Supplementary Online Figures

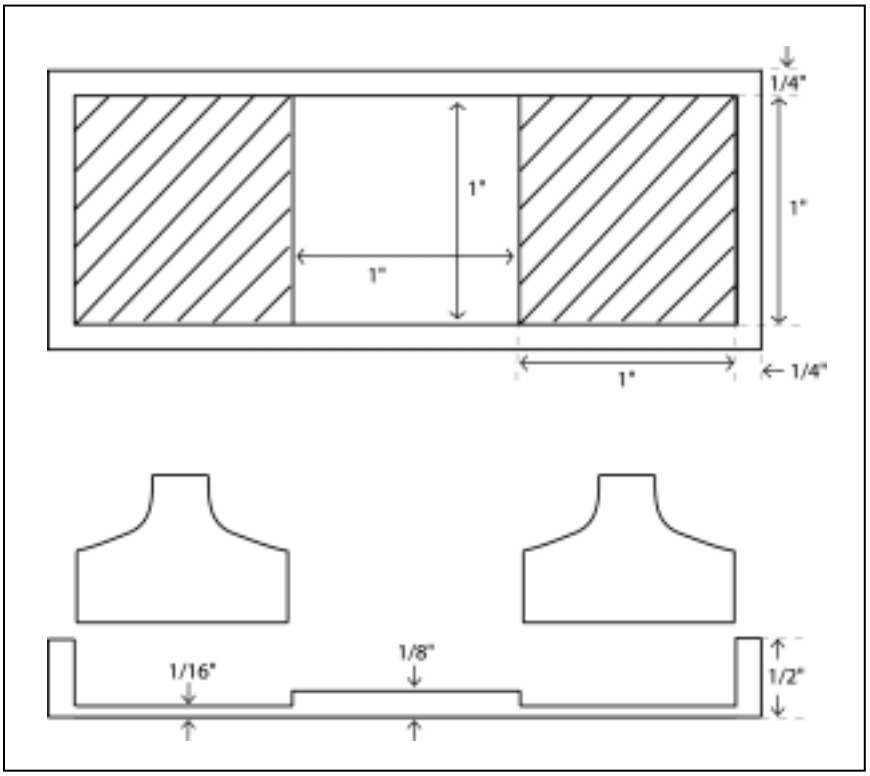

Figure 1. Design of diffusion chamber.
A diffusion chamber was fabricated from Plexiglas to provide controlled VEGF concentrations and gradients to sprouting MEC. The center portion of the bottom is transparent to allow imaging through the surface by microscopy. The removeable abutments fit tightly in the ends and are used during cross-linking of gels. The abutments are removed after the gel has set to allow for the addition of VEGF containing media on one end, and VEGF diffusion across the gel creates a gradient. Measurements are stated in inches.

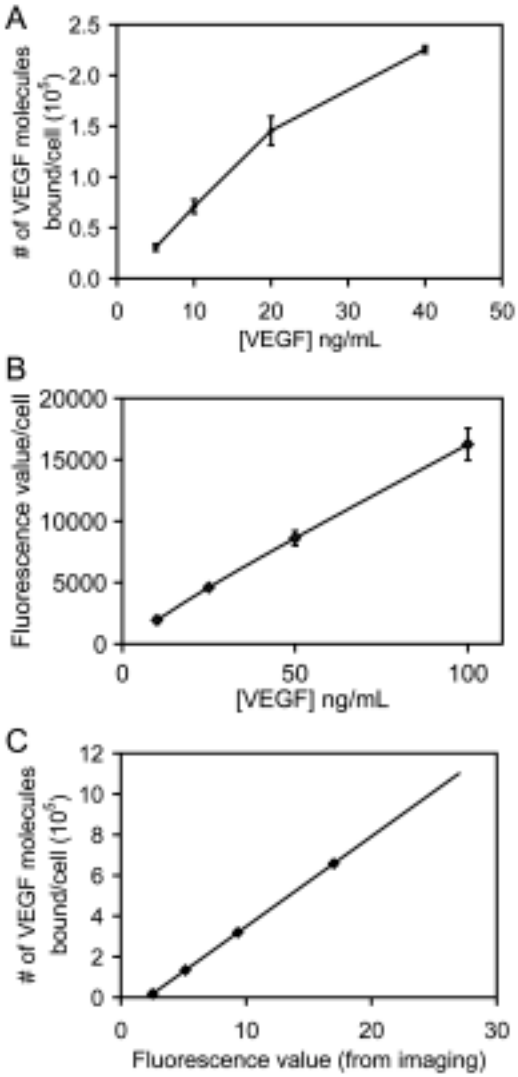

Figure 2. Development of a calibration curve for FRET measurements of VEGF binding MECs.

