenosis [44]. CEUS identified vascular heterogeneity associated with symptoms within Plaque-RADS 3 [45]. Another single-center cohort combined morphology, CEUS, and elastographic hardness against one-year stroke status, although laterality and modeling details remained unclear [20]. These studies have not tested whether acting on a combined score improves care.

Molecular Ultrasound Imaging

Target selection across vascular and intraplaque compartments

Molecular ultrasound detects selected targets through binding or accumulation of an acoustic agent. Conven-

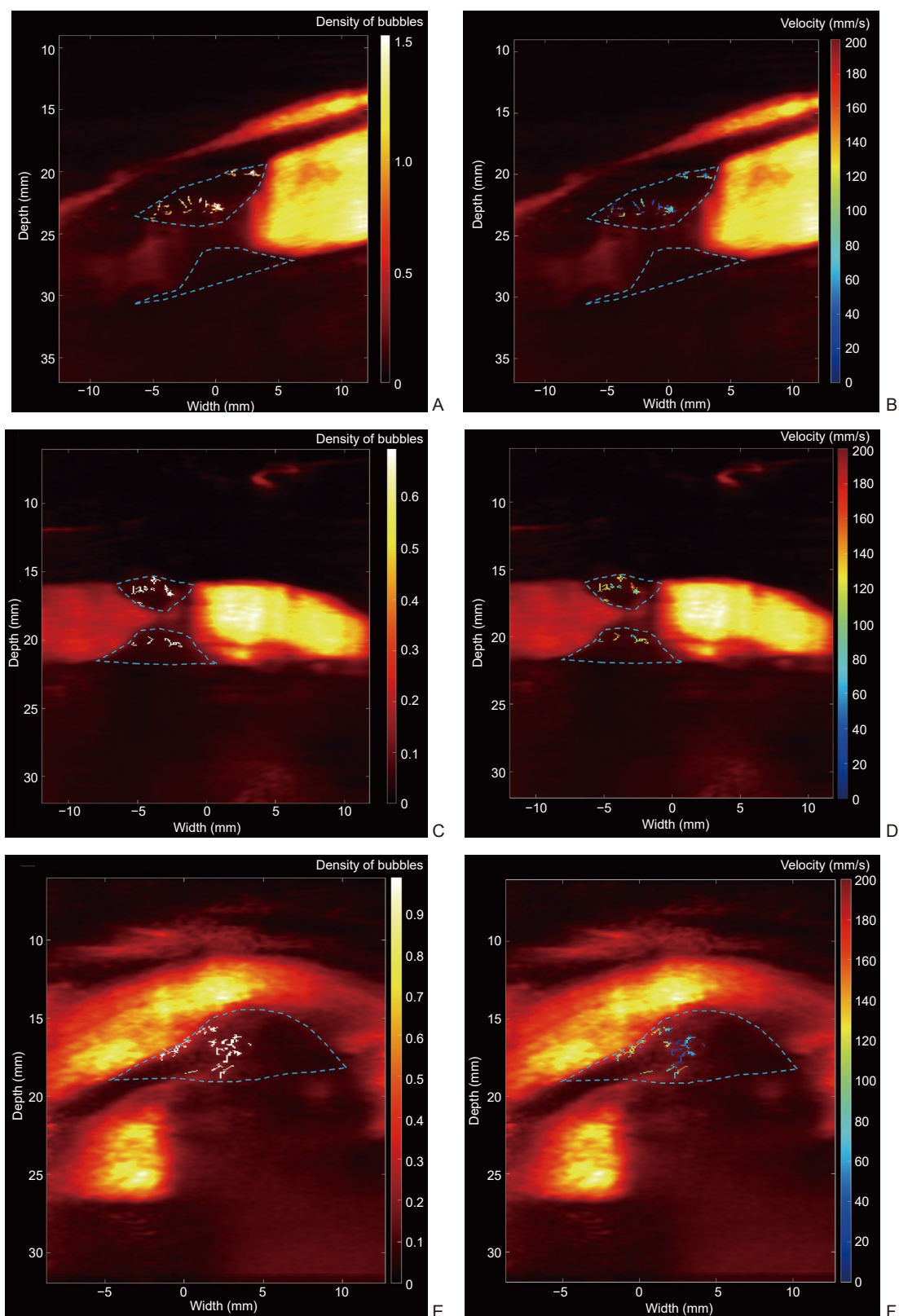


Figure 4 Ultrasound localization microscopy (ULM) density and velocity maps in three patients with histologically detected carotid plaque neovessels. (A, C, and E) show localized-bubble density; (B, D, and F) show the corresponding tracked-bubble velocity. Blue dashed contours are the source study's plaque segmentations. Adapted from Leroy et al. [34].

tional microbubbles remain intravascular. Accessible targets include endothelial vascular cell adhesion molecule-1 (VCAM-1) and P-selectin, surface-associat-

ed von Willebrand factor (vWF), activated platelet epitopes, and receptors within neovessel lumens. Detectability depends on expression above background

