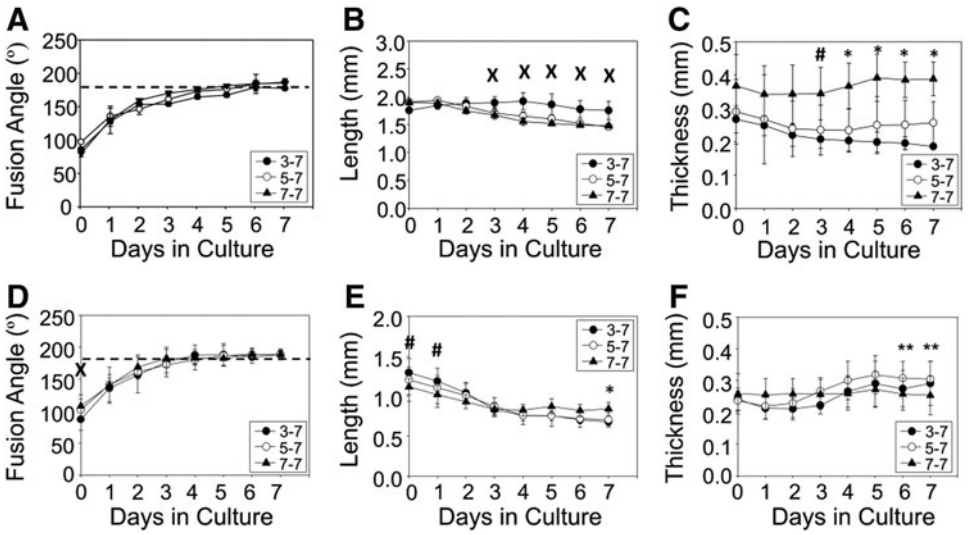
Supplementary Data

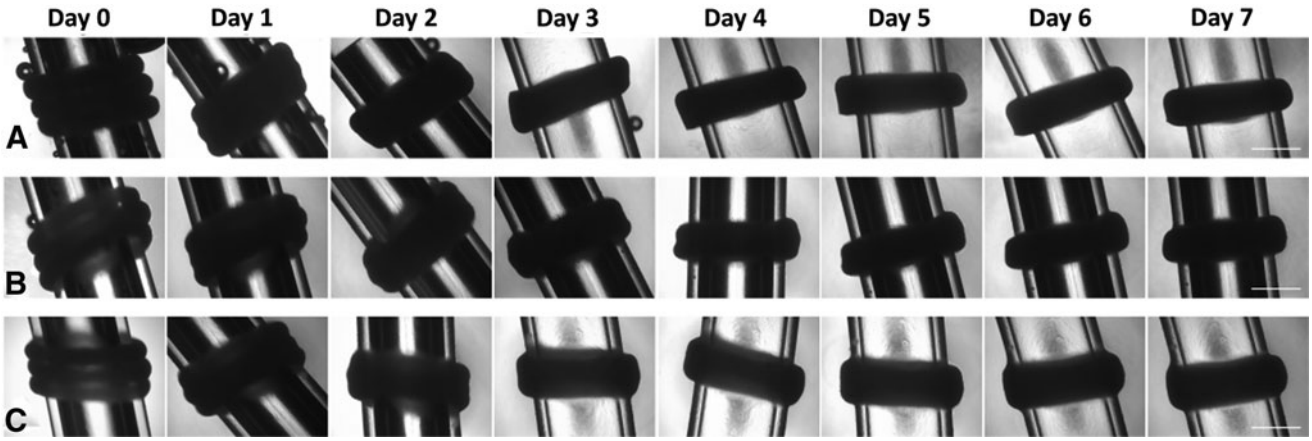
Supplementary Methods

Cell culture

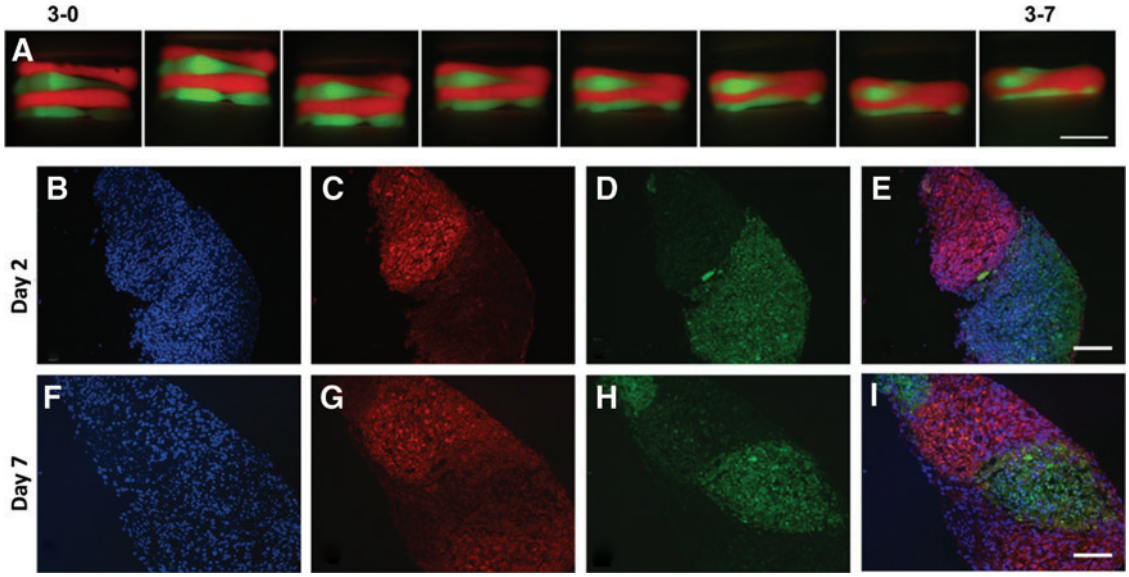
Human coronary artery smooth muscle cells (SMCs; Lonza) were cultured and maintained in complete SMC growth medium (SmGM-2; Lonza) containing 5% FBS, 2 ng/mL fibroblast growth factor-basic, 0.5 ng/mL epidermal growth factor, insulin, 30 μg/mL gentamicin, and 15 ng/mL amphotericin B. Rings were seeded as described in the Cell culture section.



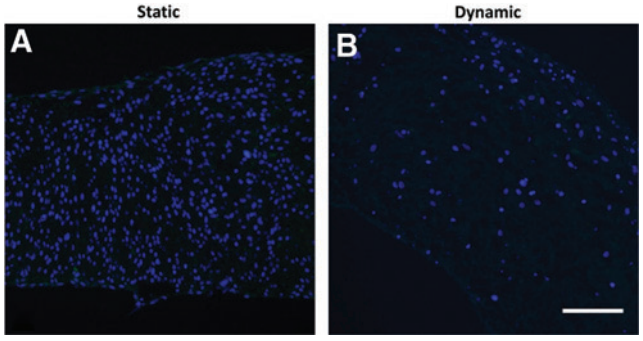
SUPPLEMENTARY FIG. S1. Fusion of human SMC rings. Fusion kinetics from duplicate experiments with human aortic SMCs shown in **(A–C)**, and with human coronary artery SMCs shown in **(D–F)**. Fusion angles **(B, D)**, tube length **(C, E),** and thickness **(D, F)** as a function of time for tubes fabricated from rings cultured for 3, 5, or 7 days before 7 days as tubes. $^{*}p<0.05$ for 7–7 versus 3–7 and 5–7, $^{**}p<0.05$ for 5–7 versus 7–7, $^{x}p<0.05$ for 3–7 versus 5–7 and 7–7, and $^{\#}p<0.05$ for 3–7 versus 7–7 groups, $n=3$. *Dashed line* = 180°. SMC, smooth muscle cell.



