

Title: A biomimetic microfluidic model to study signalling between endothelial and vascular smooth muscle cells under hemodynamic conditions

Running title: Micromimick of the hemodynamic EC-VSMC signalling niche

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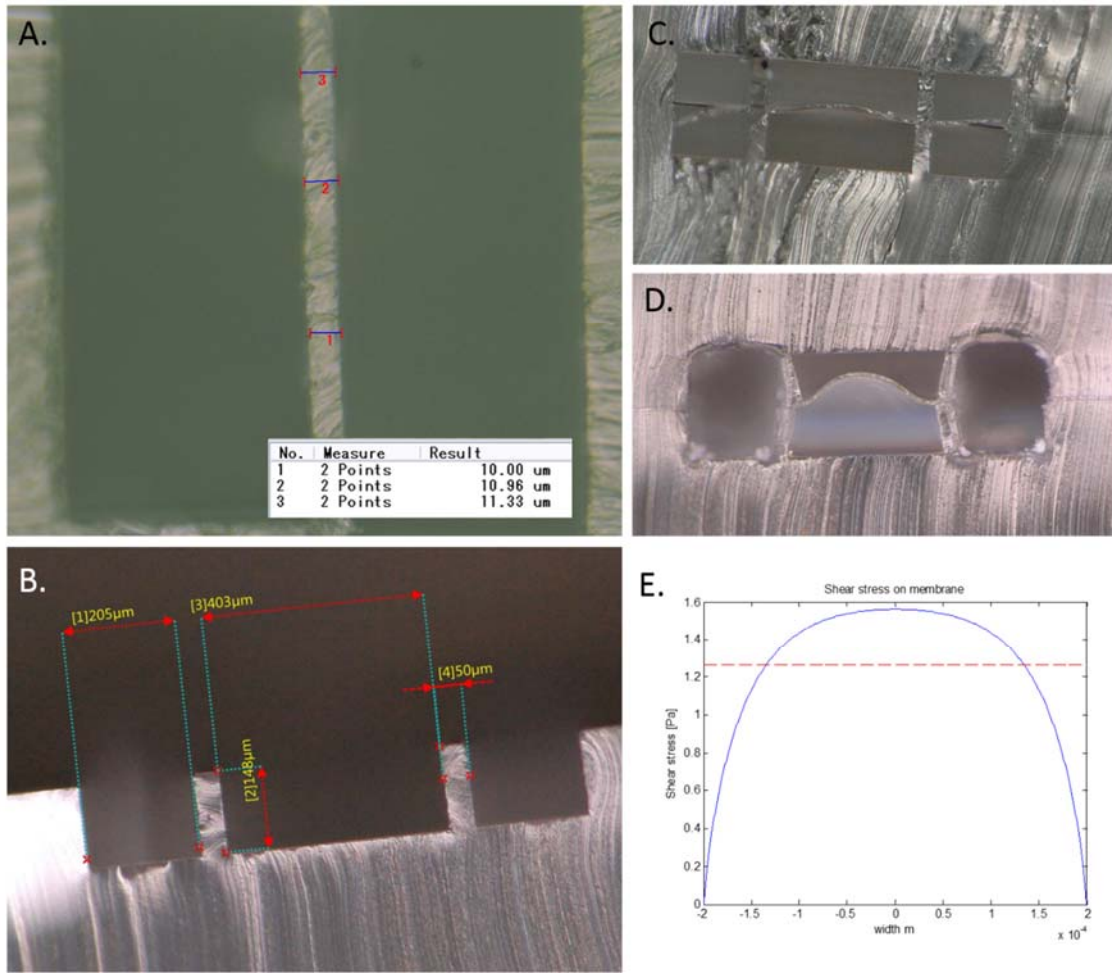
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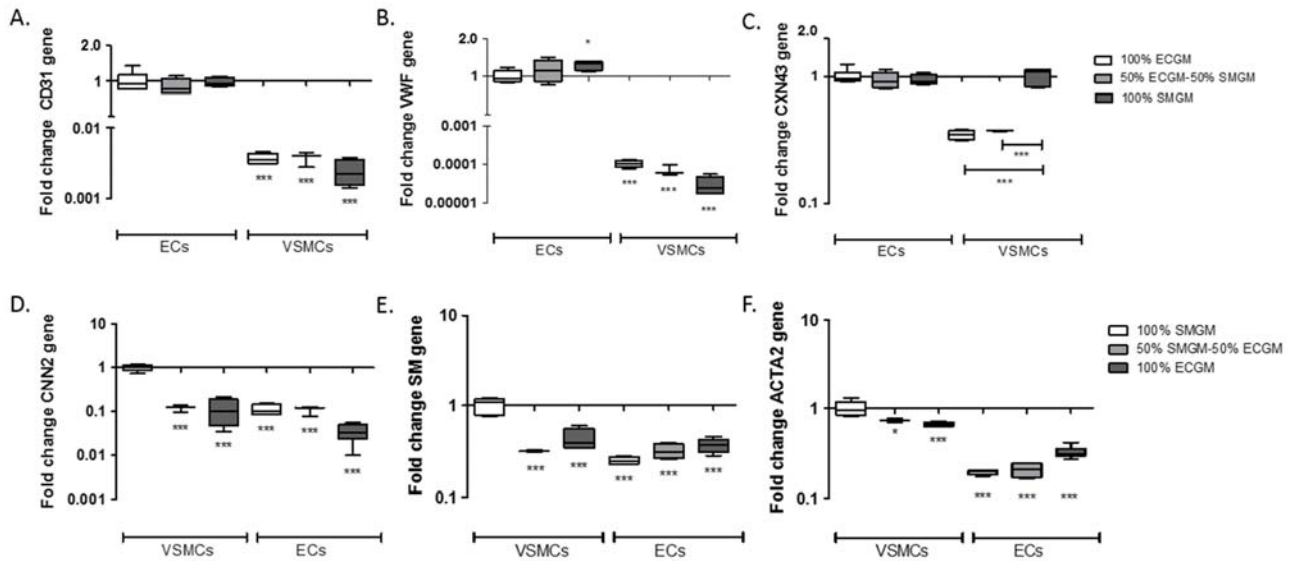
Supplemental table/figures

