Supporting Information

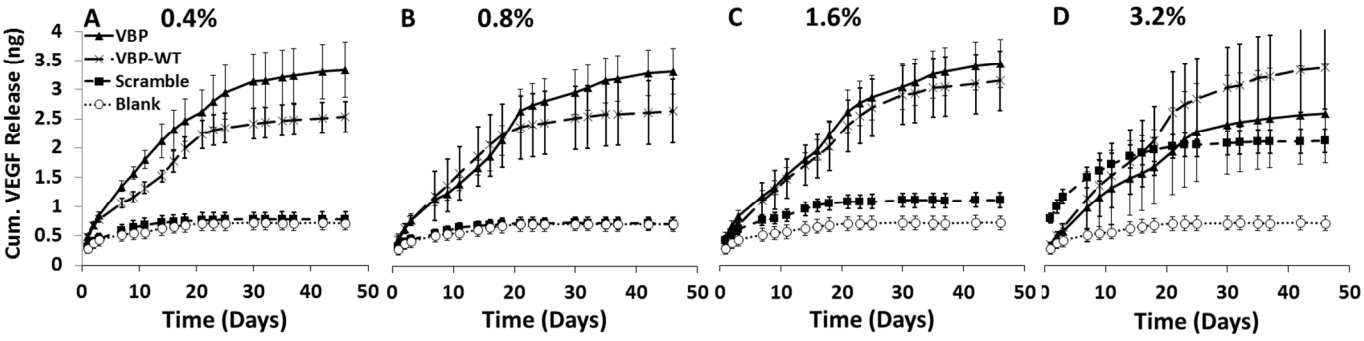

Figure 1S: Cumulative VEGF release measured from binding in 9.9 ng mL^{-1} VEGF, 0.1 ng mL^{-1} [^{125}I]VEGF in 0.1 wt.% BSA in PBS at 0.4%-3.2% peptide concentrations. Release from 0.4% (Fig. 1S A), 0.8% (Fig. 1S B), 1.6% (Fig. 1S C), and 3.2% (Fig. 1S D) peptide % microspheres is shown over time. Cumulative release from VBP and VBP_{WT} microspheres was significantly higher than Scramble and Blank microspheres at 0.4%, 0.8%, and 1.6% peptide (p-value<0.05). At 3.2% peptide, no significant difference was observed in cumulative release between Scramble, VBP, and VBP_{WT} microspheres (p-value >0.1). Cumulative release from 3.2% Scramble microspheres was significantly increased relative to 0.4%-1.6% Scramble microspheres (p-value < 0.05)