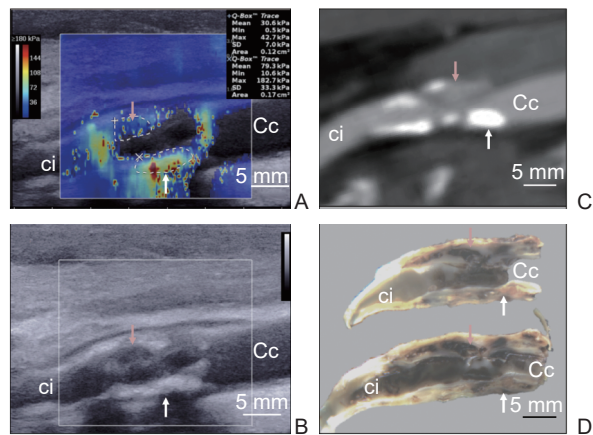


Figure 5 Multiparametric assessment of the same human carotid plaque. (A) Shear-wave elastography, (B) B-mode ultrasound, (C) computed tomography angiography, and (D) gross endarterectomy specimen in longitudinal view. In the source, the red arrow marks a hemorrhagic region corresponding to low stiffness, low echogenicity, and low computed-tomography density; the white arrow marks a fibrous calcified region corresponding to high stiffness, high echogenicity, and high density. Ci, internal carotid artery; Cc, common carotid artery. Reproduced from Goudot et al. [38].

and retention under arterial shear. Experimental nanoscale probes extend targeting into plaque tissue. Table 2 relates these targets to candidate interventions and response measures.

VCAM-1-targeted microbubbles attached under arterial flow and produced increasing signal with experimental plaque burden in hypercholesterolemic mice [46]. Dual targeting of P-selectin and VCAM-1 detected an atherogenic endothelial phenotype before obstructive

lesions and generated progressively greater signal with disease duration [47]. Human endarterectomy tissue confirms target expression at the plaque–blood interface and binding under carotid-like flow, but symptomatic and asymptomatic expression overlaps [48]. A separate study identified VCAM-1 and vWF on human plaque surfaces while demonstrating target-specific imaging in atherosclerotic mice [49].

Platelet and vWF targeting could identify a different stage of injury. In mice, a vWF-A1-based agent detected platelet adhesion that increased with lesion severity [50]. Experimental targeting of activated platelet glycoprotein IIb/IIIa likewise connected imaging signal with platelet-rich plaque, but its reported therapeutic effect varied with acoustic setting [51]. Angiogenic targets offer a route to neovessel imaging, yet deep intraplaque vessels may be less accessible to circulating agents than luminal endothelium.

Target expression varies with the biological process and timing. VCAM-1 can persist during inflammatory recruitment, whereas P-selectin is rapidly mobilized on activated endothelium and is also expressed by activated platelets. Exposed vWF A1 domains support platelet adhesion at an activated or injured surface; activated glycoprotein IIb/IIIa reflects platelet aggregation. Symptom-to-scan time and antiplatelet exposure are consequently relevant to interpreting these signals.

Nanoscale carriers may enter plaque through permeable luminal endothelium or leaky neovessels. In rabbit

Table 2 Evidence linking plaque imaging, treatment, and response assessment

Research area	Target or treatment	Measurements	Evidence	Unresolved clinical question
Endothelial inflammation	Luminal VCAM-1 and P-selectin; experimental targeted microbubbles	Targeted molecular signal, target expression, and ex vivo agent binding	Animal molecular imaging and treatment-response studies [46-47,56-57,60]; human ex vivo target-expression and binding studies [48,57]	Can a clinical-grade agent measure carotid target engagement in vivo and inform treatment?
Platelet adhesion	Accessible vWF and activated platelet epitopes; experimental targeted microbubbles	Platelet/vWF-associated imaging signal and experimental treatment effects	Human ex vivo plaque target expression [49]; animal imaging and treatment experiments [49-51]	How do timing and antiplatelet exposure affect signal interpretation and its relation to ipsilateral events?
Plaque-directed delivery	UTMD-assisted gene, antibody, siRNA, or drug delivery; EDB-fibronectin and other experimental targets	Payload delivery, target modulation, CEUS or stiffness, and histology	Animal delivery and imaging studies [54,62-66]	What human dose, biodistribution, and repeat-treatment safety permit carotid application?
Sonodynamic therapy	Sensitizers and ultrasound in vascular or macrophage-rich experimental plaque	Vascular, inflammatory, and matrix changes; early clinical imaging and functional outcomes	Animal studies [67-74]; femoropopliteal randomized trial with reduced plaque inflammation and improved walking performance [75]; mixed carotid/femoral pilots with imaging responses [76-77]; completed carotid study with no posted registry results [78]	Can carotid target engagement translate into durable benefit with acceptable neurological safety?
Treatment-response monitoring	Lipid-lowering or antithrombotic therapy	CEUS/SMI vascularity, plaque volume, and other ultrasound measurements	Human studies with treatment-related changes and neutral findings [80-82,84]	Do imaging changes predict a reduction in stroke?

CEUS, contrast-enhanced ultrasound; EDB, extra-domain B; siRNA, small interfering RNA; SMI, superb microvascular imaging; UTMD, ultrasound-targeted microbubble destruction; VCAM-1, vascular cell adhesion molecule-1; vWF, von Willebrand factor. Human ex vivo evidence refers to excised plaque studies. Imaging responses and peripheral functional outcomes are distinct from carotid stroke-prevention outcomes

atherosclerosis and an endothelialized microfluidic model, lipid–polymer nanoparticle translocation depended on vascular permeability [52]. Plaque accumulation reflects both endothelial passage and subsequent retention, influenced by particle size and surface properties.

Within plaque, inflammatory cells, lipid deposits, and extracellular matrix provide additional targets. Apolipoprotein A1-mimetic peptide nanoemulsions bound oxidized low-density lipoprotein (oxLDL), entered cultured foam cells, and accumulated in perfused mouse aortic plaques *ex vivo* [53]. Extra-domain B fibronectin (EDB-FN)-targeted biosynthetic gas vesicles have been investigated for ultrasound-guided small interfering RNA (siRNA) delivery in experimental plaques [54]. Matrix binding provides a retention mechanism distinct from intracellular uptake.