A) Radiolabeled VEGF binding assays were performed to determine the number of VEGF molecules bound per MEC (n=4). Binding was determined in subconfluent cultures of MECs with increasing quantities of ^{125}I-VEGF. Non-specific binding was determined with a 200-fold excess of unlabeled VEGF and was ~5% of total binding. B) Increasing concentrations of fluorescently labeled VEGF (acceptor) were also added to subconfluent cultures of labeled MECs (donor) for FRET measurements. Binding was allowed to equilibrate at 4^{o}C to prevent uptake of VEGF. Alexa555-VEGF binding to HEDAF-MECs was quantified by excitation at the donor excitation wavelength (488 nm) and monitoring emission at the acceptor emission wavelength (565 nm). Values were corrected for autofluorescence and non-specific binding obtained by monitoring emission in the presence of unlabeled MECs. C) The quantitative relationship between the number of molecules of VEGF bound to a sprouting MEC, as determined by iodinated VEGF binding and the FRET signal obtained from the parallel analysis, was linear: $VEGF_{cell} = 44273F_{image} - 94354$.

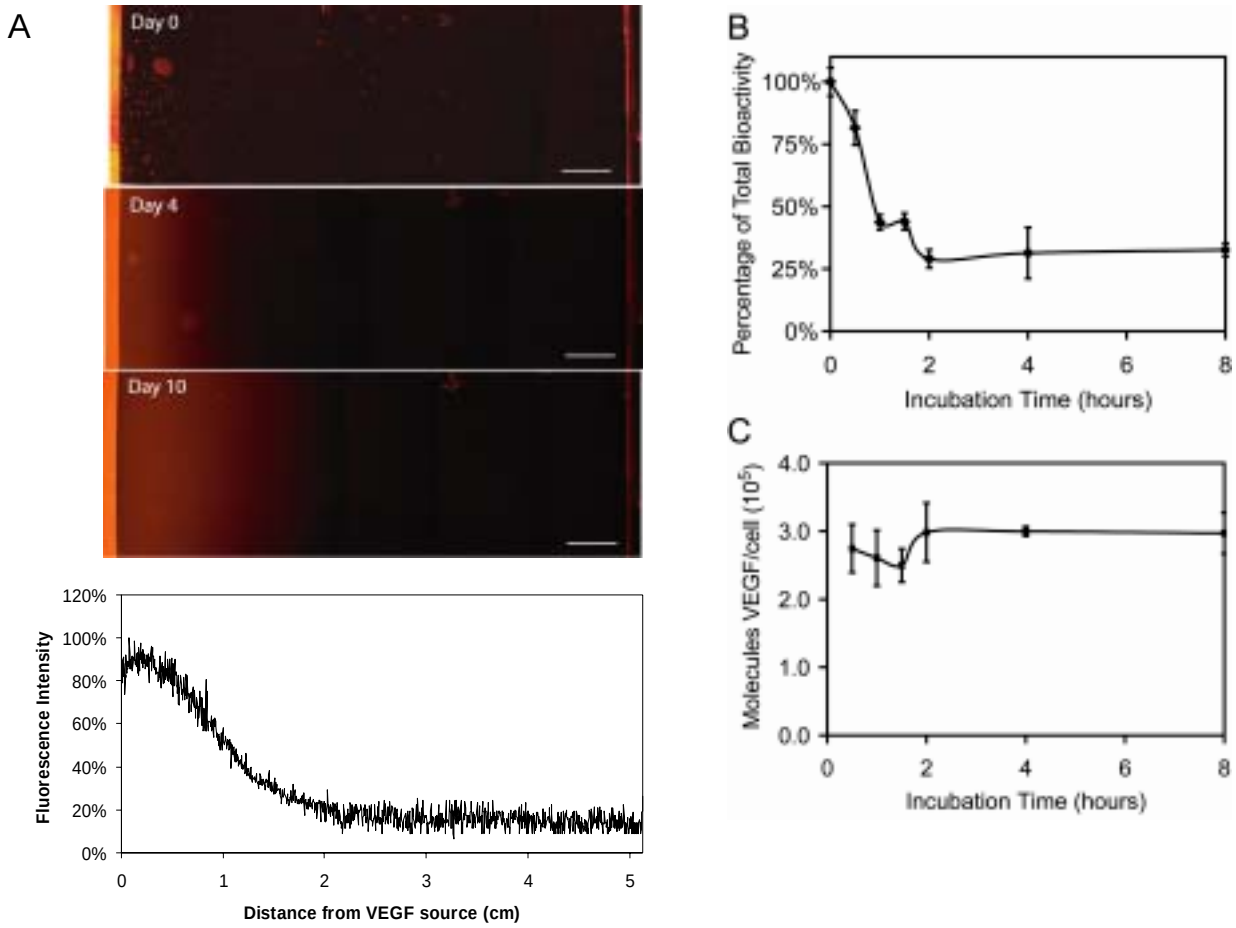

Figure 3. VEGF diffusion, bioactivity, and uptake measurements.