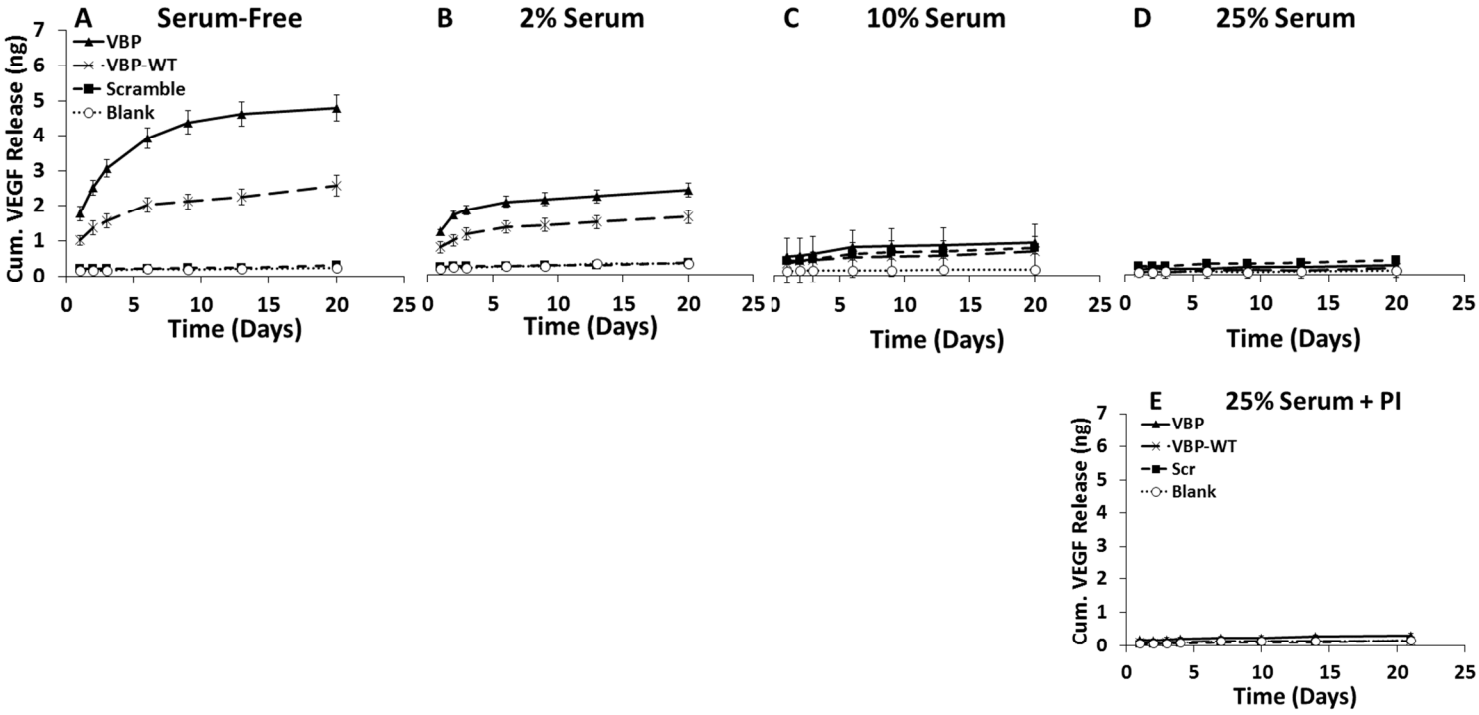

Figure 2S A-D: Cumulative release of VEGF measured from binding in 9.9 ng mL^{-1} VEGF, 0.1 ng mL^{-1} [^{125}I]VEGF in various loading solutions containing albumin-only or serum. Release of bound VEGF from 0.4% microspheres in albumin-only, 1.25 wt.% BSA in PBS solution (Fig. 2S A), 2 vol.% FBS in PBS (Fig. 2S B), 10 vol. % FBS in PBS (Fig. 2S C), and 25 vol.% FBS in PBS (Fig. 2S D). E: Cumulative VEGF release is presented for microspheres incubated in 25 vol.% serum with 1X protease inhibitor cocktail during VEGF binding and subsequent release (Fig. 2S E). Cumulative release was significantly higher for VBP and VBP_{WT} microspheres in serum-free versus all serum-containing environments (p-value < 0.05). Cumulative release from VBP and VBP_{WT} microspheres was increased at 2% serum relative to 10% and 25% serum, and increased at 10% serum relative to 25% serum (p-value < 0.05).