An activatable probe detected heterogeneous matrix metalloproteinase (MMP) activity in excised human carotid plaques using multispectral optoacoustic tomography, with histologic and zymographic comparison [55]. Optoacoustic imaging uses optical excitation and acoustic detection, whereas pulse-echo molecular ultrasound uses acoustic excitation and detection.

Vascular binding, tissue access, and agent design

Microbubble imaging depends on wall contact, adhesion under pulsatile shear, and clearance of unbound contrast. Leukocyte-mimetic constructs improved binding and detected lower signal after atorvastatin treatment in mice [56]. VCAM-1 nanobody agents showed flow-resistant binding and animal lesion signal, followed by target-dependent signal in an *ex vivo* human plaque circuit [57].

Micrometer-scale bubbles produce strong nonlinear scattering but contact the wall infrequently in fast arterial flow. Ligand density and multivalency improve attachment while also affecting shell mechanics, complement interactions, and storage stability. Slow ligand dissociation can prolong nontarget background. Peptides are compact and synthetically tractable; antibodies add size and potential immunogenicity. Nanobodies offer small size and defined specificity, with conjugation control and toxicology specific to each product.

Tissue-directed probes must cross the vascular barrier and remain acoustically detectable after diffusion, binding, or cellular uptake. Phase-change droplets are small during delivery and generate gas after activation; gas vesicles are preformed protein-based acoustic structures [53–54]. Microscopy localizes the carrier, while ultrasound measures its acoustic response.

Measured signal includes circulating agent, nonspecific retention, and target-mediated adhesion. Destruction–replenishment imaging estimates retained contrast

after controlled bubble destruction and replenishment. Blocking experiments, nontargeted agents, receptor-deficient models, and histology help distinguish target-specific binding from perfusion and background.

Blood-pool normalization reduces delivered-dose variation but remains sensitive to depth, attenuation, and nonlinear response. Retained intensity, targeted-to-control ratios, and destruction–replenishment differences measure related aspects of binding and perfusion; injection timing affects their interpretation.

Red-cell interactions, vessel diameter, bubble size, pulsatility, and bifurcation geometry affect near-wall transport and contact time. Pulsatile and oscillatory flow altered microbubble-mediated endothelial permeabilization under controlled insonation *in vitro* [58]. Arterial-waveform flow chambers can therefore characterize agent–wall interactions that static binding assays miss.

Preclinical molecular imaging

Targeted signal can precede severe luminal disease [47] and respond to treatment in animal models. Antioxidant treatment produced dose-dependent signal reductions alongside histologic changes [59]. Anti-interleukin-1 β treatment abolished post-myocardial-infarction increases in targeted signal and adverse plaque histology in mice [60]. Multitarget probes can improve capture across variable receptor expression, but their combined signal is less attributable to one receptor [56].

The cRGD-microbubble mouse study combined targeted and control agents, receptor blocking, background subtraction, pathology, and lesion-level associations to assess signal specificity (Fig. 6) [51].

Peptide nanoemulsions bound oxLDL and entered foam cells; their retention was tested in perfused mouse aortas *ex vivo*. Acoustic detection used cell-containing phantoms and model deposits beside *ex vivo* porcine coronary arteries [53]. Matrix-targeted gas vesicles have also been investigated for ultrasound-guided siRNA delivery in experimental plaques [54].

Human carotid plaques are deeper and often more calcified than superficial murine aortic lesions, complicating detection against circulating background. Animal models reproduce selected effects of diet, diabetes, renal disease, or disturbed flow, but rarely spontaneous embolic rupture.

Path to first-in-human carotid imaging

Human plaque studies demonstrate surface target expression and *ex vivo* agent binding [48–49,57], but clinical *in vivo* carotid molecular ultrasound remains unreported.

Outside atherosclerosis, a clinical-grade vascular endothelial growth factor receptor-2 (VEGFR2)-target-

