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Adhesive strength of atherosclerotic plaque in a mouse model depends on local collagen content and elastin fragmentation

Ying Wang [a,b], John A. Johnson [b], Abigail Fulp [c], Michael A. Sutton [a,d], Susan M. Lessner [a,b,*]

[a] Biomedical Engineering Program, University of South Carolina, Columbia, SC, USA
[b] Department of Cell Biology and Anatomy, University of South Carolina School of Medicine, Columbia, SC, USA
[c] Department of Biomedical Engineering, North Carolina State University, Raleigh, NC, USA
[d] Department of Mechanical Engineering, University of South Carolina, Columbia, SC, USA

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ABSTRACT

Atherosclerotic plaque rupture is a major cause of myocardial infarction and ischemic stroke. The adhesive strength of the bond between a plaque and the vascular wall, measured as local energy release rate, $\mathcal{G}$, is used for quantitative plaque stability estimation. We tested the hypothesis that adhesive strength varies with plaque composition. Matrix metalloproteinase-12 (MMP12) deficiency was previously reported to alter lesion composition. To estimate $\mathcal{G}$ values, peeling experiments are performed on aortic plaques from apolipoprotein E knockout (apoE KO) and apoE MMP12 double knockout (DKO) male mice after 8 months on high-fat diet. For plaques in apoE KO and apoE MMP12 DKO mice, experimental values for $\mathcal{G}$ differ significantly ($p < 0.002$) between genotypes, averaging 19.2 J/m^2 and 12.1 J/m^2, respectively. Histology confirms that plaques delaminate along their interface with the underlying internal elastic lamina (IEL) in both genotypes. Quantitative image analysis of stained tissue sections demonstrates a significant positive correlation ($p < 0.05$) between local collagen content of lesions and $\mathcal{G}$ values in both genotypes, indicating that adhesive strength of plaques depends on local collagen content. Surprisingly, macrophage content of aortic plaques is neither significantly correlated with $\mathcal{G}$ values nor significantly different between genotypes. The IEL underlying plaques in apoE KO mice is significantly more fragmented (number of breaks and length of breaks) than in apoE MMP12 DKO mice, suggesting that elastin fragmentation also influences adhesion strength of plaques. Overall, our results suggest that plaques adhere more strongly to the underlying IEL in apoE KO mice than in apoE MMP12 DKO mice.

1. Introduction

Atherosclerotic plaque rupture is the main cause of myocardial infarction, coronary thrombosis and ischemic stroke. In a previous study, we proposed a new plaque rupture mechanism, plaque separation at the shoulder, and developed a novel quantitative mechanical experiment to measure the adhesive strength between the atherosclerotic plaque and the underlying vascular wall in mouse models using local energy release rate, $\mathcal{G}$, as a quantifiable metric for direct comparison of plaque separation strengths (Wang et al., 2011). Previous studies focused on morphological characteristics of the entire vulnerable plaque, such as fibrous cap thickness and lipid core size, or on local features of the rupture site in human samples, such as presence of micro-calcifications or thinning of the fibrous cap (Vengrenyuk et al., 2006; Virmani et al., 2007). Since mouse plaques generally do not rupture spontaneously (Schwartz et al., 2007), studies of local morphology at the rupture site cannot readily be conducted in mouse models. However, by studying the structure–function relationships between local energy release rate and local plaque composition at non-rupture sites, we can provide quantitative comparisons of relative plaque stability and identify those plaques more likely to fail.

