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Quantifying Effect of Intraplaque Hemorrhage on Critical Plaque Wall Stress in Human Atherosclerotic Plaques Using Three-Dimensional Fluid-Structure Interaction Models

Recent magnetic resonance studies have indicated that intraplaque hemorrhage (IPH) may accelerate plaque progression and play an important role in plaque destabilization. However, the impact of hemorrhage on critical plaque wall stress (CPWS) and strain (CPWSn) has yet to be determined. The objective of this study was to assess the effect of the presence and size of IPH on wall mechanics. The magnetic resonance image (MRI) of one patient with histology-confirmed IPH was used to build eight 3D fluid-structure interaction (FSI) models by altering the dimensions of the existing IPH. As a secondary end point, the combined effect of IPH and fibrous cap thickness (FCT) was assessed. A volume curve fitting method (VCFM) was applied to generate a mesh that would guarantee numerical convergence. Plaque wall stress (PWS), strain (PWSn), and flow shear stress (FSS) were extracted from all nodal points on the lumen surface for analysis. Keeping other conditions unchanged, the presence of intraplaque hemorrhage caused a significant increase (27.5%) in CPWS; reduced FCT caused an increase of 22.6% of CPWS. Similar results were found for CPWSn. Furthermore, combination of IPH presence, reduced FCT, and increased IPH volume caused an 85% and 75% increase in CPWS and CPWSn, respectively. These results show that intraplaque hemorrhage has considerable impact on plaque stress and strain conditions and accurate quantification of IPH could lead to more accurate assessment of plaque vulnerability. Large-scale studies are needed to further validate our findings. [DOI: 10.1115/1.4007954]

Keywords: intraplaque hemorrhage, fluid-structure interactions, magnetic resonance imaging, plaque wall stress, atherosclerosis, vulnerability

1 Introduction

Acute cardiovascular syndromes are often associated with the rupture of atherosclerotic plaque and may occur without any warning [1–3]. Recently, magnetic resonance imaging techniques have been shown to be able to noninvasively characterize plaque morphology and composition [4–6]. Vulnerable plaques often have a large necrotic core covered by a thin fibrous cap and often contain intraplaque hemorrhage, calcification, and inflammation [2,3,7]. However, clinical diagnosis is still mainly based on the degree of luminal stenosis. Nevertheless, there is growing evidence suggesting that luminal stenosis alone is probably not the best criterion for stratification of disease severity [5,8–11]. It was reported that in the European Carotid Surgery Trial, 43.8% of the 3018 trial participants with symptomatic carotid disease had < 30% stenosis [10]; in the North American Symptomatic Carotid Endarterectomy Trial (NASCET), 61% of the 2226 recently symptomatic subjects had less than 50% carotid stenosis [8]. Based on these substantial observations, additional indicators need to be sought for better plaque vulnerability assessment.

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From the mechanical point of view, plaque rupture is likely to occur when the mechanical stress exceeds the material strength of fibrous cap. Therefore, mechanical factors have been considered as potential indicators for plaque vulnerability. MRI- and histology-based computational simulations have been proposed to perform mechanical analysis. Many interesting and significant results have been reported. Richardson et al. found out that high circumferential stress correlated well with the site of intimal tears using 2D finite element analysis based on the geometric information obtained from histology [12]. Since then, several groups have further investigated the relationship between stress concentration and plaque vulnerability based on 2D models [13–17]. To obtain more accurate mechanical data, 3D computational models have been introduced recently. Holzapfel et al. introduced a multilayer 3D anisotropic model for the simulation of balloon angioplasty using *in vitro* MRI [18]. Their results indicated that different layers and tissue properties displayed different mechanical behaviors, which are crucial for realistic computational models and accurate stress predictions. Tang et al. introduced the first 3D multicomponent fluid structure interactions model for image-based plaque mechanical analysis [19]. It was observed that stress concentration occurred at thin fibrous cap and large lipid-rich necrotic core correlated well with the instability of plaque. Since then, mechanical analysis by FSI model has gained popularity for providing more complete results. Several groups obtained very interesting and valuable results for the assessment of plaque

vulnerability [20–25]. Bluestein et al. investigated the effect of microcalcifications on vulnerable plaque [20]. Their results suggested that microcalcifications increase the plaque vulnerability. Tang et al. demonstrated that localized critical stress values had much better correlation with plaque morphological features known to be linked to plaque rupture risk, compared to global maximum stress conditions [21]. They further compared five plaques with rupture and seven unrupture plaques using FSI models [22]. Their results showed that plaques with prior ruptures are associated with higher critical stress conditions. Teng et al. demonstrated that 3D critical plaque wall stress is a better predictor of carotid plaque rupture sites than flow shear stress by comparing 6 plaques with prior rupture and 12 with nonrupture [23]. Leach et al. compared carotid atheroma rupture observed *in vivo* and the plaque stress distribution from FSI analysis based on prerupture medical imaging [24]. They found a region of pronounced elevation in stress within the fibrous plaque layer of the lesion with a location and extent corresponding to that of the observed site of plaque rupture [24]. Gao et al. compared 8 asymptomatic and 12 symptomatic patients; it was observed that local maximum stress values were found to be significantly higher in symptomatic patients than that in asymptomatic patients [25]. These reports support the potential link between critical plaque wall stress and plaque vulnerability.

Most recently, the role of IPH in atherosclerotic disease progression has gained serious interest. One cause of IPH is rupture of the fragile neovessels within the plaque. IPH has been recognized as a high-risk feature closely associated with subsequent cerebrovascular events. In clinical follow up studies, it has been demonstrated that IPH is associated with accelerated plaque progression and cerebrovascular events; the repeated bleeding into the plaque may produce a stimulus for the progression of atherosclerosis by increasing the size of the lipid-rich necrotic core and plaque volume and creating new destabilizing factors [26,27]. Multicontrast high resolution MRI imaging has noninvasively characterized carotid intraplaque hemorrhage [28]. However, the discussion about the impact of the IPH on critical mechanical conditions such as plaque wall stress and flow shear stress remains very limited. Sadat et al. reported that the aging of hemorrhage would help in plaque stabilization using 2D MRI-based computational modeling [29]. Huang et al. determined the potential relationship between PWS and FSS with the location of IPH using FSI models for five human carotid plaques [30]. Their results suggested that (a) IPH positions were associated with higher PWS and (b) correlation results between FSS and location of hemorrhage were mixed for the five plaques studied: Only one out of five plaques showed mean FSS value from hemorrhage nodes was higher than that from nonhemorrhage nodes; three out of five plaques showed that their mean FSS values from hemorrhage nodes were lower than those from nonhemorrhage nodes; and one plaque showed that the difference had no statistical significance.

