Supplemental Information

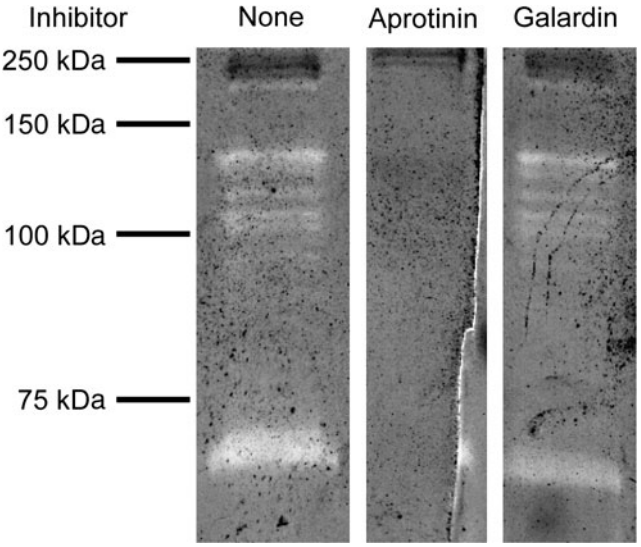
Materials and Methods

Fabrication of fibrin-based tubular constructs

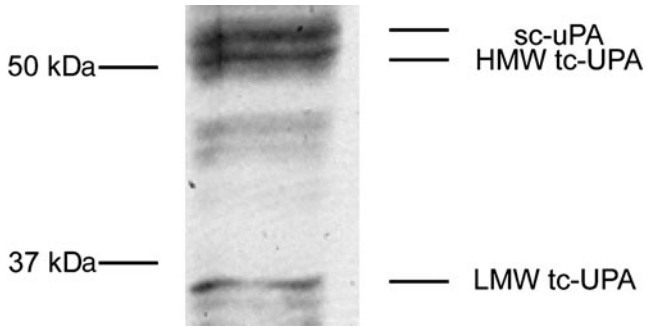
The fabrication of tubular constructs has been previously described.[1] The constructs were prepared using an identical fibrin gel mixture, as described in the primary text, and injected into a tubular mold 7.5 cm long with a 9 mm outer diameter and a 2 mm inner glass mandrel. Constructs were incubated statically on the mandrel. The culture medium was identical to that described in the primary text.

Immunoblotting

Lysates were prepared from vascular smooth muscle cells using the lysis buffer and sonication protocol previously described[2] and 20 μg of total protein (determined by BCA assay; Thermo Fisher Scientific) was analyzed. For medium samples, equal volumes were loaded in each lane. Western blotting was performed using the SNAP i.d. apparatus (Millipore), Superblock (Thermo Fisher Scientific) + 0.1% Tween-20 as blocking agent, primary antibody (mouse monoclonal to urokinase, ab8473; Abcam Inc.) diluted 1:1000 in block, and secondary antibody (horseradish-peroxidase-conjugated anti-mouse or goat immunoglobulin G; Jackson ImmunoResearch) diluted 1:5000 in TBS-T.



SUPPLEMENTAL FIG. S1. Zymographic activity is sensitive to serine protease inhibition, but not metalloproteinase inhibition. Medium samples conditioned by a construct cultured in 3 mM ε-aminocaproic acid (ACA) for 6 days were analyzed by zymography, with the indicated inhibitor added to the overnight digest buffer. The pattern of negatively staining bands was identical whether or not Galardin (25 μM) was added to the digest buffer, but was eliminated with the addition of aprotinin (1 μg/mL).



