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MECHANICAL CHARACTERIZATION OF CALCIFICAITON IN DISEASED CORONARY ARTERY WITH ATOMIC FORCE MICROSCOPE

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ABSTRACT

In this work, the mechanical propertied of calcification in diseased coronary artery was evaluated with atomic force microscope (AFM). The heavily calcified coronary artery was harvest from a cadaver's heart. The artery slices with thickness of 10 um were prepared with cryosectioning. Staining with Alizarin Red has been performed to highlight the calcification region. Results have shown that the calcified areas have a significant larger stiffness compared with the surrounding plaque and the media layer of a healthy artery. The calcification showed a heterogeneous property with larger deviation in stiffness distribution. The staining process affected the mechanical properties. Results will enhance the mechanical property database in the literature.

Keywords: calcification, coronary artery, AFM, mechanical characterization, atherosclerosis.

1. INTRODUCTION

The presence of stiff calcific deposits in an atherosclerotic coronary artery induces larger resistance during stenting, further leads to stent underexpansion and malapposition. The comprehension of mechanical properties of calcification can help to understand the stent-artery interaction with the heavily calcified coronary artery. Our previous computational studies have shown that the calcification induces a larger resistance during stenting and post-dilation, and further leads to the stent underexpansion, malapposition, and even higher risk of vessel rupture **[1–3]**. The uniaxial tension test of the calcified artery along the circumferential direction also have shown that the large volume of calcification reduces the stretch capability of the vessel and predisposes the vessel to rupture **[4]**. It has been well known that the calcification has various types and pathologies **[5]**. However, the mechanical characterization of the calcification, especially in coronary arteries, is lacking in the literature. The atomic force microscope (AFM) has shown its advantage in quantifying the regional mechanical properties of the plaque in an artery **[6]**. In this work, the local mechanical properties of the calcification will be quantified with the AFM scanning.

2. MATERIALS AND METHODS

The calcified coronary arteries were harvest from a cadaver's heart, then preserved at the -80℃ until cryosectioning for AFM scanning.

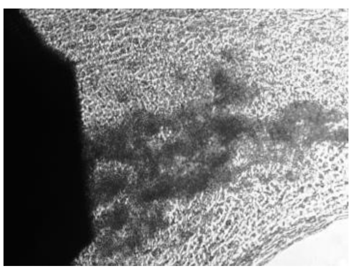
2.1 Sample preparation

The arteries were embedded in optimal cutting temperature compound (OCT, Tissue-Tek, Torrance, CA) for sectioning and immediately frozen until usage. Transverse cryostat sectioning was taken with a thickness of 10 um, selected to enhance the sample's adhesion to the microscope slide, and avoid surface corrugation. The artery slices were stored at a temperature of -20°C until further use. Before the AFM testing, the tissue sections were thawed at room temperature for 10 min, and then the coverslips were submerged in Deionized (DI) water for 30 minutes to rinse away residuals of OCT cryo-gel and glycerin. Alizarin Red staining was performed on every other slice for identifying the calcification region and serving as references for neighboring slices.

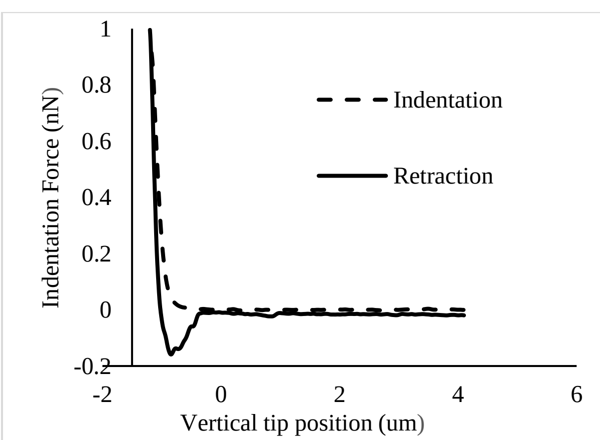
2.2 AFM scanning

The setup of AFM scanning is shown in FIGURE 1. The Axio Observer inverted microscope (Carl Zeiss, Göttingen, Germany) was used to determine the targeted location for AFM scanning. The test was performed using the atomic force microscope (AFM) (Nanowizard III, JPK Instruments AG, Germany) to determine the elastic modulus of calcification in diseased artery and media layer in healthy artery as control (FIGURE 1a). Tip radius of 5 um (JPK, SAA-SPH-5UM) was adopted for the AFM test. For each group, two locations were selected for AFM scanning and for each location, 64 (8x8) force curves were generated within scanning area of 5x5 μm^2. Tip radius of 20 nm (SAA-Fluid) was adopted to investigate the influence of scanning area on the stiffness measurement by reducing the scanning area from 20x20 μm^2 to 10x10 μm^2 and 5x5 μm^2. The stiffnesses were quantified by post processing the force-displacement curves (FIGURE 1b), using the Hertz Model:

$$E = \frac{F*3*(1-v^2)}{4\sqrt{R}\,\delta^{3/2}} \qquad (1)$$



(a)



(b)

FIGURE 1: (a) AFM scanning setup and (b) representative force-indentation curve

3. RESULTS AND DISCUSSION

The calcium deposition in the sliced artery was determined with Alizarin Red staining. Arteries with presence of darker red spots were classified as diseased arteries and arteries with no presence of red marks were characterized as healthy. Then mechanical properties were quantified with different tip size and scanning area.