To date, the impact of the presence of intraplaque hemorrhage on stress and strain has not been analyzed using 3D FSI modeling. The goal of this study is to quantify the impact of IPH on structural stress and strain and the integrating effect of IPH and reduced fibrous cap thickness by building 3D FSI models. 3D FSI models were constructed applying volume component-fitting method (VCFM) based on *in vivo* MRI data of carotid atherosclerotic plaque containing IPH.

2 Materials and Methods

2.1 MRI Acquisition. Sequential 2D *in vivo* MR images of a human atherosclerotic carotid plaque with hemorrhage were acquired from a patient scheduled for carotid endarterectomy (age 64, male) by the Vascular Imaging Laboratory (VIL) at the University of Washington (UW) using protocols approved by UW Institutional Review Board. The patient was imaged with a 1.5-T MR scanner (Signa Horizon EchoSpeed, General Electric Health Care). Precontrast MR images included double-inversion-recovery

T1W, proton density-weighted (PDW), T2W, and time-of-flight (TOF). Post-contrast double-inversion-recovery T1W MR images of carotid arteries were obtained with a previously published standardized protocol (T1W: repetition time/echo time/inversion time, 800/2010/650 ms; PDW and T2W: repetition time/echo time, 3RR, 20/40 ms; TOF: repetition time/echo time, 23/3.8 ms) [4,31,32]. The images were obtained with the following parameters: $16 \times 16 \text{ cm}^2$ field-of-view, 256×256 matrix size, and 2 mm slice thickness. After interpolation, the in-plane resolution was $0.31 \times 0.31 \text{ mm}^2$. The longitudinal coverage covered the bulk region of the lesion.

MR images were segmented using custom-made analysis tools (CASCADE developed at the Vascular Imaging Laboratory at University of Washington) to identify lipid-rich necrotic core, loose matrix, calcification, thrombus, and intraplaque hemorrhage [4,28,31–35]. Figure 1 shows a human atherosclerotic carotid plaque slice (S4) with hemorrhage validated by histology. Figure 2 gives a set of ten slices of T1-weighted MR images and segmented contour plots.

2.2 FSI Computational Model. A 3D multicomponent fluid-structure interactions model was constructed to obtain the mechanical stress/strain and flow shear stress for the plaque presented in Fig. 2. Both the artery wall and plaque components were assumed to be hyperelastic, isotropic, incompressible, and homogeneous. Blood flow was assumed to be laminar, Newtonian, viscous, and incompressible. Transient simulations were carried out with time-dependent pressure at the inlet of common carotid artery (CCA) and outlet of internal carotid artery (ICA) and external carotid arteries (ECA). Patient-specific systolic (120 mm Hg) and diastolic (96 mm Hg) pressure conditions from the last hospital admission were used in the simulation. The incompressible Navier–Stokes equations with arbitrary Lagrangian–Eulerian (ALE) formulation were used as the governing equations. A no-slip condition between all interfaces was assumed. With these assumptions, we have the following governing equations:

$$\rho \mathbf{v}_{i,t} = \sigma_{ij,j}, i, j = 1, 2, 3 \quad (\text{equation of motion for solids}) \quad (1)$$

$$\varepsilon_{ij} = (\mathbf{v}_{i,j} + \mathbf{v}_{j,i} + \mathbf{v}_{\alpha,i} \mathbf{v}_{\alpha,j})/2, \quad i, j, \alpha = 1, 2, 3 \quad (\text{strain-displacement relation}) \quad (2)$$

$$\rho (\partial \mathbf{u} / \partial t + ((\mathbf{u} - \mathbf{u}_g) \cdot \nabla) \mathbf{u}) = -\nabla p + \mu \nabla^2 \mathbf{u} \quad (\text{equation of motion for fluid}) \quad (3)$$

$$\nabla \cdot \mathbf{u} = 0 \quad (\text{equation of continuity}) \quad (4)$$

where t stands for time, i and j label spatial coordinates, $\mathbf{v}$ is the solid displacement vector, σ is the stress tensor, ε is the Green–Lagrange strain tensor, $f_{.,j}$ stands for derivative with respect to the j^{th} variable, $\mathbf{u}$ and p are the flow velocity and pressure, $\mathbf{u}_g$ is the mesh velocity, μ is the coefficient of viscosity, and ρ is the density of blood. The details of the complete fluid-structure interactions model can be found in the studies of Tang et al. [19] and Yang et al. [36]. The modified Mooney–Rivlin (M–R) model was used to describe the material properties of the components in the plaque [37]. The strain energy function for the M–R model is given by

$$W = c_1(I_1 - 3) + c_2(I_2 - 3) + D_1[e^{D_2(I_2 - 3)} - 1] \quad (5)$$

where $I_1 = \Sigma C_{ii}$, and $I_2 = \frac{1}{2}(I_1^2 - C_{ij}C_{ij})$ are the first and second strain invariants, $\mathbf{C} = [C_{ij}] = \mathbf{X}^T \mathbf{X}$ is the right Cauchy–Green deformation tensor, $\mathbf{X} = [X_{ij}] = [\partial x_i / \partial a_j]$, where x_i is the current position, a_i is the original position, and c_i and D_i are material parameters chosen to match experimental measurement and previously published studies. In this paper, the material parameters for vessel tissue and lipid-rich necrotic core were selected from Tang’s study: vessel tissue, $c_1 = 36.8 \text{ kPa}$, $D_1 = 14.4 \text{ kPa}$, $D_2 = 2$;

Fig. 1 TOF, PD, and T1-weighted MR images of a human carotid plaque sample with hemorrhage validated by histology

lipid-rich necrotic core, $c_1 = 2 \text{ kPa}$, $D_1 = 2 \text{ kPa}$, $D_2 = 1.5$ [36,38]. Aglyamov et al. applied model-based elasticity reconstruction method to obtain the Young's modulus of the blood clot during formation and aging of thrombus [39]. Based on their data, least squares fitting method were employed to obtain the material parameters for hemorrhage: chronic hemorrhage, $c_1 = 9 \text{ kPa}$, $D_1 = 9 \text{ kPa}$, $D_2 = 0.25$; fresh hemorrhage, $c_1 = 1 \text{ kPa}$, $D_1 = 1 \text{ kPa}$, $D_2 = 0.25$ [29]. The stress-stretch plots for the material of different components derived from the modified Mooney–Rivlin model were presented in Fig. 3(a).