Using transgenic models, we have now explored the correlation between $\mathcal{G}$ and plaque composition in a mouse model of diet-induced atherosclerosis. We previously showed that plaques delaminate at the IEL interface in this mouse model (Wang et al., 2011), suggesting that genetic defects that alter elastin degradation might lead to changes in plaque adhesive strength. MMP-12 (macrophage metalloelastase) is known to be important in elastin degradation (Luttun et al., 2004), promoting lesion expansion and destabilization through atherosclerotic media destruction and ectasia. Furthermore, plaque composition in MMP-12 deficient mice was reported to be significantly different from that in apoE single knockout controls

* Corresponding author at: Department of Cell Biology and Anatomy, University of South Carolina School of Medicine, 6439 Garners Ferry Rd., Columbia, SC 29209, USA. Tel.: +1 803 216 3819; fax: +1 803 216 3846.
E-mail address: susan.lessner@uscmed.sc.edu (S.M. Lessner).

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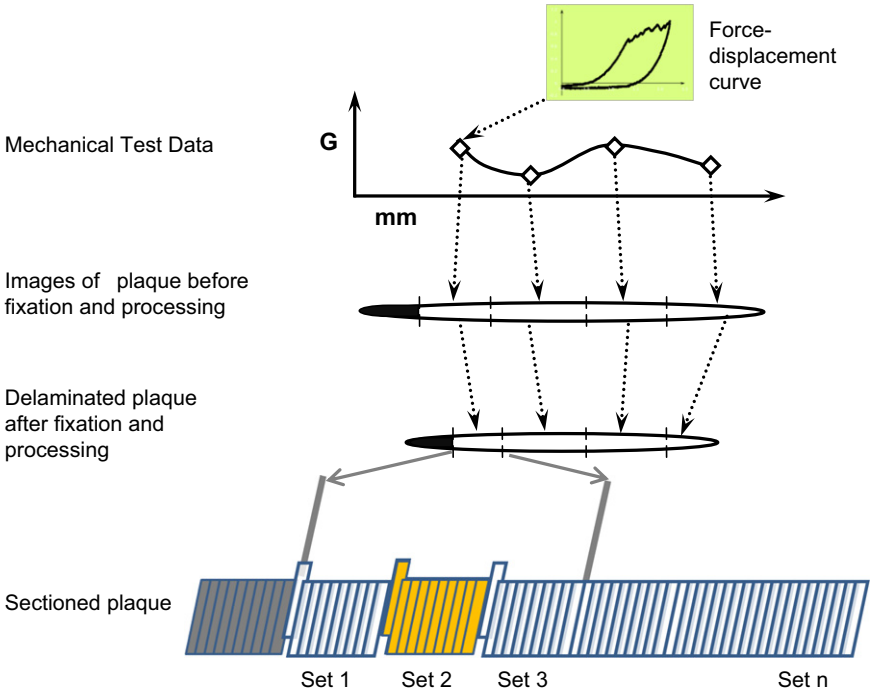

Fig. 1. Schematic of strategy for correlating experimental biomechanics data with histological sections. Experimental biomechanics data and histology are correlated by calculating the lengths of plaques before and after fixation and embedding, and aligning the data sets. Each experimental data point (diamonds in top figure) corresponds to a force–displacement curve (inset) acquired during delamination of a specific length of plaque. Total unfixed plaque length is calculated by summing individual delamination event lengths (displacements) measured by the Bose mechanical test system. Plaques shrink after fixation and embedding (schematic, third panel). After sectioning (bottom panel), embedded plaque length is calculated as total number of sections × section thickness. Each experimental biomechanics data point corresponds to several groups of sections (enclosed in gray brackets), representing a length of delaminated plaque in the fixed specimen. Both unfixed and fixed plaque lengths are corrected for the initial peel arm length (black segment at left of plaque) and aligned.

(Johnson et al., 2005). Therefore, we expect that the structure of the IEL underlying plaques will differ in apoE KO and apoE MMP-12 DKO mice, due to altered capacity to remodel elastin.

In the current work, we show for the first time that (a) local collagen content of atherosclerotic plaques is positively correlated with local adhesive strength between the plaque and the underlying vascular wall and (b) increased elastin fragmentation in the IEL underlying plaques is positively correlated with increased adhesive strength between the plaque and the underlying vascular wall.

