TiO_2-based nanotopographical cues attenuate the restenotic phenotype in primary human vascular endothelial and smooth muscle cells

Supporting Information

5 figures, 5 pages

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S1. Optimization of anodization parameters to achieve controlled nanotube diameters

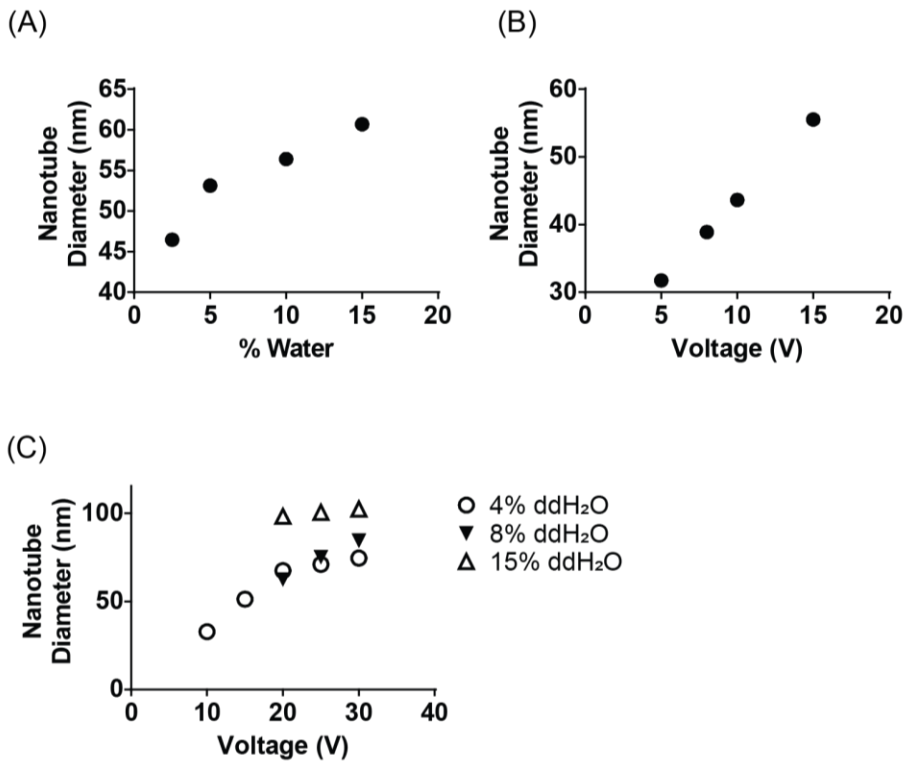

Figure S1. Voltage and electrolyte composition were adjusted to determine the optimal anodization parameters to achieve the target nanotube dimensions of 30, 50, and 90 nm. (A) Anodization voltage was kept constant at 15V, while the water content in the electrolyte was adjusted between 2.5 wt% to 15 wt%. The electrolyte contained 3 g/L ammonium fluoride, and the remainder of the electrolyte was made of up ethylene glycol (between 97.5 wt% and 85 wt% ethylene glycol). (B) Anodization voltage was tuned from 5 V to 15 V. The electrolyte composition remained constant, containing 3 g/L ammonium fluoride, 10 wt% distilled water, and 90wt% ethylene glycol. Nanotube diameter increases as voltage increases. (C) Voltage was tuned between 10 V and 30 V for anodizing TiO_2 foil in a glycerol electrolyte solution. The electrolytes contained 1.4 wt% ammonium fluoride, the indicated wt% of water, and the remaining wt% made up of glycerol.

