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EFFECTS OF STRAIN, COLLAGEN AND ELASTIN ON THE POSITION OF THE CRACK INITIATION SITE OF BIAXIALLY STRETCHED AORTAS

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INTRODUCTION

The aortic aneurysms sometimes result in a fatal rupture. It is widely accepted that aneurysms with a diameter larger than a critical value are of high risk for rupture. However, there is concern that aneurysms with a diameter smaller than the critical value could also rupture [1,2], indicating the weakening of aneurysmal wall. However, little is known about what intramural elements and their structure are responsible for the failure of the aneurysms, and how the process of aortic failure proceeds. Thus, simultaneous observation of the failure phenomenon and microstructure of the aorta may provide valuable insights into its failure mechanisms.

In this paper, a novel device and method for observation at failure site of biaxially stretched aortas [3] are introduced. The specimens at their center were thinned to induce its failure within this region. These techniques enable us to observe failure region of aortic specimens within the observable area under a microscope, and how the cracks propagate within the specimens. By applying these techniques to healthy porcine thoracic aorta as a first step, we investigated the difference between the crack initiation site and other areas with regard to local strain, collagen content, and deformation of elastin.

METHODS

To apply stretch to aortic specimens and observe their microstructure simultaneously, we performed tensile test under a microscope. Since aortic walls *in vivo* are in biaxial stretch state in the circumferential and the longitudinal directions, we developed a biaxial stretch system [3].

Porcine thoracic aortas were excised and their adventitia and surrounding tissues were trimmed away. The aortas were cut into small pieces (15 mm in the longitudinal and 20 mm in the circumferential length), sandwiched with two metal frames which have a hole of 3-mm in diameter at their center, compressed ~40% in the radial direction, frozen at -80°C, and sliced into 50 μm in thickness. After being thawed at room temperature, the specimen had a thinner region around its center. The cell nuclei were stained with Hoechst 33342 as strain markers, and α -elastin was stained with its antibody (BA-4, Santa Cruz Biotechnology).

The specimen was glued on a metal frame which has a hole of 10 mm in diameter, and a metal hollow cylinder was placed above the center of the specimen in the hole. These devices and specimens were placed under a fluorescent microscope (IX-71, Olympus). By moving the metal frame 0.5 mm to the longitudinal direction of the cylinder, the specimen was pushed onto the cylinder. This caused biaxial stretch to the specimen without moving specimen from the focal plane of the microscope. After the specimen was stretched, fluorescent images of the cell nuclei and α -elastin were captured with 2 \times objective lens and CCD-camera. To observe collagen, retardance images were also captured with birefringence imaging system, pol-scope (Abrio-LS; CRi), connected to the microscope. This system is capable of analyzing the retardance as an index of collagen density. Moving the metal frame and capturing images were repeated until specimen failure. To determine the crack initiation position, bright field images of the specimen were also captured with a high speed camera (Exilim EX-F1, Casio Computer) at rate of 300 frames/s.

The 2D strain tensors were obtained locally as reported in a previous study [4]. Briefly, local displacement was measured using particle image velocimetry (Flow-vec, Library) from the images of the cell nuclei. From positions of a measured point and its neighboring points before and after each displacement, 2D strain tensor was locally calculated assuming infinitesimal strain theory. Cumulative strain at each step from zero load state was then calculated. Finally, the maximum and the minimum principal strains, the maximum shear strain, and areal strains were determined from the strain tensor. This method was validated using the silicone rubber sheet as an example of homogeneous and isotropic material [4].

RESULTS AND DISCUSSION

Figure 1 shows typical images of the rupturing specimen of a porcine thoracic aorta. We observed the crack initiation and its propagation successfully. After the crack initiation (Fig. 1b), it expanded gradually. In this specimen, some other cracks generated (Fig. 1c) and the cracks connected each other (Fig. 1d), causing failure of the specimen.

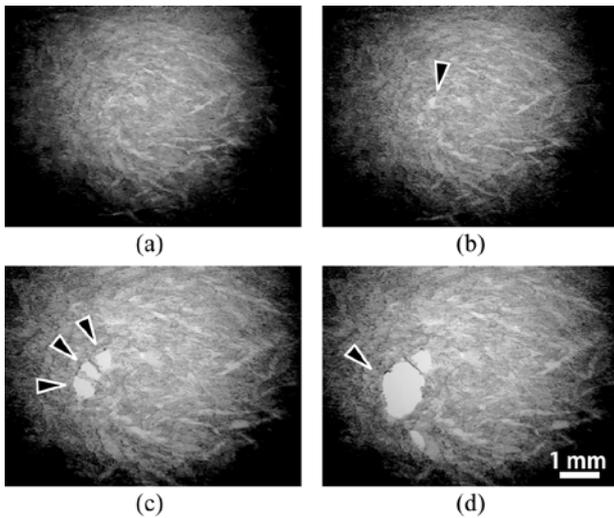


Figure 1: Typical images of a specimen of rupturing porcine thoracic aorta. After a crack generated at the center area (b), the number of cracks increased (c) and the cracks connected each other (d). Arrow heads show the cracks.