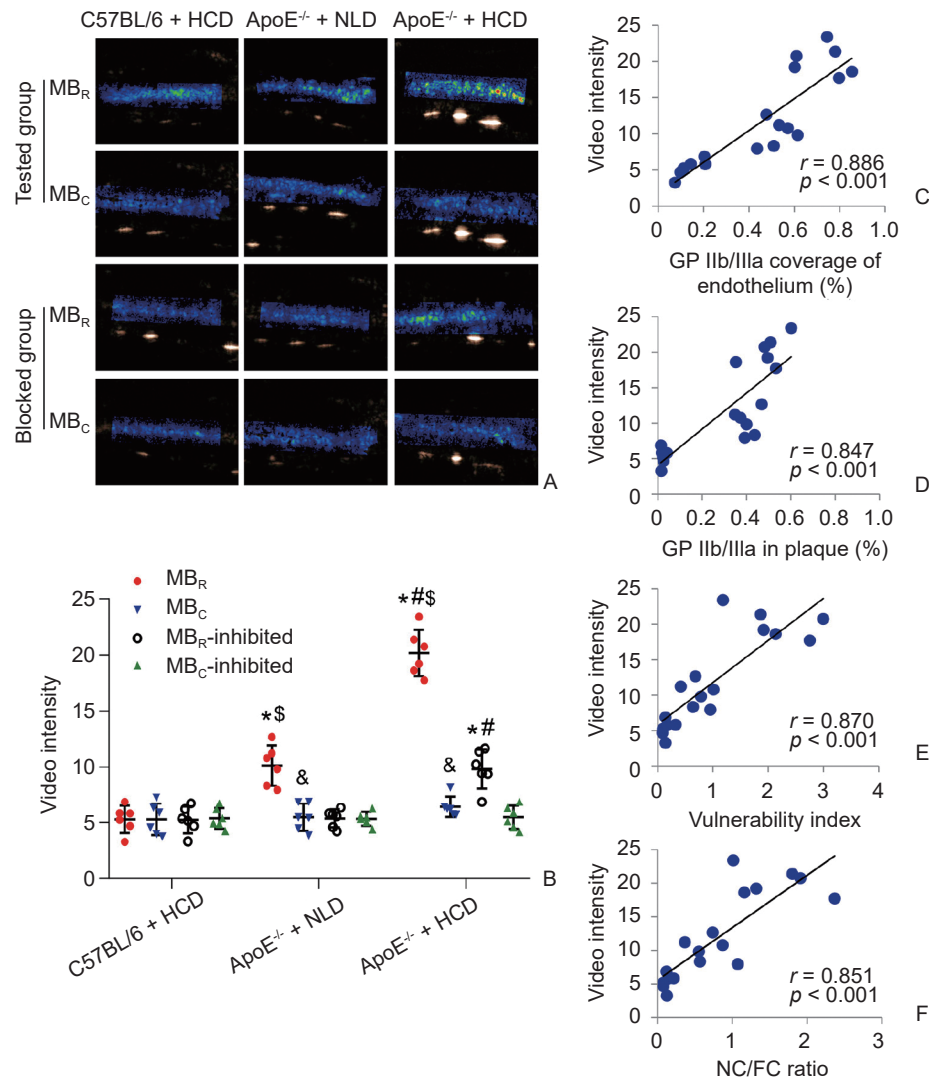


Figure 6 Experimental molecular ultrasound with cyclic Arg-Gly-Asp-modified microbubbles (MBR) in mice. (A) Background-subtracted color-coded signal after MBR or control microbubbles (MBC) in C57BL/6 mice and ApoE^{-/-} mice receiving a normal or high-cholesterol diet, with separate receptor-blocked groups; (B) Quantified video intensity ($n = 6$ per group); (C-F) Associations between MBR video intensity and endothelial or plaque glycoprotein IIb/IIIa, plaque vulnerability index, and necrotic-core/fibrous-cap ratio. Reproduced from Guo et al. [51].

ed microbubble was administered to 45 women with breast or ovarian lesions; 40 contributed to imaging analysis [61]. Signal broadly agreed with surgical immunohistology, and the agent was tolerated under protocol monitoring.

Initial carotid studies can establish dose, clearance, repeatability, and correspondence with endarterectomy tissue before evaluating performance in nonsurgical populations.

Ultrasound-Mediated and Ultrasound-Activated Plaque Modulation

Acoustic mechanisms and dose regimes

Therapeutic ultrasound acts through several mechanisms. Sustained microbubble oscillation can increase permeability, whereas inertial cavitation produces

stronger mechanical effects and greater injury potential. Radiation force changes bubble-wall contact; thermal exposure heats tissue; SDT activates a sensitizer. The dominant effect depends on the acoustic field, pulse regimen, agent, and local geometry.

The transition from stable to inertial cavitation depends on bubble size, shell, flow, confinement, and local pressure. Passive cavitation monitoring detects spectral patterns of sustained oscillation and broadband activity associated with inertial behavior [7]. Interpretation depends on detector calibration and geometry. Exposure feedback developed in other therapeutic settings has not yet established a carotid safety window.

Radiation force can move bubbles toward the wall before a permeabilizing or destructive pulse. Sonoporation ranges from transient, repairable permeability to lethal membrane injury. Under a controlled 1-MHz pro-

tocol, endothelial shear preconditioning and pulsatile bubble flow changed permeabilization [58]. Non-reversing pulsatile flow increased it by up to 2.4-fold relative to shear-matched laminar flow in vitro.

Comparing regimens requires frequency, calibrated lesion pressure, pulse duration and repetition, duty cycle, total exposure, beam dimensions, agent formulation and concentration, and injection timing. Temperature and tissue injury measurements help interpret the biological dose. Comparable plaque-specific dose-response data remain sparse [7–8].

Preclinical UTMD and targeted delivery

Ultrasound-targeted microbubble destruction (UTMD) uses acoustically responsive carriers to concentrate or release a payload and transiently alter vascular permeability. In experimental rabbit plaque, UTMD-mediated gene modulation changed stiffness and CEUS enhancement alongside histologic markers of stability [62]. Repeated UTMD delivery of an interleukin-8-directed construct reduced contrast intensity, microvessel density, and macrophage counts without reducing two-dimensional plaque thickness [63]. These findings suggest that plaque composition can change without a measurable change in thickness.

An EDB-FN-targeted gas-vesicle platform combined ultrasound imaging with siRNA delivery and favorable animal histology [54].

Payload location determines how ultrasound assists delivery. Cavitation can facilitate free-drug uptake, release shell-bound payload, or support delivery from co-

administered nanoparticles. Shell loading couples release to the bubble but limits payload mass and may allow premature release. Nanoparticles provide additional loading and targeting options.