2. Materials and methods

2.1. Animal model, specimen preparation and mechanical experiments

ApoE KO and apoE MMP-12 DKO male mice are used in this study. Six-week-old mice are fed a high-fat Western diet for 8 months to develop atherosclerosis throughout the aorta (Nakashima et al., 1994). Mice are sacrificed and dissected to expose the aorta. The descending aorta is cut open longitudinally to expose each plaque. A small initial flaw is introduced at the proximal end of the plaque to initiate delamination. Animals used in this study were euthanized by humane methods in accordance with PHS guidelines and as approved by the university Institutional Animal Care and Use Committee.

Cyclic peeling experiments are performed to obtain local $\mathcal{G}$ values. Briefly, energy used for delamination in one peeling cycle is averaged by the area newly exposed by plaque delamination in that cycle. Details of the experimental setup and data processing can be found in Wang et al. (2011). Each delamination experiment generated a series of local $\mathcal{G}$ values as delamination proceeded stepwise from the proximal edge of the plaque.

2.2. Histology

After the peeling experiment, delaminated plaques are immersion-fixed in 10% neutral-buffered formalin for at least 24 h. The small size of plaque specimens makes it difficult to directly embed them in paraffin in an appropriate orientation. Therefore, specimens are first embedded in 10% agarose gel. The agarose blocks are cut into cubes and trimmed to ensure the correct orientation for each plaque. Specimens are oriented to obtain transverse sections starting at the proximal end of the plaque. Agarose blocks are dehydrated in graded alcohols and processed using conventional methods for paraffin embedding.

The protocol for correlating experimental delamination data (hereafter referred to as experimental mechanics data) with histological data is shown schematically in Fig. 1. Embedded plaque specimens are sectioned at 5 μm along their entire length. For every 10 sections, we collected the first 5 sections as a set. Since specimens shrink during dehydration and embedding (Lowder et al., 2007), we record the total number of sets to calculate the lengths of plaques after embedding (L_f=section thickness × number of sets × sections/set), enabling us to obtain a more accurate correlation with experimental mechanics data. The first section of each set is stained with Masson's Trichrome for general morphology, and the remaining sections are stained to identify specific plaque components, as described below. Images are taken under 100X magnification with a Zeiss Axioskop 2 microscope and analyzed with image processing software ImagePro Plus (Media Cybernetics). During delamination experiments, black tissue marking dye is applied to the luminal surface of the plaque to distinguish newly exposed area under the plaque. Tissue marking dye is applied after creation of a small flaw at the proximal end of each plaque and before initiation of peeling cycles. Thus, in serial sections of delaminated plaques, tissue marking dye coating both surfaces of the "peel arm" appears in the first few images, then decreases on the abluminal surface corresponding to newly exposed area. The transition from sections with abluminal dye to those without is identified as the mechanical experiment starting point on the delaminated specimens. As shown schematically in Fig. 1, this starting point corresponds to axial position $x=0$ during the first peeling cycle. Along the remaining plaque length, the mechanical experiment data point for each cycle is correlated with plaque composition of a representative set of cross-sections taken from the plaque segment delaminated during that cycle. The length of the plaque segment corresponding to each data point can be determined from the displacement length recorded by the Bose actuator. The sum of all such displacements is the total plaque length represented by mechanical experiment data points (L_o). The total length of each delaminated plaque after fixation and embedding (L_f) can be calculated as described above from the number of sections and section thickness. The position of each histological section in the processed plaque which corresponds to an experimental mechanics data point is estimated by assuming uniform strain and multiplying the experimental displacement value by a proportionality constant, L_f/L_o, defined as total fixed length of the plaque (L_f) divided by total fresh length (L_o) (see Fig. 1).

The most important error in correlating experimental mechanics data to histological data is likely to be underestimation of the fixed plaque length due to loss of sections during paraffin sectioning; based on estimates of how many