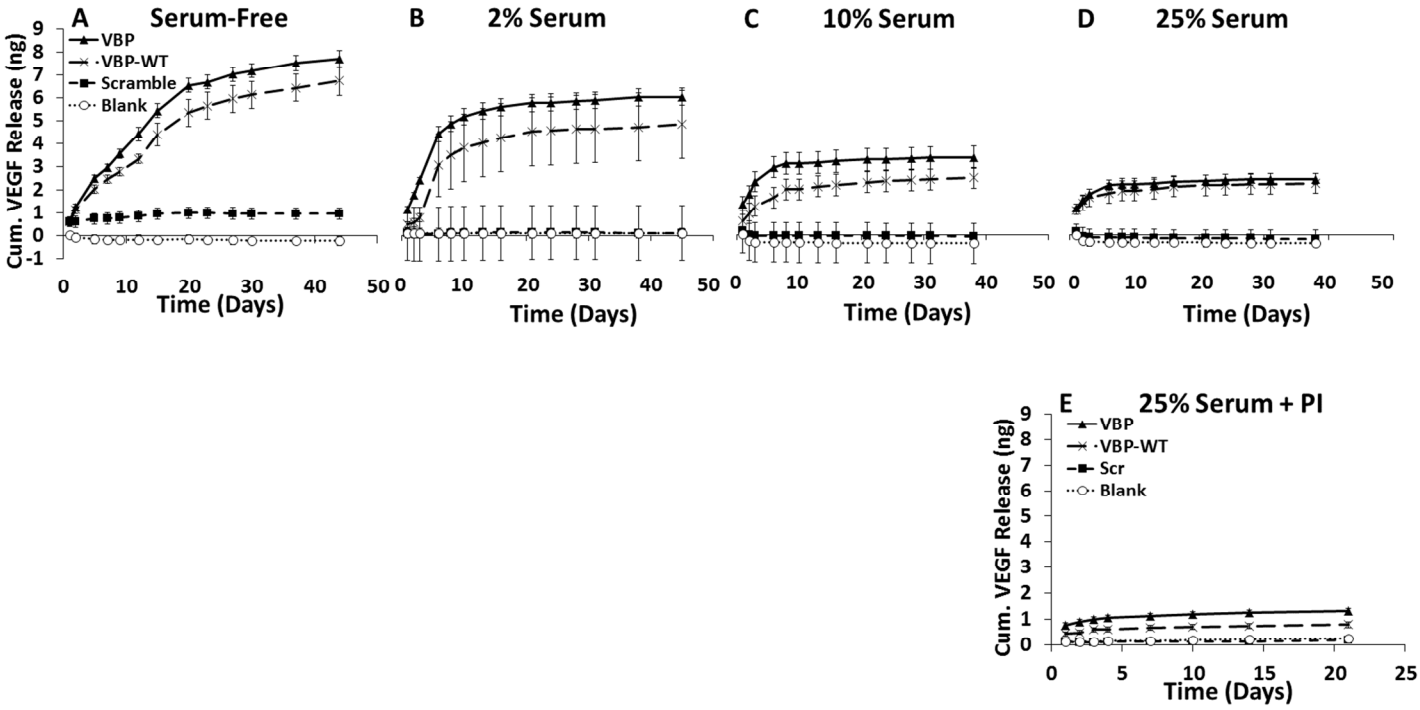

Figure 3S A-D: Cumulative release of VEGF measured from binding in 9.9 ng mL^{-1} VEGF, 0.1 ng mL^{-1} [^{125}I]VEGF in various loading solutions containing albumin-only or serum. Release of bound VEGF from 1.6% microspheres was measured in albumin-only, 1.25 wt.% BSA in PBS solution (Fig. 3S A), 2 vol.% FBS in PBS (Fig. 3S B), 10 vol.% FBS in PBS (Fig. 3S C), and 25 vol.% FBS in PBS (Fig. 3S D). F: Cumulative VEGF release is presented for microspheres incubated in 25 vol.% serum with 1X protease inhibitor cocktail during VEGF binding and subsequent release at 1.6% peptide concentration (Fig. 3S E). Cumulative release was significantly higher for VBP and VBP_{WT} microspheres in serum-free versus 10% and 25% serum (p-value < 0.05). Cumulative release from VBP microspheres was increased at 2% serum relative to 10% and 25% serum (p-value < 0.05).

Increasing serum concentration decreased the amount of cumulative VEGF release from VBP and VBP_{WT} microspheres at both low (Fig. 2S A-D) and high (Fig. 3S A-D) peptide concentrations. No significant VEGF was released from Scramble or Blank microspheres (Fig 2S A-D, Fig. 3S A-D), as expected since these materials were not designed to bind to VEGF and were therefore not initially VEGF-loaded.