Supplemental Table 1. Primer sequences used for qPCR.

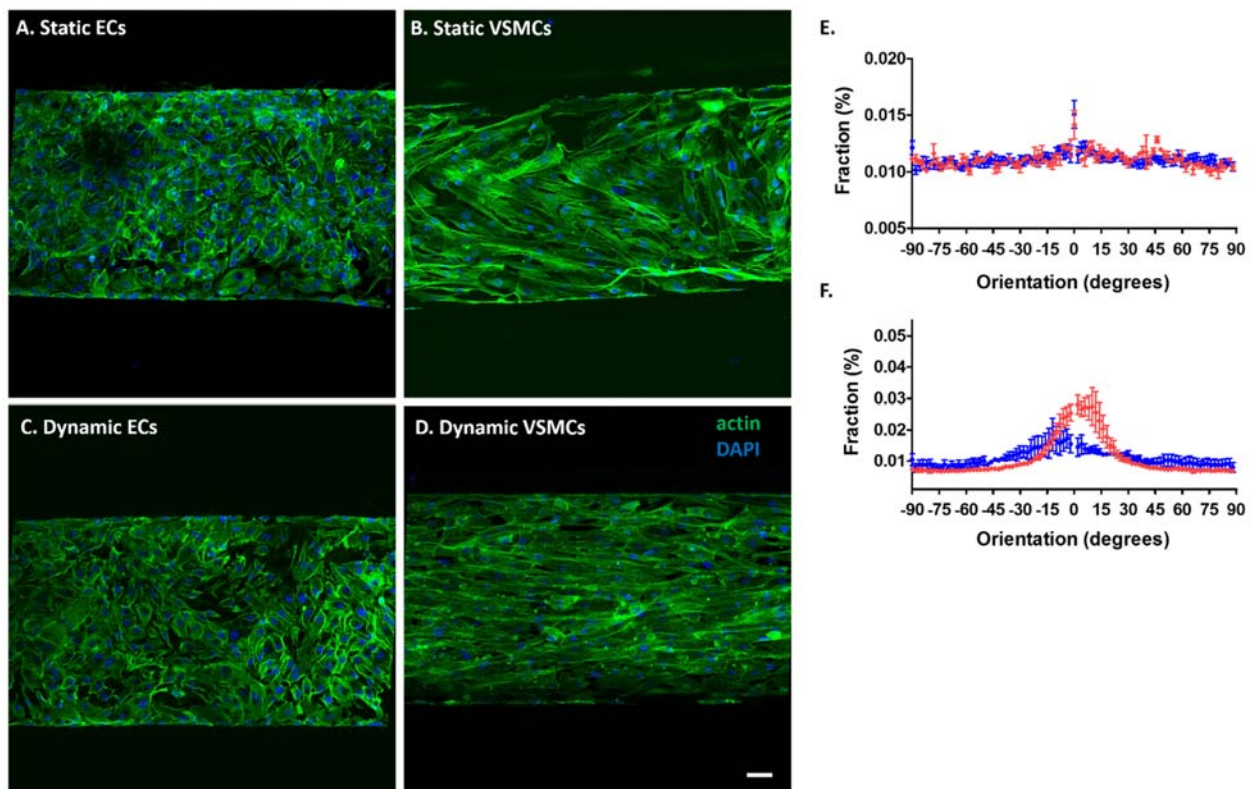
Target	Primer sequence (5' to 3')	
<i>Smooth muscle markers</i>		
α-SMA	FW=CGTGTTGCCCTGAAGAGCAT	RV=ACCGCCTGGATAGCCACATACA
Calponin	FW=TGAAGTACGCAGAGAAGCAG	RV=CAGCTTGGGGTCGTAGAG
Smoothelin	FW=CAGCCCAGAACCGAGAGTC	RV=AGCAGCCATAGGAGAATCAGAT
Connexin43	FW=TTTCTTCAAGGGCGTTAAGGATC	RV=AGGAGGAGACATAGGCGAGAG
<i>Endothelial markers</i>		
CD31	FW=AAGGAACAGGAGGGAGAGTATTA	RV=GTATTTTGCTTCTGGGGACACT
VWF	FW=CCGATGCAGCCTTTTCGGA	RV=TCTGGAAGTCCCAATAATCGAG
<i>Extracellular protein markers</i>		
FN	FW=AAGACCAGCAGAGGCATAAAGG	RV=CACTCATCTCCAACGGCATAATG
COL1	FW=TGGCTCTCCTGGTGAACAAG	RV=GCCAGGGAGACCGTTGAG
COL3	FW=ATCTTGGTCAGTCCTATGC	RV=TGGAATTTCTGGGTTGGG



Supplemental Figure 1. A. PDMS membrane thickness measurements. B. Measurements of the dimensions of a cross-section of the device half. C. Cross-section of the device before etching. D. Cross-section of the device after etching. E. Shear stress profile on the porous membrane. On the y-axis the shear stress experiences in Pa, at the x-axis the width of the cell culture channel.



Supplemental Figure 2. Gene expression analysis for different types of medium. Expression of endothelial markers *CD31* (A) and *VWF* (B), *CNX43* (C), and smooth muscle markers *CNN2* (D), *SM* (E) and *ACTA2* (F). ECs indicates ECs cultured on PDMS coated with FN (PDMS-FN) surfaces; VSMCs, VSMCs cultured on PDMS-FN surfaces; Cells were cultured with different types of medium, 100% smooth muscle growth medium (100% SMGM), 100% endothelial growth medium (100% ECGM) and 50% SMGM - 50% ECGM. For the endothelial markers, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ on each bar for comparison with the control ECs with 100% ECGM. For smooth muscle markers, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ on each bar for comparison with control VSMCs with 100% SMGM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ on the lines for comparison between groups, $n = 4-6$.



Supplemental Figure 3. Cell and nucleus orientation. A-D. Immunofluorescent staining of F-Actin (green) and DAPI (blue) for ECs static (A) and dynamic (C) and VSMCs static (B) and dynamic (D). Scalebar represents 50 μm . E and F. Nucleus orientation for ECs (E) and VSMCs (F) in static (blue) and dynamic (red) cultures.