S2. EC surface coverage on flat and NT90 surfaces over 7 days

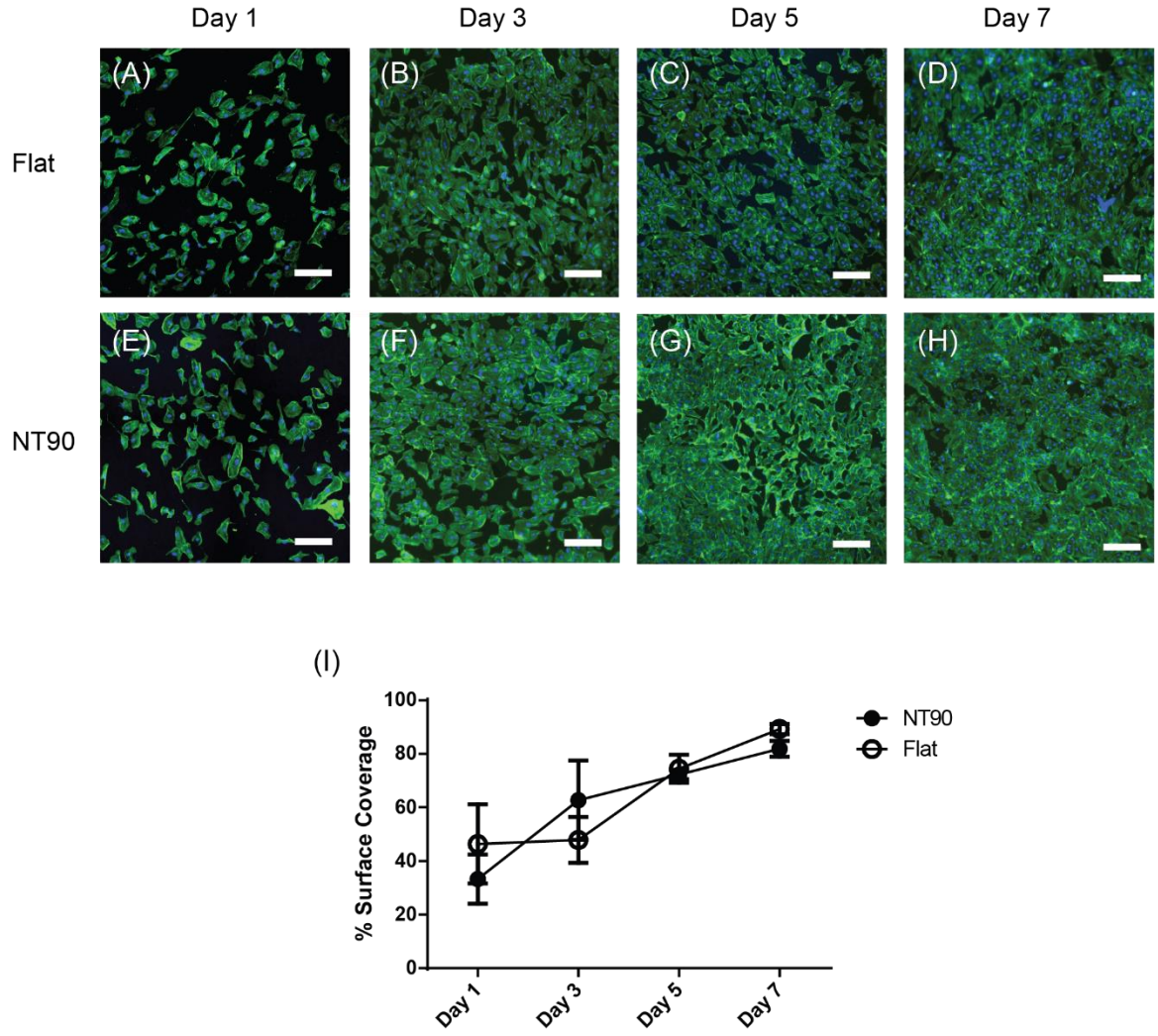

Figure S2. ECs were cultured on (A-D) flat and (E-H) NT90 surfaces for 1, 3, 5 or 7 days. At the end of each time point, the cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and stained with phalloidin (green) and DAPI (blue). Cells were imaged fluorescence microscopy. Cell coverage shown in (I) was quantified by thresholding the images in ImageJ and measuring the percentage of the field of view covered by endothelial cells. Scale bar: 200 μm.