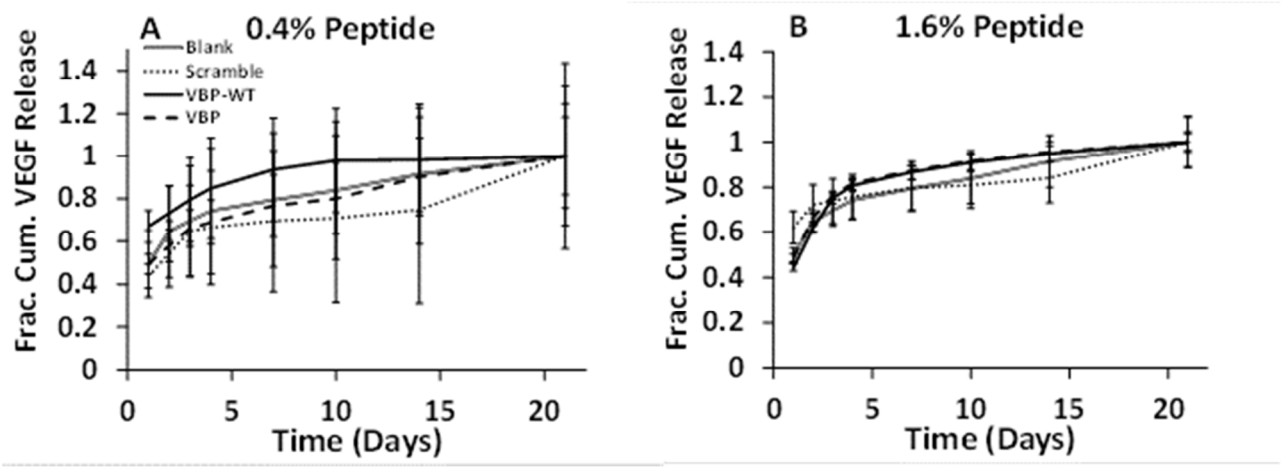

Figure 4S. Fractional cumulative release of VEGF measured from binding in 9.9 ng mL^{-1} VEGF, 0.1 ng mL^{-1} [^{125}I]VEGF in 25 vol.% serum with protease inhibitor (PI). Release of bound VEGF from 0.4% microspheres (A) and 1.6% microspheres (B) was measured in 25 vol.% serum in PBS with 1X concentration of PI. No significant differences were observed between VEGF release profiles at 0.4% or 1.6% peptide in any of the microsphere conditions tested.

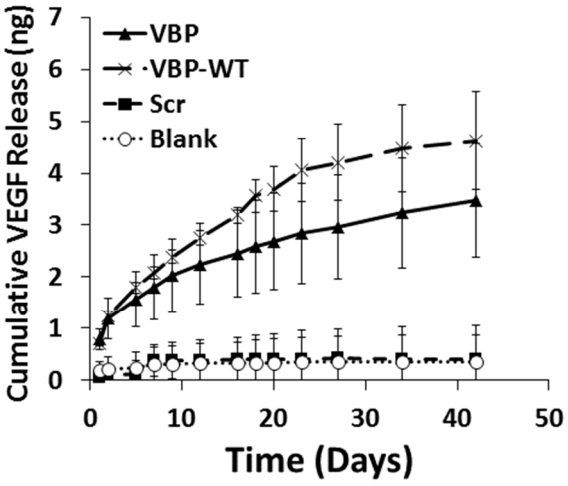

Figure 5S: Cumulative VEGF release (ng) from Blank and 1.6% VBP, VBP_{WT}, and Scramble microspheres that were incubated in 9.9 ng mL^{-1} VEGF, 0.1 ng mL^{-1} [^{125}I]VEGF in heparin-containing albumin-only solution. Subsequent release was measured in solution without VEGF supplementation in albumin-only solution with supplemented heparin. Curves represent average cumulative VEGF release (ng) +/- SD from 1.6% microspheres.

Mathematical Model

We established a mathematical model for VEGF release based on a kinetic equation describing the interaction between VEGF and peptide (Equation 1). The model is based on previous mathematical modeling of affinity-based drug release systems[1,2]. From Equation 1, we derived three differential equations to describe the rate of change of VEGF, peptide, and bound VEGF (VEGF-peptide) concentration over time. Inclusion of Fickian diffusion terms in spherical coordinates for VEGF, resulted in one coupled partial differential equation (Equation 2) and two coupled ordinary differential equations (Equations 3-4)

Coupled Partial Differential Equations Governing Reaction Kinetics and Diffusion

(1S) $$\frac{\partial C_{VEGF}}{\partial t} = \boldsymbol{\frac{D_{VEGF}}{r^2}\frac{\partial^2 C_{VEGF}}{\partial r^2}} + \boldsymbol{\frac{D_{VEGF}}{r}\frac{\partial C_{VEGF}}{\partial r}} + k_r C_{VEGF-Peptide} - k_f C_{VEGF} C_{Peptide}$$

(2S) $$\frac{\partial C_{Peptide}}{\partial t} = k_r C_{VEGF-Peptide} - k_f C_{VEGF} C_{Peptide}$$

(3S) $$\frac{\partial C_{VEGF-Peptide}}{\partial t} = k_f C_{VEGF} C_{Peptide} - k_r C_{VEGF-Peptide}$$

where D_i is the diffusion coefficient of species "i", $k_{f,i}$ and $k_{r,i}$ are the association and dissociation rate constants respectively for species "i", and C_i is the concentration of species "i". The differential equations describe the rate of change of C_i with respect to time, t, in the radial component, r, of the spherical coordinate system.

