INVESTIGATING RUPTURE CHARACTERISTICS OF TISSUE-ENGINEERED ATHEROSCLEROTIC PLAQUE CAPS

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Introduction

Stroke can be initiated by rupture of the atherosclerotic plaque fibrous cap in a carotid artery. However, cap rupture mechanisms are not well understood yet. Understanding the impact of the structural components of the cap on its local mechanics may provide critical insights into plaque rupture. Various limitations within studying plaques *in vivo* and *ex vivo* highlight the need for additional methods to investigate rupture mechanics. Therefore, we created collagenous tissue-engineered plaque cap analogs [1]. In the current study, we obtain local collagen structural parameters, local mechanical properties, and rupture characteristics of these analogs to analyze the relationship between the local collagen architecture and local rupture mechanics of cap analogs.

Methods

Nine collagenous cap analogs with a soft inclusion (SI), mimicking the plaque lipid core, were created [1]. Afterwards, the analogs were imaged with multiphoton microscopy (MPM) with second harmonic generation (SHG) to visualize the collagen architecture. From the SHG images, the local fiber orientations were measured using a fiber orientation analysis tool (FOAtool, TU/e). After imaging, the analogs were exposed to uniaxial tensile tests until full rupture. Tissue deformation, the rupture initiation location, and the rupture propagation path of the tests were recorded with a high-speed camera (PL-D725, Pixelink). Local (Green-Lagrange) strains under tensile stretching were measured through DIC analysis using the software Ncorr [2].

Results

In three samples, the rupture initiated at a clamping site whereas in the other six samples, rupture initiated in the SI or at the SI-fibrous tissue interface. The DIC-derived local tensile strain (E_{yy}) analysis at the timepoint of rupture initiation (Fig 1A) showed statistically significantly higher strain levels at the rupture initiation locations (Fig 1B).

Qualitative analysis of rupture propagation paths showed inter-sample variation. In some samples, multiple ruptures were found which were connected by the rupture propagation (Fig. 1C). Moreover, the shape of the rupture propagation path differed between samples. The local predominant fiber angles and the rupture propagation path in a cap analog are shown in Fig 1C. The figure demonstrates that at most locations within the analog, the rupture propagated parallel to the local predominant fiber angle. Similar results were found in the other samples. Fig. 1D shows the rupture path versus the local predominant fiber orientation. The linear regression curve in figure 1D ($R=0.60$, $\beta_1=1.04$, $p<0.001$) shows that the rupture path angle approximates the fiber angle, again indicating that rupture in the analogs propagated parallel to the local fiber angle.

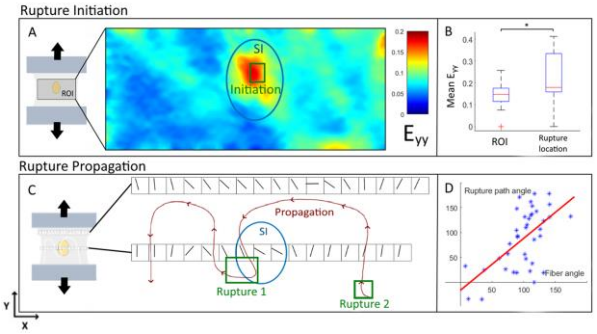

Figure 1: A) DIC-derived local tensile strains B) DIC-derived average tensile strain in the ROI vs in the rupture initiation region C) θ (black line) and rupture path (red line) in a representative analog D) Fiber angle vs rupture path angle, linear regression curve (red)

Discussion

We successfully visualized the collagen orientation and obtained local mechanical (strain) fingerprints in tissue-engineered cap analogs to study atherosclerotic plaque rupture. Local strain measurements showed that rupture in the analogs initiated at the elevated tensile strain regions. The strains found in the analog are similar to the local rupture strain found in a previous DIC-analysis of ex vivo human plaque tissue [3]. Despite the inter-sample variation in the rupture propagation path, it was found that the ruptures usually propagated parallel to the local predominant fiber angle. As a next step, we will investigate the rupture surfaces with scanning electron microscopy (SEM) to test our new hypothesis proposing that rupture propagation in the analogs is mostly parallel to the local predominant fiber direction. This data will help us to gain better understanding of the underlying failure mechanisms of plaques.

References

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