RNA delivery can alter cell phenotype after a brief exposure. In mice, miR-145-loaded cationic microbubbles with UTMD increased vascular delivery and promoted a contractile smooth-muscle phenotype [64]. Plaque area was 48.04% lower than with free miR-145. Platelet-membrane-mimetic rapamycin nanoparticles combined with microbubble destruction increased plaque localization and improved histologic stability measures in animals (Fig. 7) [65]. Ginsenoside Rb3-loaded microbubbles delivered an antioxidant payload to mouse aortic plaque, reducing oxidative and foam-cell endpoints [66].

Delivery to luminal endothelium can use modest, reversible permeability changes. Reaching deeper smooth-muscle cells or macrophages may need a smaller secondary carrier, microvascular access, or greater permeabilization, with associated risks to endothelium and cap integrity. Component controls in the platelet-membrane-mimetic nanoparticle study separated the contributions of rapamycin, microbubbles, and insonation (Fig. 7) [65].

Preclinical sonodynamic modulation of macrophages, matrix, and neovessels

In a randomized dose study of experimentally induced rabbit femoral plaques, 5-aminolevulinic-acid SDT at an optimized intensity reduced provoked plaque

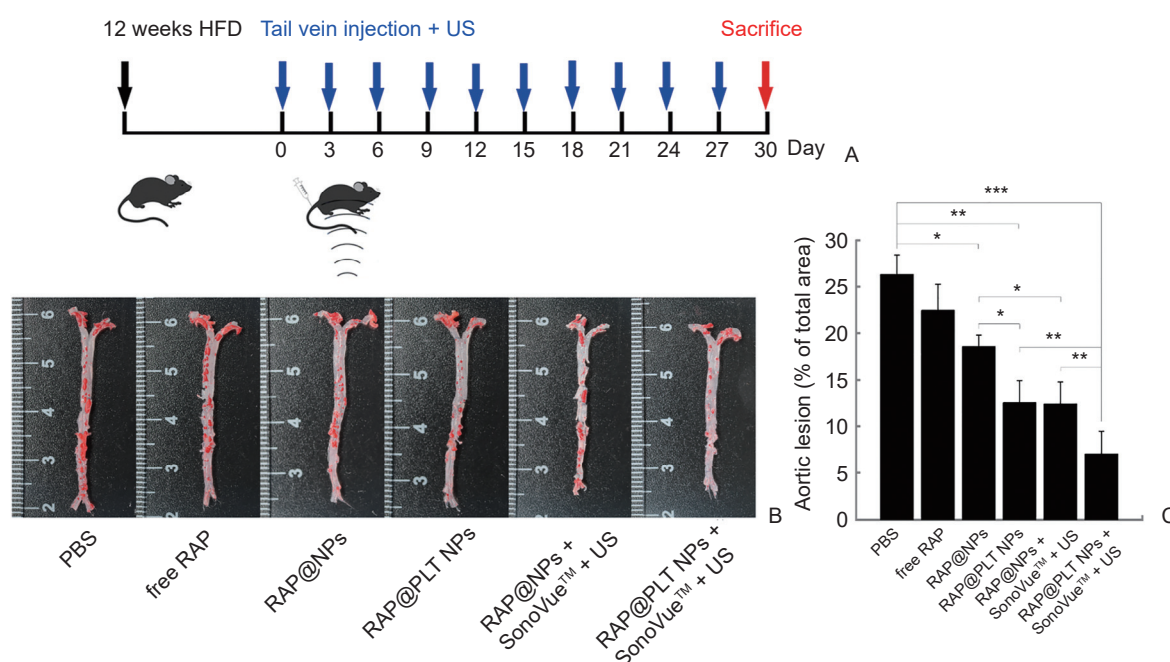


Figure 7 Plaque-directed delivery using platelet-membrane biomimetic rapamycin nanoparticles combined with ultrasound-targeted microbubble destruction (UTMD) in ApoE^{-/-} mice. (A) Thirty-day treatment schedule; (B) En face Oil Red O-stained aortas; (C) Quantified aortic plaque area. Reproduced from Zhou et al. [65].

disruption by 88% at seven days [67]. Macrophage and lipid areas fell by 94% and 60%, respectively, while collagen increased by 127%.

SDT reduced adverse iron retention through macrophage oxidative signaling and iron export in hemorrhagic plaque [68]. Other experimental work reported more collagen and smooth muscle, less macrophage burden, and altered matrix-metalloproteinase activity [69]. Angiogenesis-directed platforms have induced neovessel endothelial apoptosis and reduced experimental plaque hemorrhage and inflammation [70]. A flexible, parameter-adjustable ultrasound patch has been paired with targeted delivery in a preclinical carotid model [71].

Sonosensitizer activity depends on cellular uptake, subcellular localization, and oxygen availability. Plaque hypoxia can limit reactive oxygen species (ROS) generation. An experimental macrophage-membrane-coated manganese-dioxide/chlorin-e6 platform used plaque hydrogen peroxide to generate oxygen and imaging-active manganese, increasing sonodynamic killing of activated macrophages [72]. Its degradation products and metal exposure also affect pharmacology.

SDT can alter cell signaling without causing cell death. Non-lethal exposure changed foam-cell exosomal cargo in ApoE-deficient mice and affected endothelial

cholesterol efflux and extracellular-matrix regulation [73]. Another mouse platform combined an aggregation-induced-emission sonosensitizer with osteopontin targeting and dexamethasone release, modulating macrophage polarization and efferocytosis [74]. These studies extend the experimental effects of SDT to immunometabolic regulation.

Neovessel ablation may reduce hemorrhage and inflammatory entry, but excessive endothelial injury can promote thrombosis or impair repair. Similarly, collagen deposition may strengthen the cap or alter its mechanics through disordered fibrosis. Spatial histology and biomechanical measurements help interpret changes in CEUS perfusion after treatment.