Nonlinear Equations for Equilibrium Values

(4S) $$\mathbf{k_f C_{VEGF}^{EQ} C_{Peptide}^{EQ}} = k_r C_{VEGF-Peptide}^{EQ}$$

(5S) $$C_{VEGF}^{TOT} = C_{VEGF}^{EQ} + C_{VEGF-Peptide}^{EQ}$$

(6S) $$C_{Peptide}^{TOT} = C_{Peptide}^{EQ} + C_{VEGF-Peptide}^{EQ}$$

Solution to the coupled PDE-ODE system required solution of the nonlinear equations above (Equations 4S-6S) along with initial and boundary conditions listed in Equations 7S-10S below.

Initial and Boundary Conditions

(7S) For all r @ t=0 $C_i = C_i^{EQ}$ for i=VEGF, Peptide, VEGF-Peptide

(8S) For all t @ r=R $C_{VEGF} = 0$

(9S) For all t @ r=0, $\frac{\partial C_{VEGF}}{\partial r} = 0$

(10S) $$J_{VEGF} = D_{VEGF} \frac{\partial C_{VEGF}}{\partial r}\Big|_{r=R}$$

Flux of VEGF was normalized by the final time point and plotted as normalized VEGF flux (Fig. 7A).

Mathematical Competition Model

To understand the influence of soluble proteins on VEGF release, we established a mathematical model to describe competition between VEGF and a VEGF-binding serum protein, $Competitor_i$, where 'i' is defined as sFlt-1, sKDR, or α2-MA. Together with Equations 2S-4S 6S, and 8S-10S, the below equations describe the contribution of VEGF-competitor interactions on free VEGF concentration over time. For competition analysis, Equations 1S, 5S, and 7S were omitted and replaced with Equations 11S, 15S, and 17S respectively. In the equations below, $k_{i,r}$ and $k_{i,f}$ represent the dissociation and association rate constants for the interaction between VEGF and $Competitor_i$ (Table 1).

Coupled Partial Differential Equations Governing Reaction Kinetics and Diffusion

(11S) $$\frac{\partial C_{VEGF}}{\partial t} = \frac{\boldsymbol{D_{VEGF}}}{\boldsymbol{r^2}}\frac{\boldsymbol{\partial^2 C_{VEGF}}}{\boldsymbol{\partial r^2}} + \frac{\boldsymbol{D_{VEGF}}}{\boldsymbol{r}}\frac{\boldsymbol{\partial C_{VEGF}}}{\boldsymbol{\partial r}} + k_r C_{VEGF-Peptide} - k_f C_{VEGF} C_{Peptide} + \sum_i k_{i,r} C_{VEGF-Competitor_i} - k_{i,f} C_{VEGF} C_{Competitor_i}$$

(12S) $$\frac{\partial C_{VEGF-Competitor_i}}{\partial t} = k_{i,f} C_{VEGF} C_{Competitor_i} - k_{i,r} C_{VEGF-Competitor_i}$$

(13S) $$\frac{\partial C_{Competitor_i}}{\partial t} = k_{i,r} C_{VEGF-Competitor_i} - k_{i,f} C_{VEGF} C_{Competitor_i}$$

Nonlinear Equations for Equilibrium Values

(14S) $$\mathbf{k_{i,f}} \mathbf{C_{VEGF}^{EQ}} \mathbf{C}_{Competitor_i}^{\mathbf{EQ}} = \mathrm{k_{i,r}} \mathrm{C}_{\mathrm{VEGF-}Competitor_i}^{\mathrm{EQ}}$$

(15S) $$\mathrm{C}_{\mathrm{VEGF}}^{\mathrm{TOT}} = \mathrm{C}_{\mathrm{VEGF}}^{\mathrm{EQ}} + \mathrm{C}_{\mathrm{VEGF-Peptide}}^{\mathrm{EQ}} + \sum_i C_{VEGF-Competitor_i}^{EQ}$$

(16S) $$\mathrm{C}_{Competitor_i}^{\mathrm{TOT}} = \mathrm{C}_{Competitor_i}^{\mathrm{EQ}} + \mathrm{C}_{\mathrm{VEGF-}Competitor_i}^{\mathrm{EQ}}$$

The solution to the competition modeled required Equations 2S-4S, 6S, and 8S-10S from above in combination with Equations 11S-16S above. With the additional boundary condition provided below in Equation 17S, the VEGF flux was calculated as above, normalized to the final time point in each condition, and plotted as normalized VEGF flux over time (Fig. 7B).