2.3 Volume Component-Fitting Mesh Generation Method. Due to the complicated irregular geometry of atherosclerotic plaque and the highly nonlinear 3D FSI computational model, it is very difficult to obtain convergent solutions. The complexity increases significantly when hemorrhage is included within the lipid-rich necrotic core. Therefore, a proper mesh is very critical for the convergence of the 3D fluid-structure interaction model.

The VCFM method was proposed to generate mesh for such a 3D multicomponent FSI model. The general idea of this technique is to divide the plaque geometry into thousands of small “volumes” to curve-fit the very irregular plaque geometry and all the components, which are “inclusions” in the structure. Each small volume contains only one component and has a more regular shape for mesh/element generation.

Figure 4 gives a simple illustration of the method. The IPH was contained within the lipid-rich necrotic core. Therefore, to create such proper small volumes, four artificial contours were added to help divide each slice into component-fitting areas (called “surfaces” in ADINA; see Fig. 4(b)). To generate the volumes between two neighboring slices, two types of volumes (see Fig. 4(c)) were employed. Using this technique, the 3D plaque domain was divided into thousands of small “volumes” to curve-fit the irregular plaque geometry with plaque component inclusions. After generation of the volumes, the mesh density was then assigned by specifying the proper numbers of division along the edges of the volumes. The computational mesh was made under ADINA

Fig. 2 PD-weighted MR images and segmented contour plots showing Hemorrhage. (a) *in vivo* MR-images, (b) segmented contour plots showing plaque components, and (c) 3D geometry showing hemorrhage and other components.

Fig. 3 Material curves and pressure conditions used in the multicomponent plaque model. (a) Material curves derived from the modified Mooney–Rivlin model; (b) Pressure conditions specified at the inlet (CCA) and outlet (ICA and ECA).

computing environment. For the plaque sample shown in Fig. 2, there were 2556 volumes and 47,008 elements generated for the solid model and 468 volumes and 28,944 elements generated for the fluid model.

Fig. 4 Component-fitting mesh-generation process. (a) Segmented contour data showing the components, (b) created lines connecting data points and dividing the slice into component-fitting areas, (c) two types of volumes to curve-fit components and complex geometry, and (d) component-fitting volumes formed by connection corresponding areas from stacking adjacent slices.

2.4 Pre-Shrink Process. Under the physiological condition (*in vivo* state), the arteries are axially stretched and pressurized. Therefore, axial and circumferential shrinking should be applied to generate the no-load starting shape as the numerical starting geometry for the computational simulations. The shrinkage in the axial direction was 9% so that the vessel would regain its *in vivo* length with a 10% axial stretch. Lumen shrinkage was chosen first (8%) and outer wall shrinkage was calculated so that (1) total mass volume was conserved and (2) plaque geometry after 10% axial stretch and pressurization had the best match with the original *in vivo* geometry [40]. Figure 5 presents the effect of shrink-stretch process. The difference between pressurized and stretched morphology (Fig. 5(c)) and *in vivo* morphology (Fig. 5(a)) was 1.6% (vessel area was 57.4 versus 56.5 mm²).

2.5 Models Considered. To quantify the effect of the IPH and FCT, eight 3D multicomponent FSI models were constructed from the base plaque geometry with the following specified features (Table 1): Model 1 (Base Model), IPH was replaced by a lipid-rich necrotic core; Model 2, fresh IPH; Model 3, the lipid-rich necrotic core was replaced by fresh IPH to increase the volume of IPH; Model 4, chronic IPH; Models 5–8, models with reduced FCT. To implement reducing thinnest FCT from 0.27 mm to 0.1 mm, the whole intraplaque hemorrhage and lipid-rich necrotic core were translated along the direction shown in Fig. 6. The lumen and wall were fixed. Intraplaque hemorrhage usually occurs within a lipid-rich necrotic core due to the bleeding from fragile neovessels within the plaque. Therefore, the model with a lipid-rich necrotic core replacing hemorrhage was used as our Base Model to simulate the prehemorrhage state of the plaque.

2.6 Solution Method. The coupled FSI plaque models were solved by a commercial finite-element package ADINA. ADINA uses unstructured finite element methods for both fluid and solid models. Nonlinear incremental iterative procedures are used to handle fluid-structure interactions. At each time step, the fluid model and solid model were solved individually using the latest information provided by the other part. The governing finite element equations for both the solid and fluid models were solved by the Newton–Raphson iteration method. That is, the solution process continues until the changes of solutions between two

Fig. 5 Illustration of the preshrink process to match *in vivo* MRI images: (a) segmented *in vivo* contour of Slice 7 (ICA), (b) the no-load contour after 8% shrinking in lumen and 4% expanding in outer-boundary (the expansion of outer-boundary was needed because the vessel was shortened), and (c) contour after stretch and pressurization

iterations become less than the specified tolerance, and a steady solution is obtained.

The simulation starts from a no-load state (no pressure/no stretch) whose geometry was obtained by shrinking the *in vivo* geometry. The prestretch stage (before the periodic cardiac cycles) used 60 time steps (time step = 0.005 s) to stretch the artery by 10% in the axial direction and raise the inlet and outlet pressures from zero to the specified pressure boundary conditions (Fig. 3(b)). A total of 200 time steps was used in the solid and fluid simulation for every cardiac cycle (1 s). The results of the third cardiac cycle were used for data analysis. Mesh analysis was performed for each model by reducing the mesh density in each dimension by 10% until differences between solutions from two consecutive meshes were negligible (less than 1% in L_2 -norm). More details of the models and methods can be found at Tang et al. [19,41] and Bathe [37].