A VEGFR2-targeted nanoplateform combined magnetic resonance, photoacoustic, and ultrasound imaging with SDT in rabbit femoral plaques [70]. This formulation-level theranostic study assessed neovascularization, hemorrhage, inflammation, lipid, collagen, and smooth muscle together (Fig. 8).

Early clinical evidence for SDT

Randomized Femoropopliteal Trial A double-blind, randomized, sham-controlled phase 2 study in 32 people with femoropopliteal peripheral artery disease reported a treatment-control difference of -0.53 in

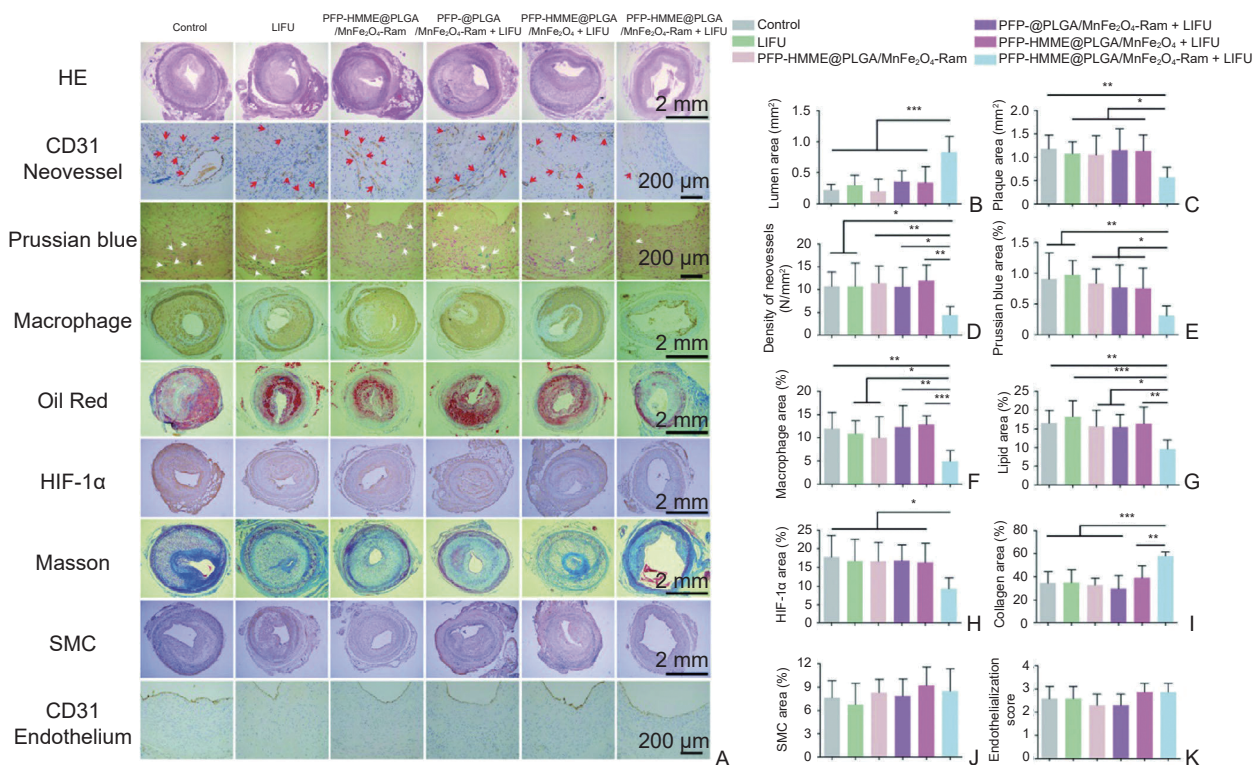


Figure 8 Low-intensity focused-ultrasound-responsive nanoplateform for sonodynamic modulation of advanced rabbit femoral plaque. (A) Representative histopathology 28 days after treatment, including hematoxylin and eosin, CD31, Prussian blue, macrophage, Oil Red O, HIF-1α, Masson, smooth-muscle-cell, and endothelial staining. Red arrows in the source mark abnormal adventitial neovessels and white arrows mark intraplaque hemorrhage; (B-K) Quantification ($n = 7$; mean $\pm$ SD; analysis of variance with Dunnett post hoc comparisons as defined in the source). Reproduced from Yao et al. [70].

positron-emission-tomography target-to-background ratio and an improvement of 118.6 seconds in peak walking time [75].

Carotid and Mixed-Territory Studies Sun et al. combined rabbit and mouse experiments with a pilot clinical comparison in 16 patients with femoral or carotid atherosclerotic lesions [76]. Eight received atorvastatin alone (19 lesions); eight received atorvastatin plus 5-aminolevulinic-acid SDT (40 lesions). At four weeks, diameter stenosis decreased in the combination group but not with atorvastatin alone. Follow-up at 24–40 weeks included only six patients: two receiving atorvastatin with seven lesions and four receiving combination therapy with 22 lesions. Stenosis continued to decline in combination-treated lesions, with no significant change in atorvastatin-only lesions. A separate single-arm pilot in 24 patients with carotid or femoral disease reported a four-week reduction in mean stenosis from 57.2% to 50.1% [77]. This overall estimate describes a mixed-territory imaging response, and its uncontrolled design limits attribution to SDT.

The SMART-C phase 1 carotid study (NCT03871725) is registered as completed, with 12 participants in an open-label, single-group design. Magnetic-resonance maximum wall thickness was the primary response measure and CEUS enhancement a secondary measure; no summary results were posted in the registry as of 28 September 2026 [78].