A) Fluorescently labeled VEGF was allowed to diffuse through Matrigel (left to right) over time and monitored by fluorescence imaging (A-top). The diffusion coefficient of VEGF was quantified from the change in fluorescence intensity across the gel over time. A representative plot of the fluorescence intensity across the gel at day 10 (A-bottom) is shown. Scale bar equals 500 μm. B) VEGF was incubated with MECs *in vitro* and the bioactivity of VEGF decreased rapidly within the first hour. The half-life of VEGF was calculated to be ~50 minutes based on the time required for VEGF to lose half its bioactivity. Values were normalized to the control condition containing 50 ng/mL VEGF without pre-incubation with MECs. C) MECs in subconfluent monolayer were incubated with 100 ng/mL of ^{125}I-VEGF. Uptake was determined to be less than 1% of total VEGF in the solution, and therefore negligible.

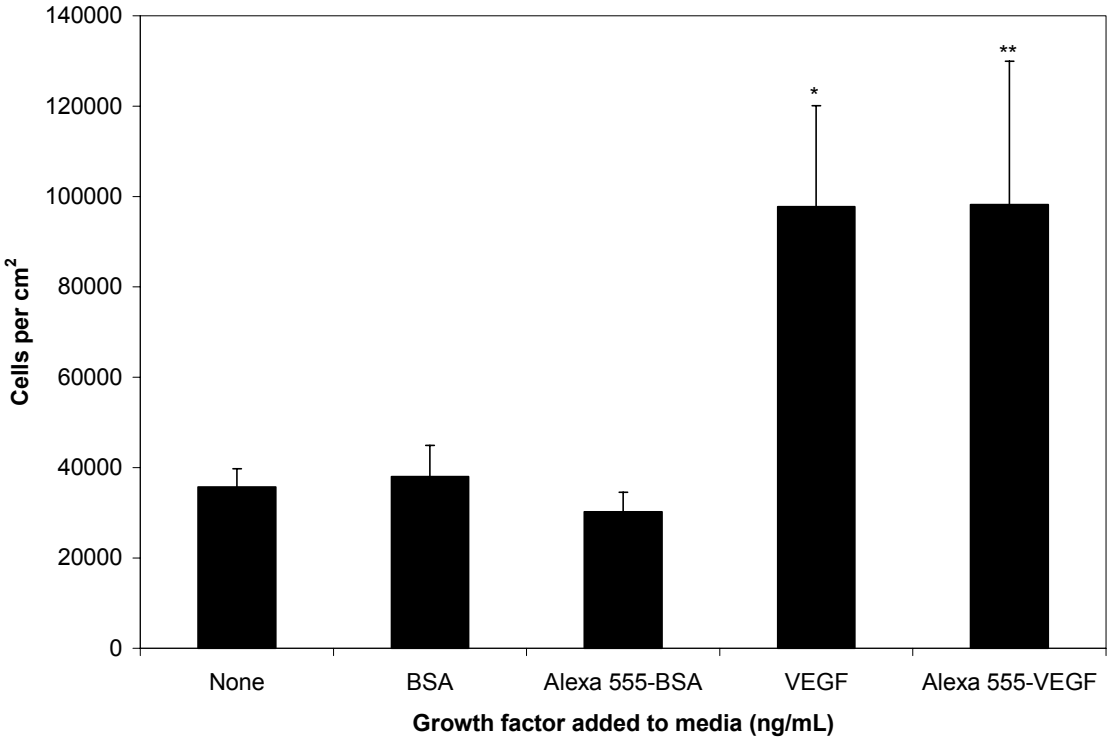

Figure 4. Bioactivity of fluorescently labeled VEGF.

A proliferation assay was performed with HDMECs to confirm the bioactivity of VEGF after fluorescence labeling with Alexa Fluor 555. BSA was also labeled to provide a control molecule to test for non-specific binding. Labeled VEGF maintained full bioactivity compared to unlabeled VEGF. *, **$p<0.05$