3.1 Calcified region determined by Alizarin Red staining.

The presence of calcification in the sliced diseased artery was determined and compared with the control group i.e., healthy artery (FIGURE 2). Calcification was clearly detected in the diseased artery, while minimal calcification was observed in the healthy artery. The intensity of the red color is heterogeneous across the vessel, which indicates the calcification is not a uniform component. In addition, the lipid pool was detected at this cross section. By comparing the stained cross sections with their unstained neighbor cross sections, we can easily determine the calcified region in the unstained one for AFM scanning.

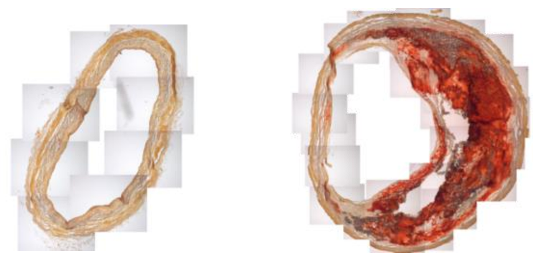


FIGURE 2: Alizarin red staining of the healthy artery (left) and calcified artery (right).

3.2 Comparation between calcification in diseased artery and media layer in healthy artery.

With a tip size of 5 um, the measured stiffness of the calcification in a diseased artery and the media layer in a healthy artery are depicted in FIGURE 3. The media layer in a healthy artery has a stiffness of 12.0 ± 4.4 kPa and the calcification in a diseased artery is 61.5 ± 35 kPa. Even the calcification showed a significant higher stiffness compared with the media layer in healthy artery, which is smaller than the reported value in a previous study, which ranges from 17 to 25 GPa [7]**.** This huge difference may result from the test protocol, the previous study tested the fully mineralized calcification after chemical fixation and dehydration, while our work tested the untreated tissue at the liquid environment. We also noticed that the staining process will affect the measurement. Following staining, the stiffness of the calcification reduced to 1.8 ± 1.1 kPa, while stiffness of media layer in healthy artery increased to 47.0 ± 18.5 kPa.

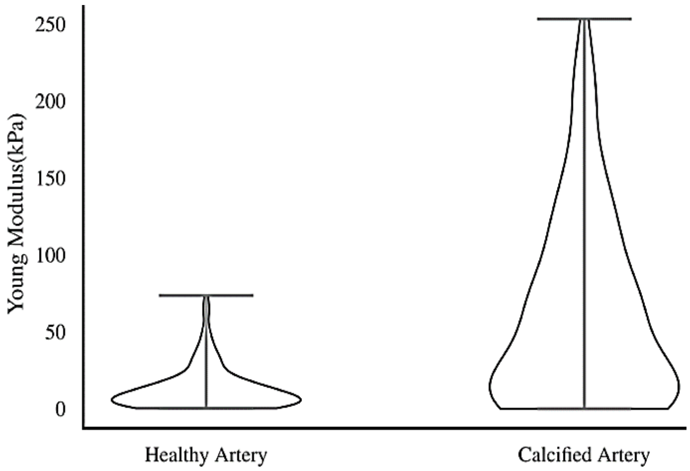


FIGURE 3: Stiffness of the calcification in diseased artery and media layer in healthy artery.

3.3 Heterogeneity quantified with AFM

With a tip size of 20 nm, the heterogeneity of the calcification was quantified by changing the scanning area from 20 um to 10 um and 5 um. For each scanning area, more than 50 force curves were generated. The histogram of the measured stiffness for each scanning area is shown in FIGURE 4. The stiffness interval with the highest frequency varied for different scanning area which ranges from 127 kPa to 743 kPa. It is observed that the averaged measurement significantly varied site by site and was depended on the scanning size.

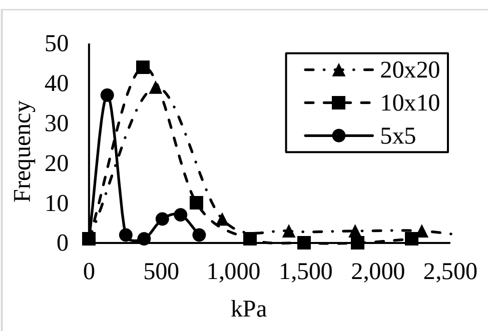


FIGURE 4: Measured stiffness with scanning area of 20x20 μm^2, 10x10 μm^2, 5x5 μm^2.

4. CONCLUSION

The local mechanical properties of the calcification in diseased coronary arteries were quantified with AFM scanning. The calcification showed a significantly higher stiffness compared with the media layer in healthy artery. The effects of the tip size and scanning area on the stiffness measurement were quantify. This study can provide new insight in future practices and treatments for heavily calcified arteries.

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