Figure 2 shows distributions of various strain measures just before failure. The maximum principal strain, the areal strain, and the maximum shear strain were higher around the crack initiation position. We cannot say that those strain parameters are critical factors for aortic rupture because the crack did not initiate at the position where strain parameters were highest. However, those higher strains may be a candidate for cause of rupture.

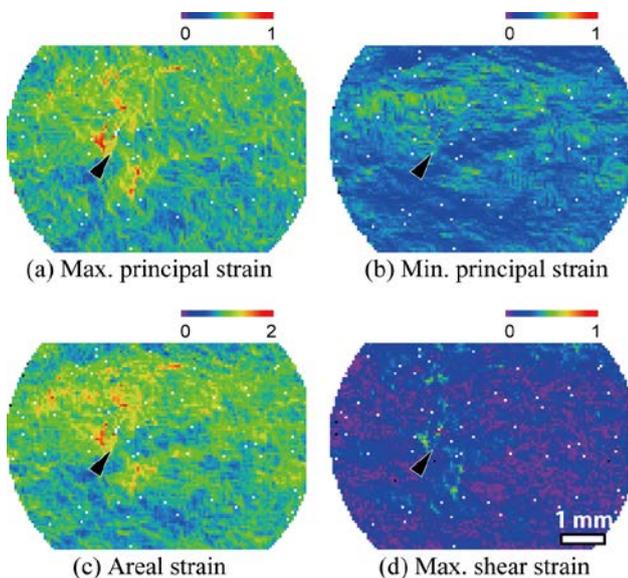


Figure 2: Strain distributions in the specimen shown in Fig. 1(a). Arrow heads show the crack initiation position.

Figures 3a and 3c show retardance images, which is an index of collagen density. Since collagen fibers have high tensile strength in the aorta, we had expected that crack would initiate at low retardance areas. However, unlike our expectation, the retardance value at the crack initiation position was not so low compared with adjacent areas (Fig. 3c). Figures 3b and 3d show the fluorescent images of α -elastin. Around the crack initiation position, we found the slight failure of elastin network shown in black area in Fig 3b and 3d (white arrows). This failure of elastin network might have caused higher values of the maximum principal

strain, areal strain, and the maximum shear strain in Fig. 2. Since the composition of elastin in aneurysms was reported to be low [5], strength of the aorta might be determined by local elastin volume.

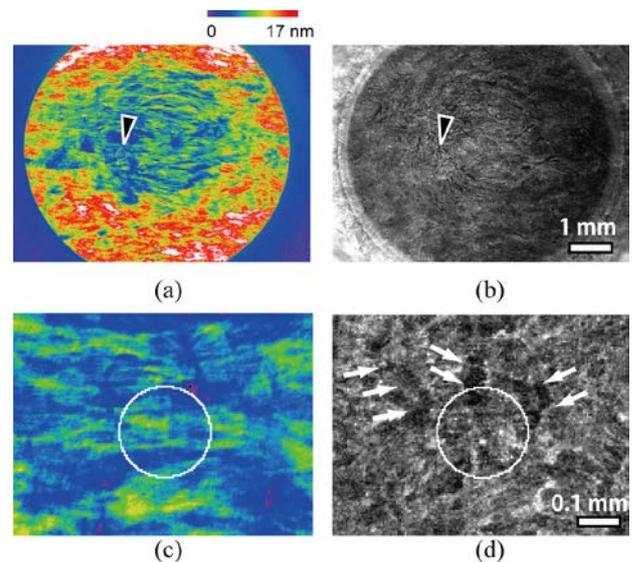


Figure 3: Typical images of retardance (a) and elastin (b) and their enlarged view around crack initiation position (c and d) in the specimen shown in Figs. 1(a) and 2. Arrow heads in (a) and (b) and the center points of the white circles in (c) and (d) show the crack initiation position. White arrows show failure of elastin network.

CONCLUSIONS

In order to study the crack initiation process in detail in the aorta, we developed biaxial stretch system placed on a microscope, and applied it to healthy porcine thoracic aortas. The maximum principal strain, areal strain, and the maximum shear strain were higher around the crack initiation position. The cracks of elastin were also observed there, which might be a cause of aortic failure.

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