These carotid and mixed-territory studies provide early imaging-response data; clinical benefit in carotid disease requires evaluation against carotid-specific outcomes.

Safety considerations and negative evidence

In diabetic atherosclerotic mice, mild targeted cavitation left plaque size and inflammation unchanged at 4 weeks but transiently increased inflammatory monocytes [79].

Embolization and arterial-wall injury dominate carotid safety assessment. Diffusion-weighted brain MRI, transcranial Doppler, and neurological examination assess ischemic lesions, microembolic signals, and clinical deficits. Timing relative to insonation helps distinguish treatment-associated injury from background risk. Small cohorts cannot resolve uncommon events, particularly in asymptomatic disease.

A neutral result can arise from inadequate delivery or an ineffective target. Cavitation recordings, tissue drug measurements, and histology help distinguish these explanations before acoustic exposure is increased; a higher dose can add injury without improving delivery.

Repeated exposure raises questions about carrier accumulation, immune clearance, and delayed tissue

responses. Sensitizers may also cause systemic toxicity without acoustic activation. Follow-up should cover delayed arterial injury, nearby neck structures, and systemic effects.

Linking Plaque Imaging to Treatment and Response Assessment

Linking plaque phenotype to treatment

Serial carotid imaging measures within-patient plaque changes during lipid-lowering treatment [80–82]. Human plaque studies establish target expression and ex vivo binding, while treatment-related molecular imaging changes have mainly been demonstrated in animals [48–49,56–57,60]. Table 2 summarizes the evidence for these applications.

Luminal markers of endothelial inflammation and platelet adhesion are accessible to circulating microbubbles [46–51,56–57,60]. Targeted probes have measured molecular responses to treatment in animals [56,60]. High perfusion or platelet-associated signal alone does not identify which therapy a patient should receive.

A preclinical intravascular ultrasound (IVUS)-guided system paired osteopontin-targeted piezoelectric nanoparticles with a catheter that provided imaging and delivered pulses to activate SDT [83]. This agent–device theranostic approach requires intravascular access.

Response assessment and surrogate validation

Serial ultrasound detects changes during conventional therapy. A systematic review associated lipid lowering with IPN regression in several small studies with heterogeneous acquisition and analysis [80]. In a randomized placebo-controlled study of 82 patients, atorvastatin reduced SMI-detected IPN prevalence from 69.23% to 48.72%; CEUS changes were smaller [81]. In a prospective single-arm study, adding evolocumab to statin therapy reduced plaque-to-lumen contrast-curve area from 0.27 to 0.19 [82]. Low-density-lipoprotein cholesterol fell by approximately 57%.

In contrast, a double-blind randomized study found no change in grayscale median, plaque volume, or IPN over 12 months with added low-dose rivaroxaban [84]. Both groups received aspirin and statin therapy.

A treatment-related fall in enhancement can reflect fewer perfused vessels or altered flow, as well as differences in contrast delivery and analysis. Standardized serial measurements are therefore candidate response markers. Establishing them as stroke surrogates would require evidence that marker changes capture treatment effects on stroke outcomes; that relationship remains unproven for enhancement, volume, or stiffness.

Serial CEUS in the evolocumab study illustrates a perfusion change despite little visible change on B-mode imaging (Fig. 9) [82].

Clinical Translation and Standardization

Acquisition and measurement harmonization

Multicenter comparison depends on consistent acquisition and analysis. For CEUS, contrast administration, acoustic settings, timing, motion correction, and reference selection affect quantification. SMI varies with clutter filtering and proprietary processing; elastography with probe contact, blood pressure, cardiac phase, geometry, and inversion. ULM additionally depends on localization and tracking settings.

Repeat scans quantify acquisition variability, blinded rereads assess interpretation, and phantoms characterize system differences. Retaining raw data and software versions allows measurement changes to be traced across sites and visits.

Phantom or volunteer scans support site qualification and core-laboratory review. Reporting technical failures alongside evaluable scans clarifies the population covered by an imaging result. Plaque-RADS provides morphological definitions, and CEUS guidance addresses acquisition and safety [3,85].

Endpoint and trial design

Histology studies test tissue correspondence, prospective cohorts test prediction beyond symptoms

