

Biological Ferroelectricity uncovered in Aortic Walls by Piezoresponse Force Microscopy

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Supplementary Information

This document includes 4 figures (Fig. S1-S4)

Experimental Methods

Fresh porcine descending thoracic aortas were harvested from a slaughterhouse and transported on ice to lab. Immediately after arrival, the aortas were cleaned off adherent tissues and fat, dissected, and rinsed in DI water. Samples of about 2cm×1cm were cut from the middle section of the aorta. For histological studies, samples were fixed in 10% formalin buffer and then embedded in paraffin. Sections of about 6μm in thickness were cut and stained. Movat's pentachrome stain stains were performed to examine the presence of cells, elastin and collagen. To prepare the sample for PFM, tissue samples were fixed with 4% paraformaldehyde in PBS for 1 hour at room temperature. The fixed samples were dehydrated in a graded distilled water/ethanol series: 30%, 50%, 70%, and 100% for 15 minutes each, followed by washes with a graded ethanol/HMDS series: 30%, 50%, 70%, and 100% for 15 minutes each, and finally allowed to dry overnight. It was then cut to 8mm×8mm piece and glued on silicon substrate sputtered with 100nm thick gold using silver paint for PFM studies.

Asylum Research MPF-3D atomic force microscope was used for PFM studies. The probe was made by silicon, with the lever coated by Al and the tip coated with Ti/Ir (3/15), and the radius of tip is 23 ± 10 nm. The resonance frequency of the lever in air is 70 kHz and the spring constant of lever is 2 N/m. All the topography and PFM mappings were acquired using contact mode. During the switching PFM and SSPFM, two cycles of DC bias were applied, starting from negative maximum towards to the positive one, with the period set to be 5s and each 'on' and 'off' steps maintained at 50ms. All the data shown in the paper were obtained in the second cycle.

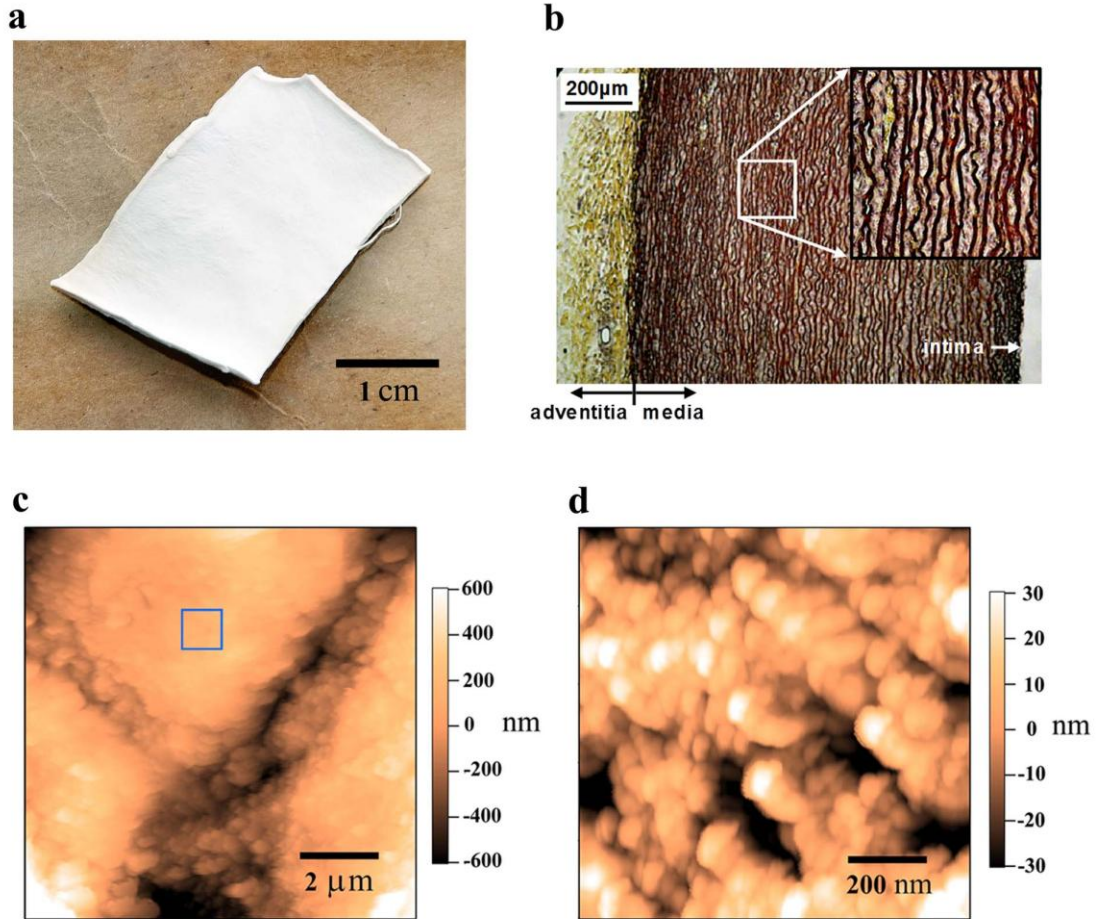


Figure S1 Structure of aortic wall; (a) photo of the aortic tissue sample with its inner wall on top; (b) histological image of the cross-section of artery; (c) AFM topography mapping of inner wall over a $10 \times 10 \mu\text{m}^2$ area; and (d) AFM topography mapping of inner wall zoomed-in over a $1 \times 1 \mu\text{m}^2$ area.