2.7 Data Analysis. Data for plaque wall stress, plaque wall strain, and flow shear stress (FSS) were extracted from 3D FSI solutions for all integral nodes (total: structure: 448; fluid: 576) on all lumen surfaces of each slice for analysis. For each nodal point, the maximum principal stress (Stress- P_1) and maximum principal strain (Strain- P_1) were selected for PWS and PWSn, respectively, and maximum flow shear stress was used for FSS. Several rays, which began at the center of the lumen and tangent to the contour of either lipid-rich necrotic core or hemorrhage, were used to segment the lumen to three zones: (1) Lipid-rich necrotic core zone, the fibrous cap overlying the lipid-rich necrotic core, which does not contain hemorrhage; (2) Hemorrhage zone, the fibrous cap overlying hemorrhage, which was contained in a lipid-rich necrotic core; and (3) Wall zone, normal blood vessel. Applying this method, the node type of each node on the lumen surfaces was defined as shown in Fig. 7. The data of PWS, PWSn, and FSS of each node type group were analyzed. The student paired *t*-test was used for comparison of PWS and PWSn of the hemorrhage nodes

in different models. A *P*-value of less than 0.05 was deemed a significant difference.

3 Results

Simulations were conducted based on the plaque sample presented in Fig. 2. The distribution of PWS and FSS on stacked cross-section slices and a bifurcation cut surface were presented in Fig. 8. The mean PWS, PWSn, and FSS of hemorrhage nodes and the CPWS (PWS value from vulnerable site shown in Fig. 9), CPWSn, and critical FSS for eight models were summarized in Table 2. There were a total of 110 nodes of hemorrhage. The units for PWS and FSS were kPa and dyn/cm², respectively (1 kPa = 10⁴ dyn/cm²).

3.1 Presence of Hemorrhage Caused Higher Critical PWS and PWSn. Comparing the Base Model with Model 2, it was observed from Table 2 that CPWS increased from 116.0 kPa to 147.9 kPa, CPWSn increased from 0.197 to 0.240, and critical FSS did not change much (35.3 versus 35.6 dyn/cm²) by comparing the models with and without IPH. This indicated that the presence of fresh IPH caused a 27.5% and 21.8% increase in CPWS and CPWSn, respectively. The differences of the mean PWS (2.3% increase) and mean PWSn (6.3% increase) of this comparison were much smaller ($P < 0.0001$). This indicated that the impact of IPH on CPWS and CPWSn was of localized nature. Impact of IPH on other lumen nodes damped out quickly, as the location moved away from the IPH.

3.2 Reduced Fibrous Cap Thickness Caused Higher PWS, PWSn, and FSS. Although it has been well accepted that thin FCT should be considered as a destabilizing factor, to our knowledge, the impact of FCT of plaque with IPH on biomechanical stress and strain has not been investigated yet. To investigate the effects of plaque FCT on PWS of hemorrhage nodes, cap thickness was numerically reduced. The distributions of PWS on Slice 4 and Slice 7, on which showing the position of critical point, for

Table 1 Summary of the features of the models: (a) thinnest fibrous cap thickness was 0.27 mm for Models 1–4 and 0.1 mm for Models 5–8, respectively; (b) Base Model and Model 5: hemorrhage was replaced with lipid, therefore, the material of the plaque is lipid; Models 2 and 6: the plaque with the fresh hemorrhage within the lipid; Models 3 and 7: increasing the volume of hemorrhage (151.2 mm³) by replacing lipid (233.2 mm³) with fresh hemorrhage; Models 4 and 8: the fresh hemorrhage was replaced with chronic hemorrhage

	Fibrous cap thickness (mm)	Components		Fibrous cap thickness (mm)	Components
Base Model	0.27	Whole Lipid	Model 5	0.1	Whole Lipid
Model 2	0.27	Fresh Hemorrhage	Model 6	0.1	Fresh Hemorrhage
Model 3	0.27	Whole Hemorrhage	Model 7	0.1	Whole Hemorrhage
Model 4	0.27	Chronic Hemorrhage	Model 8	0.1	Chronic Hemorrhage

Fig. 6 Schematic drawing demonstrating the method for reducing the fibrous cap thickness. (a) Original image and (b) plaque with reduced fibrous cap thickness.

the models with reduced FCT were presented in Fig. 9. When FCT decreased, an increase of 36.8% in the CPWS, 38.6% in the CPWSn, and 33.7% in critical FSS were found (Base Model versus Model 5). This indicated that the reduced FCT caused significant increase in PWS/PWSn and FSS. A more significant effect on CPWS (56.3% increase) and CPWSn (56.3%) were found due to the integration of reduced FCT and presence of IPH by comparing Model 6 with Base Model.

3.3 Volume Increase of Hemorrhage Caused Higher PWS. To investigate the effect of the size of hemorrhage, the lipid-rich necrotic core pool was replaced by fresh IPH, the plaque then turned to be whole fresh IPH. The volume of IPH was then increased from 151.2 mm³ to 384.4 mm³. The mean PWS (77.1 kPa) of hemorrhage nodes from larger IPH (Model 3) is 17.2% higher than that (65.8 kPa) from Model 2 ($P < 0.0001$). The CPWS increased from 147.9 to 168.4 kPa, an increase of 13.9% was found (Model 2 versus Model 3). It was observed that the increases of CPWSn (6.25%) and mean PWSn (6.5%) were not as significant as CPWS. When FCT was numerically decreased, the mean PWS from Model 7 was 95.0 kPa, which was 14.5% higher than that (83.0 kPa) from fresh IPH model ($P < 0.0001$); the CPWS increased to from 181.3 kPa to 214.4 kPa, which was an 18.3% increase. These results indicate that the increasing volume of IPH will increase the vulnerability of plaque. It should be noted that the integration of the presence of IPH, reduced FCT, and increasing volume of IPH caused an 84.8% increase in critical PWS and a 75.1% increase in critical PWSn.