S3. The effect of NT90 surfaces on CCL-2 expression and collagen I production in smooth muscle cells

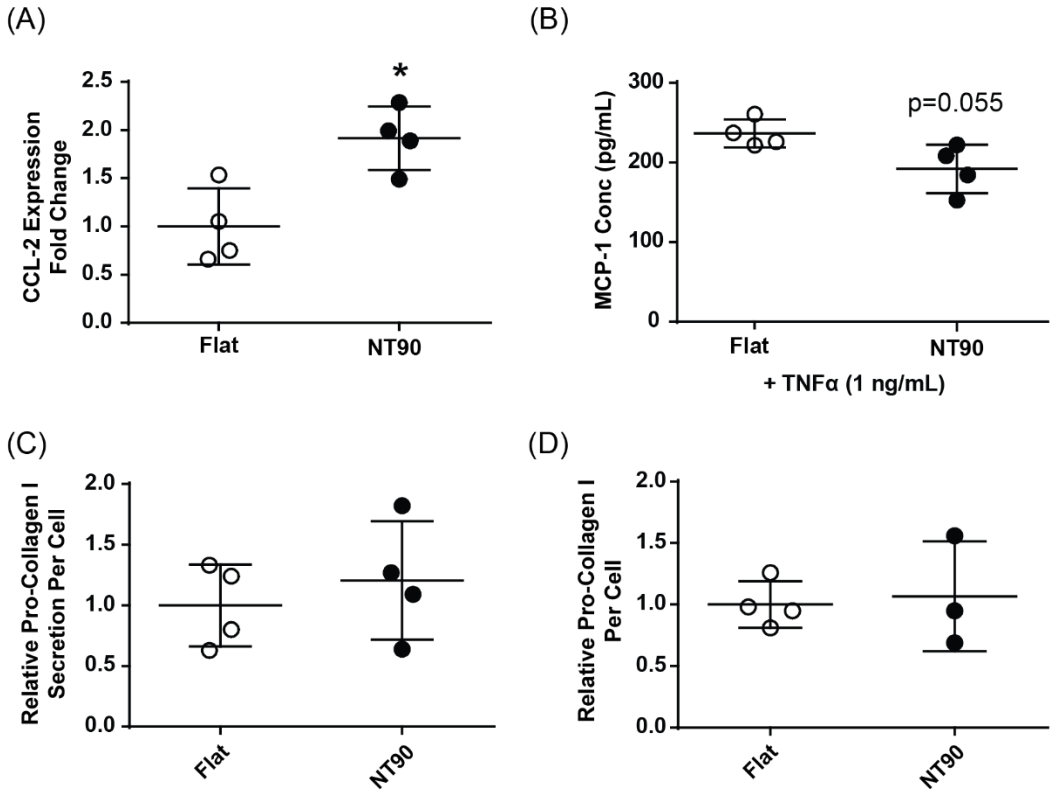

Figure S3 (A) SMCs cultured on NT surfaces increases the gene expression of CCL-2, which encodes the protein MCP-1. (B) Secretion of MCP-1 from SMCs cultured on flat or NT surfaces, in the presence of inflammatory cytokine stimulation (1 ng/mL TNFα). Pro-collagen I content of SMCs cultured on flat or NT90 surfaces was quantified using ELISA. Pro-collagen I concentration in (C) conditioned media, (D) cell extract are shown. Data are expressed as mean ± SD.