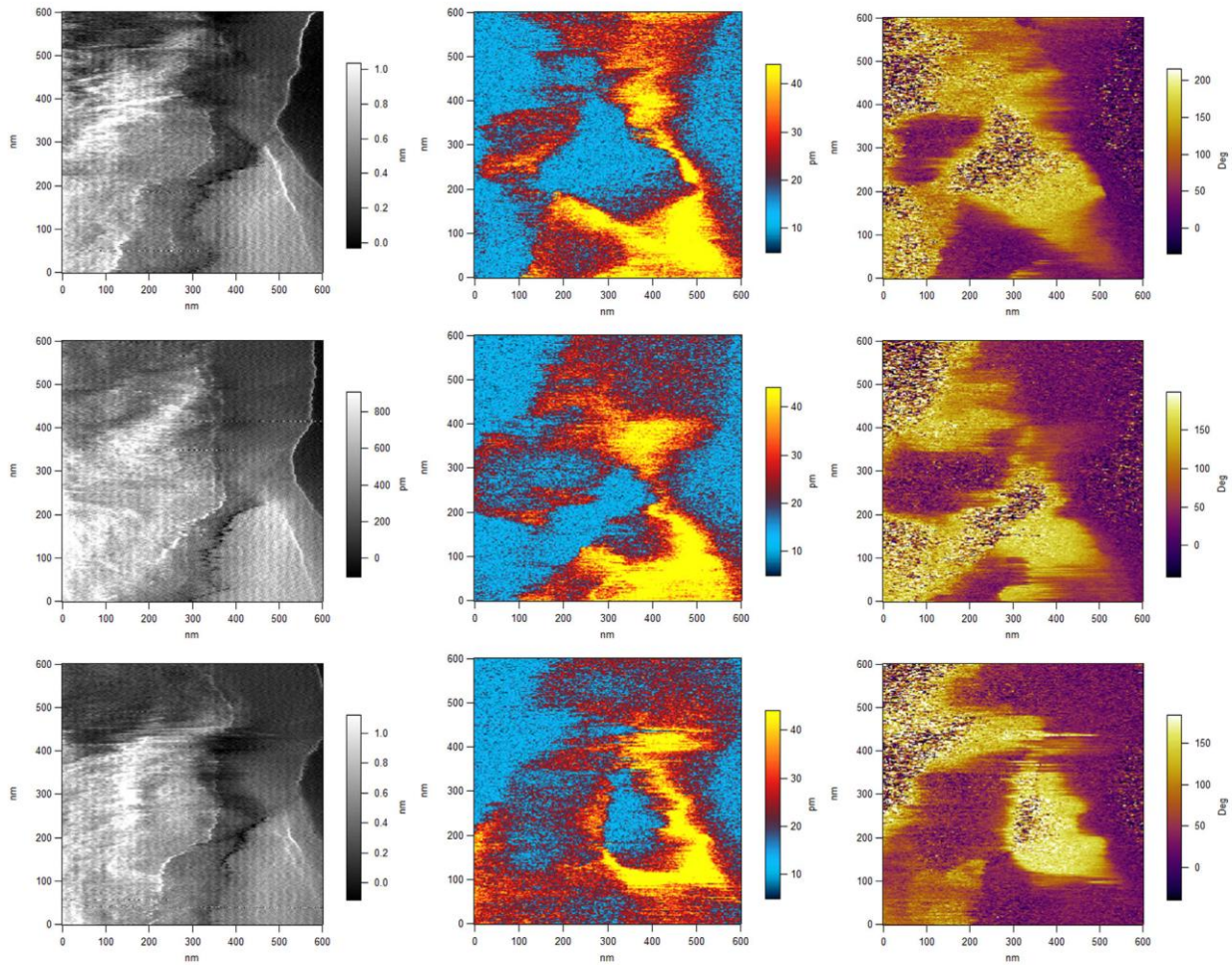


Figure S2 Three consecutive vertical PFM scans in a $600 \times 600 \text{ nm}^2$ area using a 15V AC drive voltage of 261.65kHz. The deflection images on the left indicate that there is slight right shift in topography during scanning. The amplitude mappings in the middle are stable with small evolution during different scanning. Contrast of approximately 140° is observed in the phase mappings on the right, indicating different orientations of polarization. Between different scans, small evolution in phase contrast is also observed, indicating possible ferroelectric switching.