4 Discussion

4.1 Importance of IPH for Assessing Plaque Vulnerability. It has been reported that the increase in volume of IPH may accelerate the plaque progression of atherosclerosis and suggested that the repeated intraplaque hemorrhage maybe a

marker for plaque instability [26]. Our study shows that the increase of the volume brought an 18.3% increase and 13.9% increase in CPWS for the plaque with thin and thick FCT, respectively. This suggests that the increase volume of IPH will increase the plaque instability, especially for the unstable plaques.

Our previous study using *in vivo* MRI-based models indicated that a plaque could be classified as “highly vulnerable” if its critical stress was higher than 142 kPa [21]. The threshold stress value 142 kPa should be used with caution since it was dependent on (1) the resolution of MRI data, which directly determined plaque cap thickness of the segmented plaque data and (2) material parameters used in the study. It was observed that the presence of IPH increase CPWS from 116.0 kPa to 147.9 kPa (Base Model versus Model 2), which was greater than the threshold. This indicated that the presence of IPH may change plaque vulnerability status from stable to unstable, suggesting that IPH may be used as a marker for plaque instability. When plaque FCT was thin (0.1 mm), the presence of IPH also increase (22.6 kPa) the vulnerability of the plaque. IPH has been considered in some very recent research as a trigger of plaque vulnerability [42,43].

4.2 Aging of IPH May Help the Stabilization of Plaque. It was worth noticing that, due to the replacement of fresh IPH by chronic IPH, there was a 31.4% and 24.1% decrease of CPWS and a 25.8% and 25.0% decrease of CPWSn for the plaque with thick and thin FCT, respectively. This indicates that both plaque stress and strain are high at the early stage of IPH development and turns to be stable for chronic IPH. When FCT was thick, CPWS was only 101.4 kPa even less than CPWS at the prehemorrhage stage (116.0 kPa). However, when FCT was thin, CPWS for the plaque with chronic IPH was still high (137.6 kPa). This indicated that when FCT was thick, the chronic IPH might be considered as stable plaque; for chronic IPH with thin FCT, it might still be vulnerable plaque. Therefore, it might not be proper to investigate the stability of chronic IPH without the consideration of the thickness of fibrous cap.

4.3 Critical PWS and PWSn Might be Good Indicators for Plaque Vulnerability. It was observed that the presence of IPH, reduced FCT, and increase volume of IPH brought 85% and 75% increases in CPWS and CPWSn, respectively. It was demonstrated that the MRI-based finite element analysis has the ability to integrate the effect of the plaque morphology (geometry of the lipid-rich necrotic core and IPH, the volume of IPH, and FCT, etc.) and different plaque components. In addition to morphological features, biomechanical stress and strain may provide quantitative and comprehensive information, which could help the clinician to better assess the plaque vulnerability.

4.4 Association Between Critical FSS and Plaque Vulnerability. There has been great interest and a considerable effort spent investigating the relationship between FSS and the progression and rupture of atherosclerotic plaque [44,45]. In our

Fig. 7 Schematic drawing demonstrating the node-type assignment method for nodal point on lumen surfaces. Zone 1 and 3: Lipid nodes; Zone 2: Hemorrhage nodes; Zone 4: Normal Wall nodes.

Fig. 8 Band plots of plaque wall stress (maximum principle stress) and flow maximum shear stress. (a) Wall stress on stacked cross-section slices, (b) wall stress on bifurcation cut surface, and (c) flow shear stress on bifurcation cut surface.

Fig. 9 Comparison of PWS on slice 4 and slice 7 showing critical point from the models with lipid, fresh hemorrhage, the model where Lipid was replaced by fresh hemorrhage, and chronic hemorrhage

Table 2 Summary of plaque wall stress (Stress-P₁), plaque wall strain (Strain-P₁) and flow maximum shear stress results for hemorrhage nodes from eight models

	Stress (kPa)				Strain				FSS (dyn/cm ²)			
	Mean	% of Base	Critical	% of Base	Mean	% of Base	Critical	% of Base	Mean	% of Base	Critical	% of Base
Base Model	64.3	100%	116.0	100%	0.144	100%	0.197	100%	10.9	100%	35.3	100%
Model 2	65.8	102%	147.9	128%	0.153	106%	0.240	122%	10.8	99%	35.6	101%
Model 3	77.1	120%	168.4	145%	0.163	113%	0.255	129%	11.1	101%	35.9	102%
Model 4	60.7	94%	101.4	87%	0.139	97%	0.178	90%	10.3	95%	33.0	93%
Model 5	70.5	110%	158.7	137%	0.157	109%	0.273	139%	10.4	96%	47.2	134%
Model 6	83.0	129%	181.3	156%	0.181	126%	0.308	156%	10.2	93%	48.0	136%
Model 7	95.0	148%	214.4	185%	0.200	139%	0.345	175%	10.1	93%	48.3	137%
Model 8	62.1	97%	137.6	119%	0.139	97%	0.231	117%	10.6	97%	46.8	133%

study, the reduced FCT caused more than 35% increase in critical FSS, which support the hypothesis that a high flow shear stress region is associated with plaque vulnerability. However, the presence of IPH, the volume increase of IPH, and aging of IPH did not affect the mean and critical FSS value much (less than 10%). Keep in mind that the mean value of FSS is about 11 dyn/cm², which is less than 0.001% of mean value of PWS (greater than 60 kPa), contributions from flow shear stress acting as a rupture trigger may be much smaller than that from structural stress.

4.5 Limitations of the Study. The following limitations should be acknowledged: (1) Patient-specific material properties were not included due to the inability to measure these material properties using current techniques. The material properties of various plaque components used in this research were the best available data from the literature. (2) Arm systole and diastole pressures taken at the past scan visit were used to scale the pressure profile used in the simulations since pressure conditions at the location of the plaque were not available. (3) “No slipping” boundary conditions were assumed for all interfaces between plaque components. In reality, some degree of slipping between plaque components may be present, which should be taken into consideration for more accurate representations, especially for impact of calcification on plaque stress distributions. (4) This was not a longitudinal study. Changes of thin FCT and hemorrhage were numerically obtained. Because the resolution of *in vivo* MRI images was $0.31 \times 0.31 \text{ mm}^2$, it is impossible to obtain 0.1 mm (or less) cap thickness based on MRI images. This limitation could be improved when MRI images with better resolution become available.