S4. Focal adhesion staining of SMC and ECs on flat and NT90 surfaces

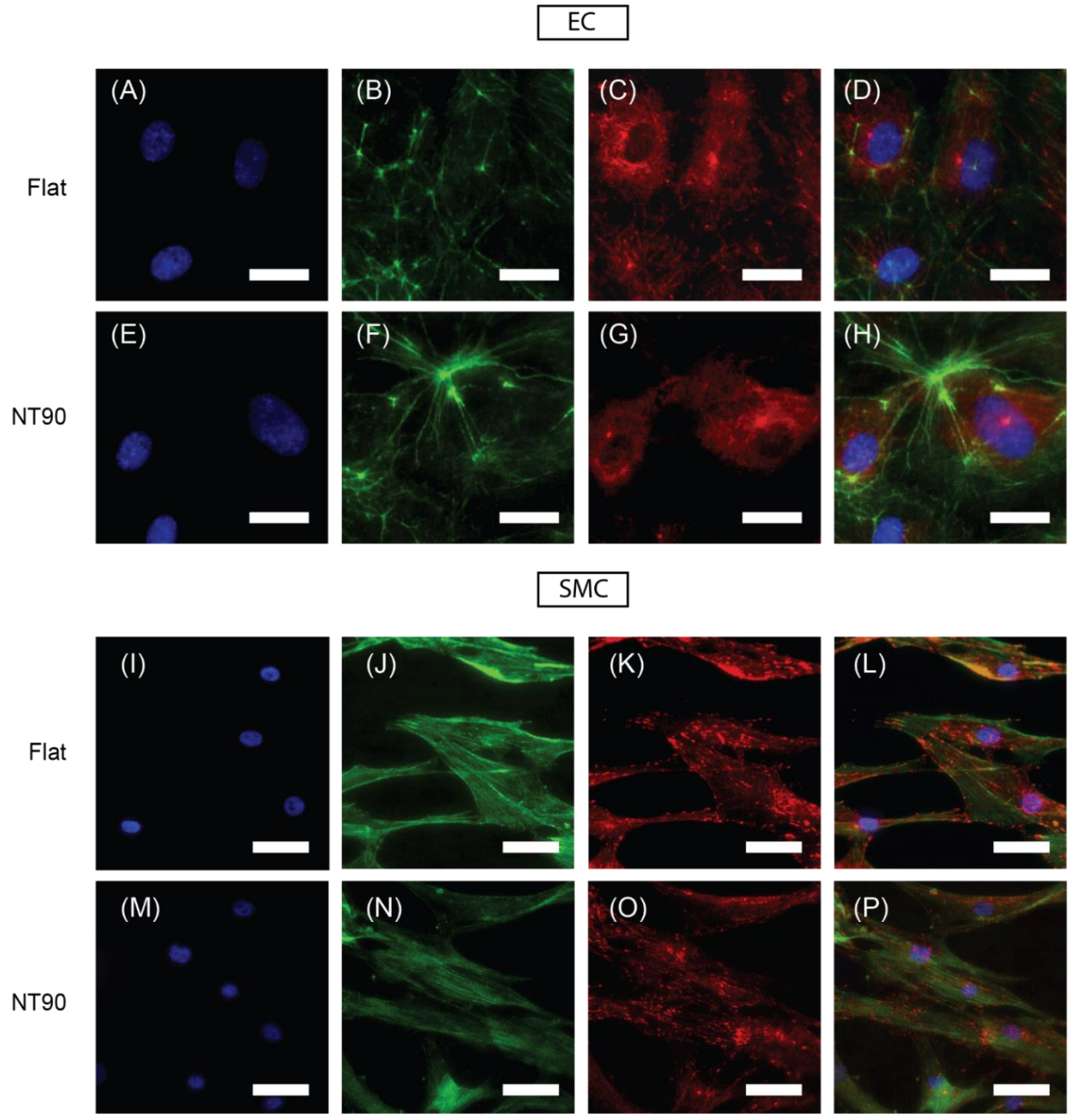

Figure S4. (A-H) ECs were cultured on (A-D) flat or (E-H) NT90 surfaces for 1 day, and then the cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and stained with (A, E) DAPI (blue), (B,F) phalloidin (green), (C, G) anti-vinculin (red). Merged images are shown in (D, H). (I-P) HCASMCs were cultured on (I-L) flat or (M-P) NT90 surfaces for 1 day, then fixed and permeabilized as described for HCAECs. SMCs were then stained with (I, M) DAPI (blue), (J, N) phalloidin (green), (K, O) anti-paxillin (red). Merged images are shown in (L, P). (A-H) scale bar: 25 μm. (I-P) scale bar: 50 μm.