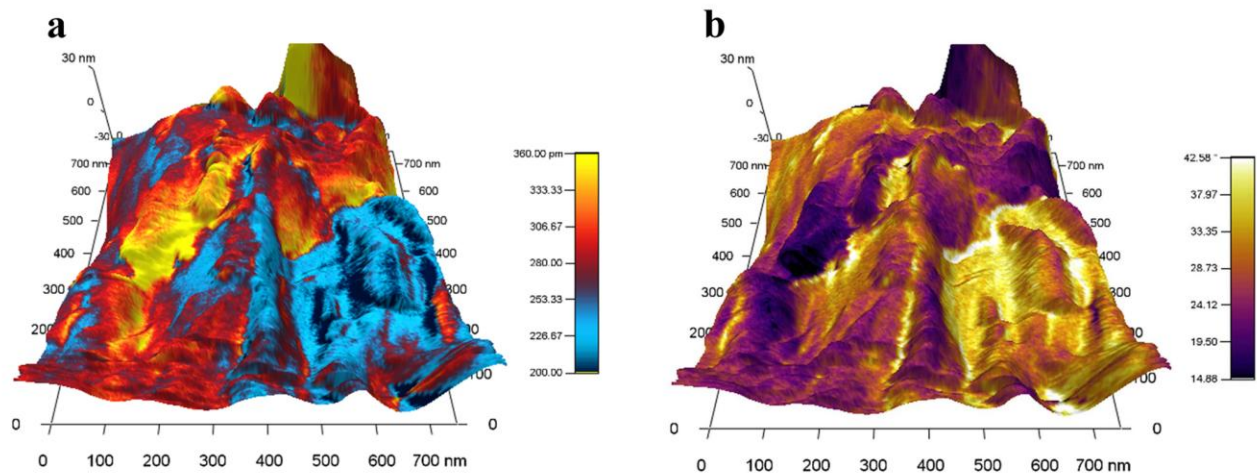


Figure S3 Additional PFM mappings corresponding to those shown in Fig. 3; (a) PFM amplitude mapping at resonance before corrected by quality factor, showing substantially higher response; (b) PFM phase mapping at resonant frequency; little variation is observed in phase contrast, indicating small variation in polar orientation distribution in the probed area.

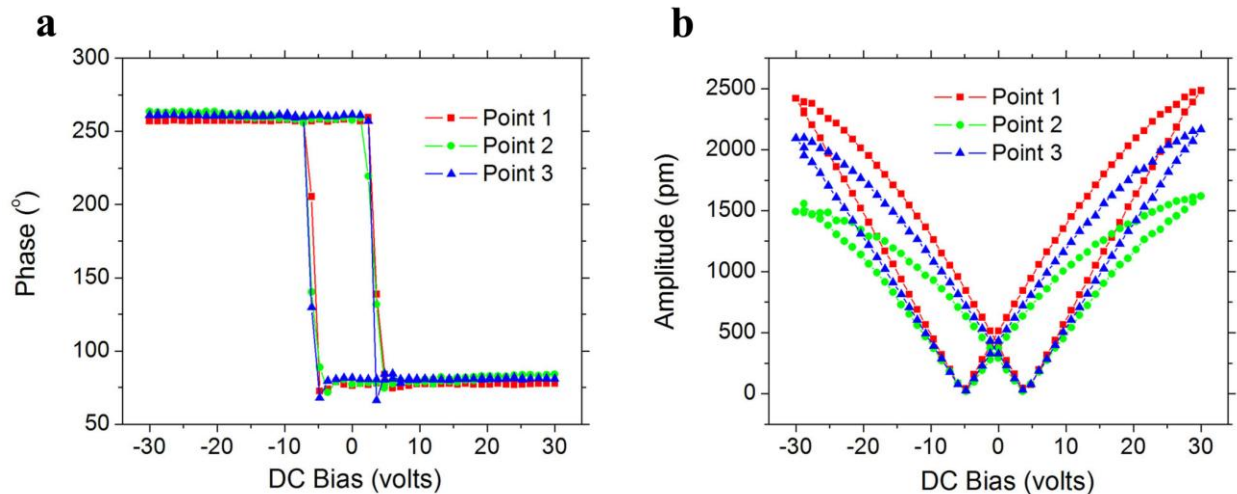


Figure S4 PFM phase-voltage hysteresis loop (a) and amplitude-voltage butterfly loop measured during “on” state, corresponding to those loops shown in Fig. 4. The coercive voltage is observed to be much smaller, and the PFM responses are more than 150% higher and do not saturate at high voltage, suggesting that the response measured during “on” state is dominated by electrostatic interactions.