5 Conclusion

In this study, the impact of the presence of intraplaque hemorrhage, the increasing volume of IPH, and fibrous cap thickness on biomechanical stress and strain was quantified based on the constructed FSI models. The results show that IPH has considerable impact on critical plaque stress and strain conditions, and accurate quantification of IPH could lead to more accurate assessment of plaque vulnerability. Large-scale studies are needed to further validate our findings.

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References

- [1] Carr, S., Farb, A., Pearce, W. H., Virmani, R., and Yao, J. S., 1996, “Atherosclerotic Plaque Rupture in Symptomatic Carotid Artery Stenosis,” *J. Vasc. Surg.*, **23**(5), pp. 755–765.
- [2] Naghavi, M., Libby, P., Falk, E., Casscells, S. W., Litovsky, S., Rumberger, J., Badimon, J. J., Stefanadis, C., Moreno, P., Pasterkamp, G., Fayad, Z., Stone, P. H., Waxman, S., Raggi, P., Madjid, M., Zarabi, A., Burke, A., Yuan, C., Fitzgerald, P. J., Siscovick, D. S., de Korte, C. L., Aikawa, M., Juhani Airaksinen, K. E., Assmann, G., Becker, C. R., Chesebro, J. H., Farb, A., Galis, Z. S., Jackson, C., Jang, I. K., Koenig, W., Lodder, R. A., March, K., Demirovic, J., Navab, M., Priori, S. G., Rekhter, M. D., Bahr, R., Grundy, S. M., Mehran, R., Colombo, A., Boerwinkle, E., Ballantyne, C., Insull, W., Jr., Schwartz, R. S., Vogel, R., Serruys, P. W., Hansson, G. K., Faxon, D. P., Kaul, S., Drexler, H., Greenland, P., Muller, J. E., Virmani, R., Ridker, P. M., Zipes, D. P., Shah, P. K., and Willerson, J. T., 2003, “From Vulnerable Plaque to Vulnerable Patient: A Call for New Definitions and Risk Assessment Strategies: Part I,” *Circulation*, **108**(14), pp. 1664–1672.
- [3] Naghavi, M., Libby, P., Falk, E., Casscells, S. W., Litovsky, S., Rumberger, J., Badimon, J. J., Stefanadis, C., Moreno, P., Pasterkamp, G., Fayad, Z., Stone, P. H., Waxman, S., Raggi, P., Madjid, M., Zarabi, A., Burke, A., Yuan, C., Fitzgerald, P. J., Siscovick, D. S., de Korte, C. L., Aikawa, M., Juhani Airaksinen, K. E., Assmann, G., Becker, C. R., Chesebro, J. H., Farb, A., Galis, Z. S., Jackson, C., Jang, I. K., Koenig, W., Lodder, R. A., March, K., Demirovic, J., Navab, M., Priori, S. G., Rekhter, M. D., Bahr, R., Grundy, S. M., Mehran, R., Colombo, A., Boerwinkle, E., Ballantyne, C., Insull, W., Jr., Schwartz, R. S., Vogel, R., Serruys, P. W., Hansson, G. K., Faxon, D. P., Kaul, S., Drexler, H., Greenland, P., Muller, J. E., Virmani, R., Ridker, P. M., Zipes, D. P., Shah, P. K., and Willerson, J. T., 2003, “From Vulnerable Plaque to Vulnerable Patient: A Call for New Definitions and Risk Assessment Strategies: Part II,” *Circulation*, **108**(15), pp. 1772–1778.
- [4] Saam, T., Ferguson, M. S., Yarnykh, V. L., Takaya, N., Xu, D., Polissar, N. L., Hatsukami, T. S., and Yuan, C., 2005, “Quantitative Evaluation of Carotid Plaque Composition by *In Vivo* MRI,” *Arterioscler., Thromb., Vasc. Biol.*, **25**(1), pp. 234–239.
- [5] Yuan, C., Mitsumori, L. M., Beach, K. W., and Maravilla, K. R., 2001, “Special Review: Carotid Atherosclerotic Plaque: Noninvasive MR Characterization and Identification of Vulnerable Lesions,” *Radiology*, **221**, pp. 285–299.
- [6] Yuan, C., Zhang, S. X., Polissar, N. L., Echelard, D., Ortiz, G., Davis, J. W., Ellington, E., Ferguson, M. S., and Hatsukami, T. S., 2002, “Identification of Fibrous Cap Rupture With Magnetic Resonance Imaging is Highly Associated With Recent Transient Ischemic Attack or Stroke,” *Circulation*, **105**(2):181–185.
- [7] Fuster, V., Cornhill, J. F., Dinsmore, R. E., Fallon, J. T., Insull, W., Libby, P., Nissen, S., Rosenfeld, M. E., and Wagner, W. D., eds., 1998, *The Vulnerable Atherosclerotic Plaque: Understanding, Identification, and Modification*, AHA Monograph Series, Futura Publishing, Armonk, NY.
- [8] Barnett, H. J., Taylor, D. W., Eliasziw, M., Fox, A. J., Ferguson, G. G., Haynes, R. B., Rankin, R. N., Clagett, G. P., Hachinski, V. C., Sackett, D. L., Thorpe, K. E., Meldrum, H. E., and Spence, J. D., 1998, “Benefit of Carotid Endarterectomy in Patients With Symptomatic Moderate or Severe Stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators,” *N. Engl. J. Med.*, **339**(20), pp. 1415–1425.
- [9] Hatsukami, T. S., and Yuan, C., 2010, “MRI in the Early Identification and Classification of High-Risk Atherosclerotic Carotid Plaques,” *Imaging Med.*, **2**(1), pp. 63–75.
- [10] Rothwell, P. M., Gutnikov, S. A., and Warlow, C. P., 2003, “European Carotid Surgery Trialists’ Collaboration Reanalysis of the Final Results of the European Carotid Surgery Trial,” *Stroke*, **34**(2), pp. 514–523.
- [11] Underhill, H. R., Hatsukami, T. S., Fayad, Z. A., Fuster, V., and Yuan, C., 2010, “MRI of Carotid Atherosclerosis: Clinical Implications and Future Directions,” *Nat. Rev. Cardiol.*, **7**(3), pp. 165–173.
- [12] Richardson, P. D., Davies, M. J., and Born, G. V., 1989, “Influence of Plaque Configuration and Stress Distribution on Fissuring of Coronary Atherosclerotic Plaques,” *Lancet*, **2**(8669), pp. 941–944.
- [13] Cheng, G. C., Loree, H. M., Kamm, R. D., Fishbein, M. C., and Lee, R. T., 1993, “Distribution of Circumferential Stress in Ruptured and Stable