SUPPLEMENTARY FIG. S2. Fusion of human coronary artery SMC rings. Phase contrast images of 3–7 **(A)**, 5–7 **(B)**, and 7–7 **(C)** tubes over a 7-day fusion period. Scale = 1 mm. Images representative of $n=3$ samples per group.



SUPPLEMENTARY FIG. S3. Fluorescent images of human coronary artery SMC ring fusion. Rings with alternating *red* and *green* cell tracker were allowed to fuse for 7 days **(A)** before sectioning and Hoechst staining. Samples were sectioned after 2 **(B–E)** or 7 **(F–I)** days to determine whether cells within ring units maintain their spatial position. *Blue* = nuclei **(B, F)**. *Red* = CellTracker *Red* **(C, G)**, *green* = CellTracker *Green* **(D, H)**, and merged image shown in **(E, I)**. Lumen on *left*. Scale = 1 mm **(A)** or 100 μm **(B–I)**. Images representative of $n = 3$ samples per group per time point.



SUPPLEMENTARY FIG. S4. Contractile protein expression in aortic SMC tubes. Tubes fabricated from human aortic SMCs were either kept in static conditions for 7 days **(A)**, or kept in static conditions for 7 days followed by 7 days of dynamic culture with ~ 12 dyne/cm^2 of applied shear stress **(B)**. *Green* = smooth muscle alpha actin, *blue* = nuclei. Lumen on *bottom* of image. Scale = 100 μm.