Modulation of Endothelial Cell Response Through Surface Patterning of VEGF

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Statement of Purpose: Vascular endothelial growth factor (VEGF) is a key regulator of both physiological and pathological angiogenesis. In recent years, anti-angiogenesis agents targeting VEGF have become an integral component of anti-cancer regimens for many tumor types. In comparison, there has been less progress in the reverse scenario, where angiogenesis is desired to overcome tissue ischemia. A major challenge has been our inability to generate mature and stable vasculatures that are physiologically normal in terms of vessel organization, structure and function. Our lab has recently shown that covalently bound VEGF not only triggers phosphorylation of the key VEGF receptor involved in angiogenesis (VEGFR-2), but also alters the phosphorylation pattern and duration of signaling [1]. In addition, the covalently bound VEGF is not internalized and remains available for repeated phosphorylation events [2]. Spatial patterning of covalently bound VEGF can thus be used as a strategy for triggering sustained VEGFR-2 phosphorylation and downstream signaling at designated positions, especially since VEGF clustering has been shown to promote vessel branching [3]. In particular, we hypothesize that endothelial cell phenotype can be modulated to favor the tip cell phenotype when VEGF is bound and clustered, creating a means to control vessel morphology. Following exposure of human umbilical vein endothelial cells (HUVECs) to surfaces with VEGF clusters, short-term cell response (VEGFR-2 phosphorylation and downstream signaling) was analyzed using Western blotting. Mid-term cell response (reprogramming of gene expression) was analyzed using quantitative real-time PCR. Mid- to long-term cell response (changes in cell morphology) was evaluated using immunofluoresence staining of cells.

Methods: *Gold surfaces and nanopatterns*. Gold substrates were generated by e-beam evaporation resulting in 10 nm gold on 1 nm titanium over standard glass slides. Gold nanopatterned surfaces with varying cluster sizes and densities were synthesized as previously reported [4]. *VEGF immobilization*. Self-assembled monolayers (SAMs) were created with 1% EG-NH_2 and 99% EG-OH. Heparin modified with the photoreactive group p-azidobenzoyl hydrazide (ABH) was conjugated to the NH_2-functionalized SAM surfaces. VEGF was then crosslinked to the heparin-functionalized surfaces as previously described [1]. *PDMS cell sheets*. HUVECs were grown to confluency on fibronectin-coated PDMS sheets prepared as previously reported [2]. *Western blot analysis*. Following exposure of the PDMS cell sheets to the VEGF coated surfaces, the cells were lysed and harvested from the PDMS sheet. The lysate was run on 8% SDS-PAGE gels and transferred onto nitrocellulose membranes. The membranes were blotted with antibodies against VEGFR-2-pY1175, VEGFR-2-pY1214, VEGFR-2, p-p38 (T180/Y182), p38, p-ERK1/2 (T202/Y204), ERK1/2, p-Akt (S473), Akt.

Results: Fluorescently labeled VEGF (VEGF-AF488) was successfully conjugated to gold nanoparticles (300 nm diameter with a 1 μm spacing) on glass slides. Using direct ELISA [1], the amount of VEGF attached to the uniform VEGF (Vc) surfaces and clustered VEGF nanoparticle (cVc) surfaces were verified to be approximately 500 pg/cm^2 and 1500 pg/cm^2 respectively. PDMS cell sheets were exposed to Vc, cVc and soluble VEGF (Vs) for 5, 10, 30, 60 and 120 min. The positive control, Vs, was at 2 ng/mL in PBS [2]. PBS was the negative buffer control. Controls employing non-VEGF conjugated gold surfaces produced results similar to the PBS control (data not shown). The cell lysates harvested were electrophoresed, transferred to nitrocellulose, then blotted for the various phosphorylated proteins to obtain a phosphorylation profile of the HUVECs at each timepoint and condition.

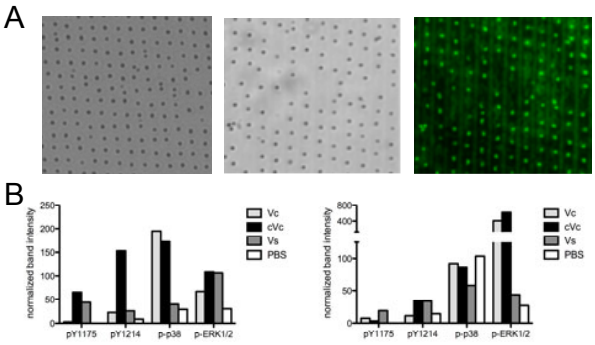

Figure 1. (A) Phase images of gold nanoparticles on slides before (left) and after (middle) VEGF-AF488 conjugation. Overlay of phase and fluorescent images (right). (B) Phosphorylation profile of HUVECs following a 5-min (left) and 30-min (right) exposure.

The results suggest that the cVc surfaces were more effective at phosphorylating VEGFR-2 at both the Y1175 and Y1214 positions, resulting in increased downstream signaling (p-p38 and p-ERK1/2) by the 5-min timepoint. Importantly, the downstream activation of p38 and ERK1/2 on the VEGF-bound surfaces was sustained at the later timepoints, such as the 30-min timepoint shown.

Conclusions: Covalently-bound VEGF resulted in sustained signaling downstream of VEGFR-2 activation. This suggests that mid- and long-term consequences of VEGFR-2 activation that take place at hours to days following stimulation will be enhanced on these surfaces.

References: [1] Anderson SM. Biomaterials. 2009;30:4618-4628. [2] Anderson SM. Integr Biol. 2011;3:887-896. [3] Anderson SM. Biomaterials. 2011;32:7432-7443. [4] Xiao F. Appl Phys Lett. 2010;97:031112.