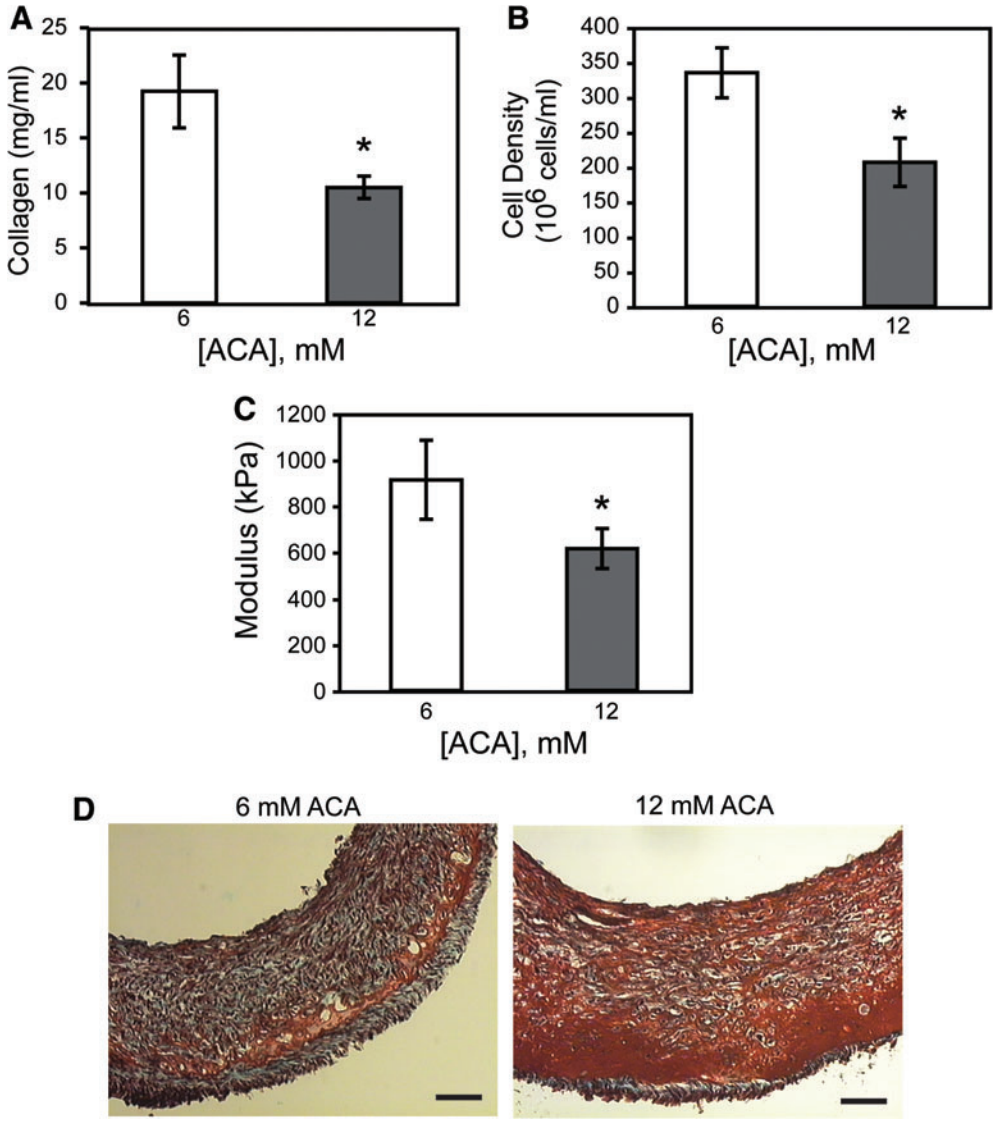
SUPPLEMENTAL FIG. S2. Urokinase is present in the vascular smooth muscle cell culture system. Vascular smooth muscle cells express urokinase plasminogen activator (uPA). Bands of representative molecular weights for the single chain (sc-) and high (HMW) and low (LMW) molecular weight two-chain (tc-) uPA isoforms are indicated.

Mechanical testing

Tubular constructs were sectioned into 2–3-mm-long rings and mechanically tested using a Microbionix mechanical testing system (MTS Systems, Eden Prairie, MN). Before testing, tissue thickness was measured using a 50-*g* force probe attached to a displacement transducer. Rings were mounted and tested as previously described.[1] True strain was calculated based on the change in length of the tissue over time. The stress was calculated as force divided by the initial cross-sectional area. The elastic modulus was determined by linear regression of the linear region of the stress–strain curve.

References

1. Isenberg, B.C., Williams, C., and Tranquillo, R.T. Endothelialization and flow conditioning of fibrin-based media-equivalents. Ann Biomed Eng **34,** 971, 2006.
2. Syedain, Z.H., Weinberg, J.S., and Tranquillo, R.T. Cyclic distension of fibrin-based tissue constructs: evidence of adaptation during growth of engineered connective tissue. Proc Natl Acad Sci U S A **105,** 6537, 2008.



SUPPLEMENTAL FIG. S3. ACA decreases collagen and cell density, as well as the stiffness, of tubular fibrin-based tissue constructs after 5 weeks in culture. (**A**) Collagen density and (**B**) cell density of constructs were decreased with high ACA treatment. (**C**) High ACA decreased the Young's modulus of tubular tissue constructs. Values are mean ± standard deviation for six samples. *Indicates a significant difference ($p < 0.05$) compared to the 6 mM ACA constructs. (**D**) Lillie's trichrome staining of tubular constructs shows decreased fibrin remodeling with high ACA treatment. The luminal surface of the tube is oriented toward the upper left. Collagen is stained green, residual fibrin and other noncollagenous proteins are red, and nuclei are black. The low ACA construct has visible collagen staining and more extensive remodeling than the high ACA constructs. Scale bars = 20 μm.