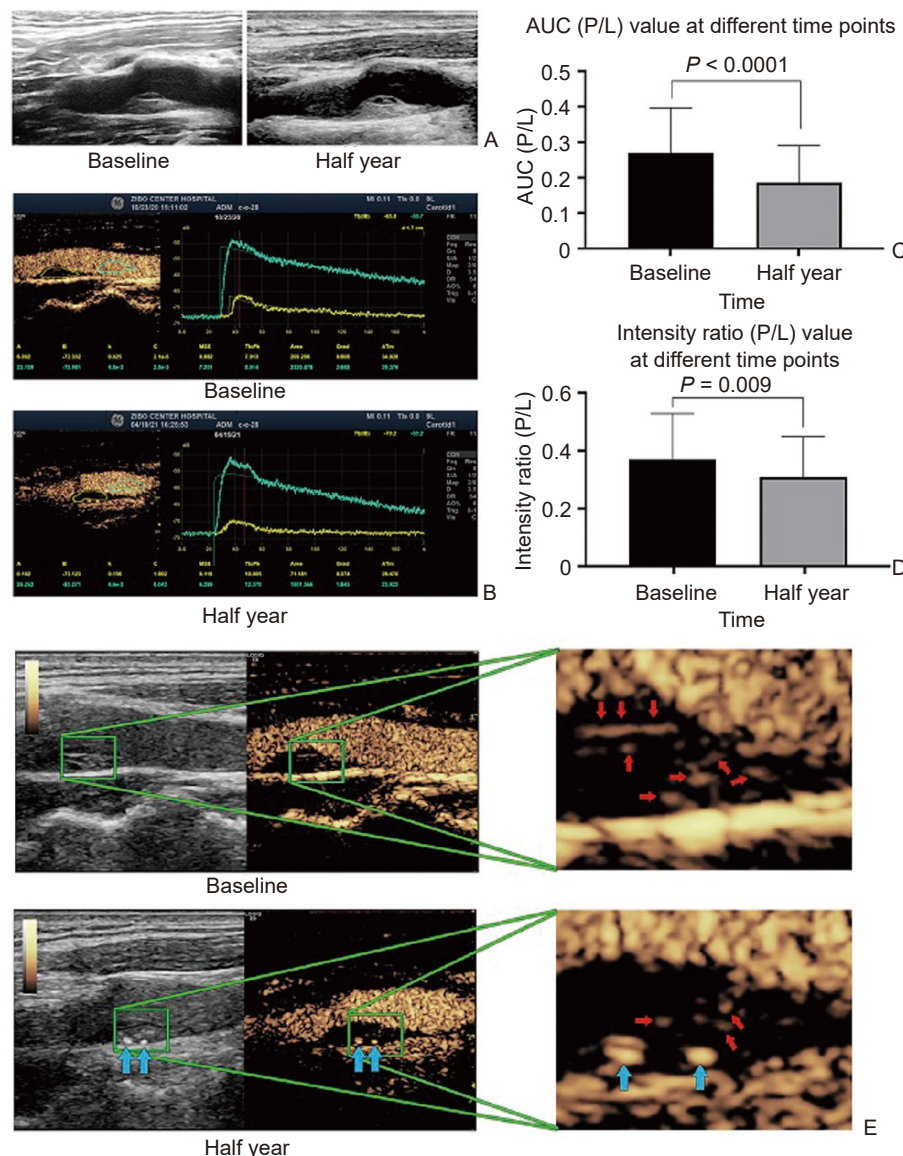


Figure 9 Serial carotid ultrasound during lipid-lowering therapy in a prospective single-arm study. (A) Representative B-mode images at baseline and 6 months show little visible change in plaque morphology; (B) CEUS images and time-intensity curves from the same representative patient; (C, D) Cohort-level reductions in plaque-to-lumen area under the curve and enhanced-intensity ratios at 6 months; (E) Magnified plaque regions in the representative CEUS images; red arrows mark intraplaque vessels and blue arrows mark calcification in the source. Reproduced from Chen et al. [82].

and stenosis, and randomized strategy trials test whether imaging-guided care improves outcomes. Co-registration and a defined imaging-to-surgery interval strengthen tissue comparisons. Representative nonsurgical cohorts are needed to assess performance beyond the endarterectomy population.

Ipsilateral stroke is the principal disease-specific outcome for carotid interventions. Early trials can assess target engagement and biological response; longer follow-up with adjudicated events tests clinical benefit.

Bilateral plaques and repeated scans share patient factors and treatment, so plaque-level analyses need to account for clustering. A defined index lesion, laterality, competing embolic sources, and interval therapy are central to attributing stroke to the imaged plaque.

The clinical question determines the trial design: surveillance, treatment selection, and response-guided management involve different actions [5]. Imaging-guided revascularization requires a strategy trial with contemporary event rates and independently adjudicated outcomes. Local acoustic treatment additionally requires neurological and embolic monitoring.

Standardization, manufacturing, and regulation

Agent composition, sterility, stability, binding potency, and calibrated acoustic exposure determine the delivered dose. Product changes therefore need comparability testing. Diagnostic CEUS safety experience [85] does not cover new ligands, sensitizers, or therapeutic cavitation regimens.

A targeted agent with a prescribed ultrasound exposure may be regulated as a drug–device or biologic–device combination, depending on its configuration and primary mode of action. FDA guidance addresses constituent interactions, nonclinical safety, manufacturing changes, and the proposed use [86–87].

Conclusions

Ultrasound adds measurements of plaque vascularity and mechanics to established assessment of stenosis and symptoms. CEUS has the strongest human evidence for microvascular plaque characterization; SMI, elastography, and ULM provide complementary information. Molecular ultrasound has demonstrated target-specific imaging in animals, supported by target-expression and binding studies in human excised plaques.

Ultrasound-targeted delivery remains preclinical. SDT has progressed from animal studies to early human investigations, with reduced plaque inflammation and improved walking performance in a femoropopliteal randomized trial and imaging responses in carotid or mixed-territory pilots. A reduction in carotid-related stroke has

yet to be demonstrated.

Serial ultrasound measures plaque changes during conventional therapy. Prospective studies can test whether these measurements improve treatment selection and predict treatment effects on stroke outcomes.

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Authors' Contributions

Yang Sun and Wang Luo reviewed the literature and drafted the manuscript. Haitao Ran supervised the work and critically revised the manuscript. Qunxia Zhang, Jianli Ren, and Zhigang Wang contributed to literature organization and manuscript revision.

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Data Availability

Data sharing is not applicable to this article, as no new datasets were generated or analyzed.

Declarations

Ethics approval and consent to participate

Not applicable.

Conflict of interest

The authors have no conflict of interest to declare.

Consent for publication

Not applicable.

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