- Atherosclerotic Lesions. A Structural Analysis With Histopathological Correlation," *Circulation*, **87**(4), pp. 1179–1187.
- [14] Lee, R. T., Grodzinsky, A. J., Frank, E. H., Kamm, R. D., and Schoen, F. J., 1991, "Structure-Dependent Dynamic Mechanical Behavior of Fibrous Caps From Human Atherosclerotic Plaques," *Circulation*, **83**(5), pp. 1764–1770.
- [15] Li, Z. Y., Howarth, S., Trivedi, R. A., U-King-Im, J. M., Graves, M. J., Brown, A., Wang, L. Q., and Gillard, J. H., 2006, "Stress Analysis of Carotid Plaque Rupture Based on *In Vivo* High Resolution MRI," *J. Biomech.*, **39**, pp. 2611–2622.
- [16] Loree, H. M., Kamm, R. D., Stringfellow, R. G., and Lee, R. T., 1992, "Effects of Fibrous Cap Thickness on Peak Circumferential Stress in Model Atherosclerotic Vessels," *Circ. Res.*, **71**, pp. 850–858.
- [17] Teng, Z., Sadat, U., Li, Z. Y., Huang, X., Zhu, C., Young, V. E., Graves, M. J., and Gillard, J. H., 2010, "Arterial Luminal Curvature and Fibrous-Cap Thickness Affect Critical Stress Conditions Within Atherosclerotic Plaque: An *in vivo* MRI-Based 2D Finite-Element Study," *Ann. Biomed. Eng.*, **38**(10), pp. 3096–3101.
- [18] Holzapfel, G. A., Stadler, M., and Schulze-Bause, C. A. J., 2002, "A Layer-Specific Three-Dimensional Model for the Simulation of Balloon Angioplasty Using Magnetic Resonance Imaging and Mechanical Testing," *Ann. Biomed. Eng.*, **30**(6), pp. 753–767.
- [19] Tang, D., Yang, C., Zheng, J., Woodard, P. K., Sicard, G. A., Saffitz, J. E., and Yuan, C., 2004, "3D MRI-Based Multicomponent FSI Models for Atherosclerotic Plaques," *Ann. Biomed. Eng.*, **32**(7), pp. 947–960.
- [20] Bluestein, D., Alemu, Y., Avrahami, I., Gharib, M., Dumont, K., Ricotta, J. J., and Einav, S., 2008, "Influence of Microcalcifications on Vulnerable Plaque Mechanics Using FSI Modeling," *J. Biomech.*, **41**(5), pp. 1111–1118.
- [21] Tang, D., Teng, Z., Canton, G., Hatsukami, T. S., Dong, L., Huang, X., and Yuan, C., 2009, "Local Critical Stress Correlates Better Than Global Maximum Stress With Plaque Morphological Features Linked to Atherosclerotic Plaque Vulnerability: An *In Vivo* Multi-Patient Study," *Biomed. Eng. Online*, **8**:15.
- [22] Tang, D., Teng, Z., Canton, G., Yang, C., Ferguson, M., Huang, X., Zheng, J., Woodard, P. K., and Yuan, C., 2009, "Sites of Rupture in Human Atherosclerotic Carotid Plaques are Associated With High Structural Stresses: An *In Vivo* MRI-Based 3D Fluid-Structure Interaction Study," *Stroke*, **40**(10), pp. 3258–3263.
- [23] Teng, Z., Canton, G., Yuan, C., Ferguson, M., Yang, C., Huang, X., Zheng, J., Woodard, P. K., and Tang, D., 2010, "3D Critical Plaque Wall Stress is a Better Predictor of Carotid Plaque Rupture Sites Than Flow Shear Stress: An *In Vivo* MRI-Based 3D FSI Study," *J. Biomech. Eng.*, **132**(3), p. 031007.
- [24] Leach, J. R., Rayz, R., Soares, B., Wintermark, M., Mofrad, M. R., and Saloner, D., 2010, "Carotid Atheroma Rupture Observed *In Vivo* and FSI Predicted Stress Distribution Based on Pre-Rupture Imaging," *Ann. Biomed. Eng.*, **38**(8), pp. 2748–2765.
- [25] Gao, H., Long, Q., Kumar Das, S., Halls, J., Graves, M., Gillard, J. H., and Li, Z. Y., 2011, "Study of Carotid Arterial Plaque Stress for Symptomatic and Asymptomatic Patients," *J. Biomech.*, **44**(14), pp. 2551–2557.
- [26] Takaya, N., Yuan, C., Chu, B., Saam, T., Polissar, N. L., Jarvik, G. P., Isaac, C., McDonough, J., Natiello, C., Small, R., Ferguson, M. S., Hatsukami, T. S., 2005, "Presence of Intraplaque Hemorrhage Stimulates Progression of Carotid Atherosclerotic Plaques: A High-Resolution Magnetic Resonance Imaging Study," *Circulation*, **111**(21), pp. 2768–2775.
- [27] Takaya, N., Yuan, C., Chu, B. B., Saam, T., Underhill, H., Cai, J., Tran, N., Polissar, N. L., Isaac, C., Ferguson, M. S., Garden, G. A., Cramer, S. C., Maravilla, K. R., Hashimoto, B., and Hatsukami, T. S., 2006, "Association Between Carotid Plaque Characteristics and Subsequent Ischemic Cerebrovascular Events: A Prospective Assessment With MRI—Initial Results," *Stroke*, **37**(3), pp. 818–823.
- [28] Chu, B., Kampschulte, A., Ferguson, M. S., Kerwin, W. S., Yarnykh, V. L., O'Brien, K. D., Polissar, N. L., Hatsukami, T. S., and Yuan, C., 2004, "Hemorrhage in the Atherosclerotic Carotid Plaque: A High-Resolution MRI Study," *Stroke*, **35**(5), pp. 1079–1084.