S5. The effect of TiO_2 nanotube surface roughness on EC and SMC phenotype

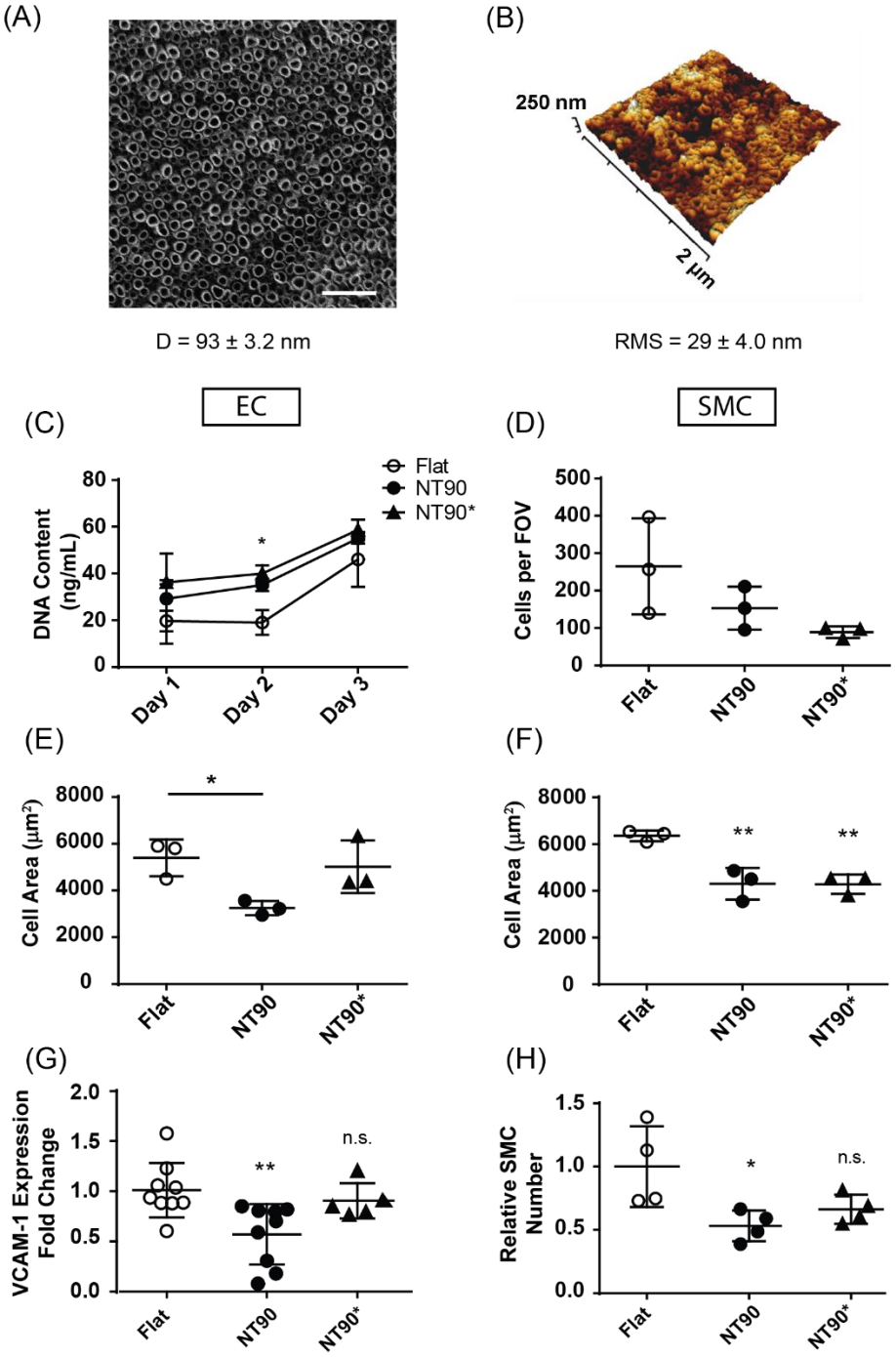

Figure S5. The effect of varying TiO_2 nanotube surface roughness on EC and SMC phenotype. NT90* surfaces were characterized using (A) helium ion microscopy and (B) AFM. (C) EC surface coverage was measured using CyQUANT over 3 days. (D) SMC cell number on day 1 was quantified by fluorescence microscopy and ImageJ. (E) EC and (F) SMC cell area were quantified by fluorescence microscopy and ImageJ. (G) EC VCAM-1 expression after 2 ng/mL TNFα stimulation was measured by qPCR. (H) SMC numbers after stimulation with 2 ng/mL TNFα was measured by CYQUANT.