- [29] Sadat, U., Teng, Z., Young, V. E., Zhu, C., Tang, T. Y., Graves, M. J., and Gillard, J. H., 2011, "Impact of Plaque Hemorrhage and its Age on Structural Stresses in Atherosclerotic Plaques of Patients With Carotid Artery Disease: An MR Imaging-Based Finite Element Simulation Study," *Int. J. Cardiovasc. Imaging*, **27**(3), pp. 397–402.
- [30] Huang, X., Teng, Z., Canton, G., Ferguson, M., Yuan, C., and Tang, D., 2010, "Intraplaque Hemorrhage is Associated With Higher Structural Stresses in Human Atherosclerotic Plaques: An *In Vivo* MRI-Based 3D Fluid-Structure Interaction Study," *Biomed. Eng. Online*, **9**:86.
- [31] Mitsumori, L. M., Hatsukami, T. S., Ferguson, M. S., Kerwin, W. S., Cai, J., and Yuan, C., 2003, "In *Vivo* Accuracy of Multisequence MR Imaging for Identifying Unstable Fibrous Caps in Advanced Human Carotid Plaques," *J. Magn. Reson. Imaging*, **17**(4), pp. 410–420.
- [32] Yuan, C., Mitsumori, L. M., Ferguson, M. S., Polissar, N. L., Echelard, D., Ortiz, G., Small, R., Davies, J. W., Kerwin, W. S., and Hatsukami, T. S., 2001, "In *Vivo* Accuracy of Multispectral Magnetic Resonance Imaging for Identifying Lipid-Rich Necrotic Cores and Intraplaque Hemorrhage in Advanced Human Carotid Plaques," *Circulation*, **104**(17), pp. 2051–2056.
- [33] Chu, B., Yuan, C., Takaya, N., Shewchuk, J. R., Clowes, A. W., and Hatsukami, T. S., 2006, "Images in Cardiovascular Medicine. Serial High-Spatial-Resolution, Multisequence Magnetic Resonance Imaging Studies Identify Fibrous Cap Rupture and Penetrating Ulcer Into Carotid Atherosclerotic Plaque," *Circulation*, **113**(12), pp. e660–e661.
- [34] Hatsukami, T. S., Ross, R., Polissar, N. L., and Yuan, C., 2000, "Visualization of Fibrous Cap Thickness and Rupture in Human Atherosclerotic Carotid Plaque *In Vivo* With High-Resolution Magnetic Resonance Imaging," *Circulation*, **102**(9), pp. 959–964.
- [35] Yuan, C., Mitsumori, L. M., Beach, K. W., and Maravilla, K. R., 2001, "Carotid Atherosclerotic Plaque: Noninvasive MR Characterization and Identification of Vulnerable Lesions," *Radiology*, **221**(2), pp. 285–299.
- [36] Yang, C., Tang, D., Yuan, C., Hatsukami, T. S., Zheng, J., and Woodard, P. K., 2007, "In *Vivo/Ex Vivo* MRI-Based 3D Non-Newtonian FSI Models for Human Atherosclerotic Plaques Compared With Fluid/Wall-Only Models," *Comput. Model. Eng. Sci.*, **19**(3), pp. 233–246.
- [37] Bathe, K. J., 2002, *Theory and Modeling Guide*, ADINA R&D, Inc., Watertown, MA.
- [38] Tang, D., Yang, C., Mondal, S., Liu, F., Canton, G., Hatsukami, T. S., and Yuan, C., 2008, "A Negative Correlation Between Human Carotid Atherosclerotic Plaque Progression and Plaque Wall Stress: *In Vivo* MRI-Based 2D/3D FSI Models," *J. Biomech.*, **41**(4), pp. 727–736.
- [39] Aglyamov, S. R., Skovoroda, A. R., Xie, H., Kim, K., Rubin, J. M., O'Donnell, M., Wakefield, T. W., Myers, D., and Emelianov, S. Y., 2007, "Model-Based Reconstructive Elasticity Imaging Using Ultrasound," *Int. J. Biomed. Imaging*, **2007**, Paper No. 35830.
- [40] Huang, X., Yang, C., Yuan, C., Liu, F., Canton, G., Zheng, J., Woodard, P. K., Sicard, G. A., and Tang, D., 2002, "Patient-Specific Artery Shrinkage and 3D Zero-Stress State in Multi-Component 3D FSI Models for Carotid Atherosclerotic Plaques Based on *In Vivo* MRI Data," *Mol. Cell Biomech.*, **6**(2), pp. 121–134.
- [41] Tang, D., Yang, C., Kobayashi, S., Zheng, J., and Vito, R. P., 2003, "Effect of Stenosis Asymmetry on Blood Flow and Artery Compression: A Three-Dimensional Fluid-Structure Interaction Model," *Ann. Biomed. Eng.*, **31**(10), pp. 1182–1193.
- [42] Michel, J. B., Virmani, R., Arbustini, E., and Pasterkamp, G., 2011, "Intraplaque Haemorrhages as the Trigger of Plaque Vulnerability," *Eur. Heart J.*, **32**(16), pp. 1977–1985.
- [43] Yuan, C., Mitsumori, L. M., Beach, K. W., and Maravilla, K. R., 2011, "Carotid Atherosclerotic Plaque: Noninvasive MR Characterization and Identification of Vulnerable Lesions," *Radiology*, **221**(2), pp. 285–299.
- [44] Groen, H. C., Gijzen, F. J., van der Lugt, A., Ferguson, M. S., Hatsukami, T. S., van der Steen, A. F., Yuan, C., and Wentzel, J. J., 2007, "Plaque Rupture in the Carotid Artery is Localized at the High Shear Stress Region: A Case Report," *Stroke*, **38**, pp. 2379–2381.
- [45] Ku, D. N., Giddens, D. P., Zarins, C. K., and Glagov, S., 1985, "Pulsatile Flow and Atherosclerosis in The Human Carotid Bifurcation: Positive Correlation Between Plaque Location and Low and Oscillating Shear Stress," *Arterioscl., Thromb., Vasc. Biol.*, **5**, pp. 293–302.