Competition Model Initial and Boundary Conditions

(17S) For all r @ t=0 $C_j = C_j^{EQ}$ for j=VEGF, Peptide, VEGF-Peptide, Competitor$_i$, VEGF-Competitor$_i$

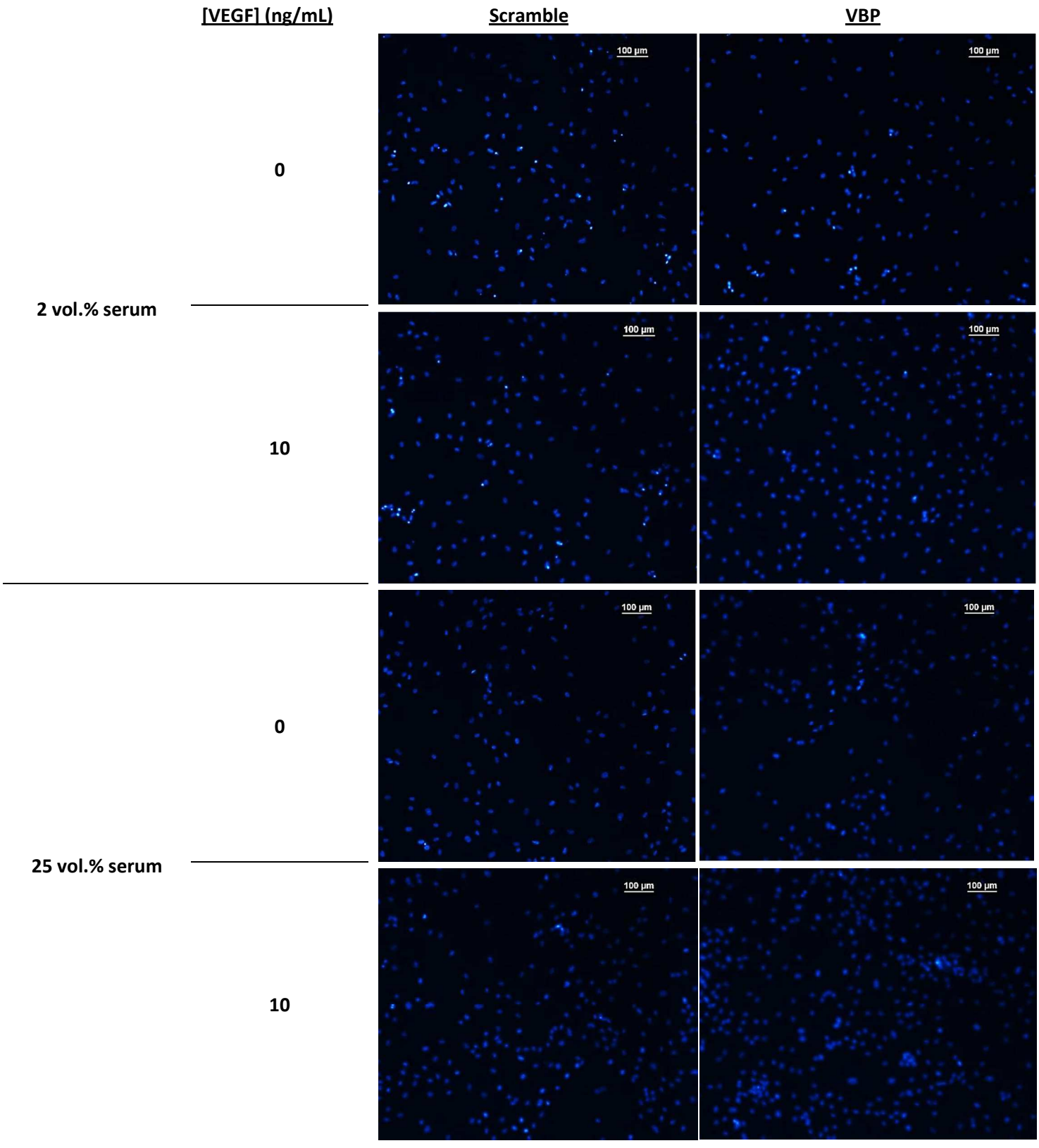
[VEGF] (ng/mL)
Scramble
VBP
2 vol.% serum
0
10
25 vol.% serum
0
10
100 µm

Figure 6S. Fluorescent micrographs showing DAPI-stained HUVECs in culture with the specified serum concentrations and the specified microsphere types (1.6% Scramble or VBP microspheres). Microspheres were first pre-incubated in the VEGF concentration described in the second column, and after 48 hours in culture, cells were fixed, stained with DAPI, and counted. Scale bar = 100µm.

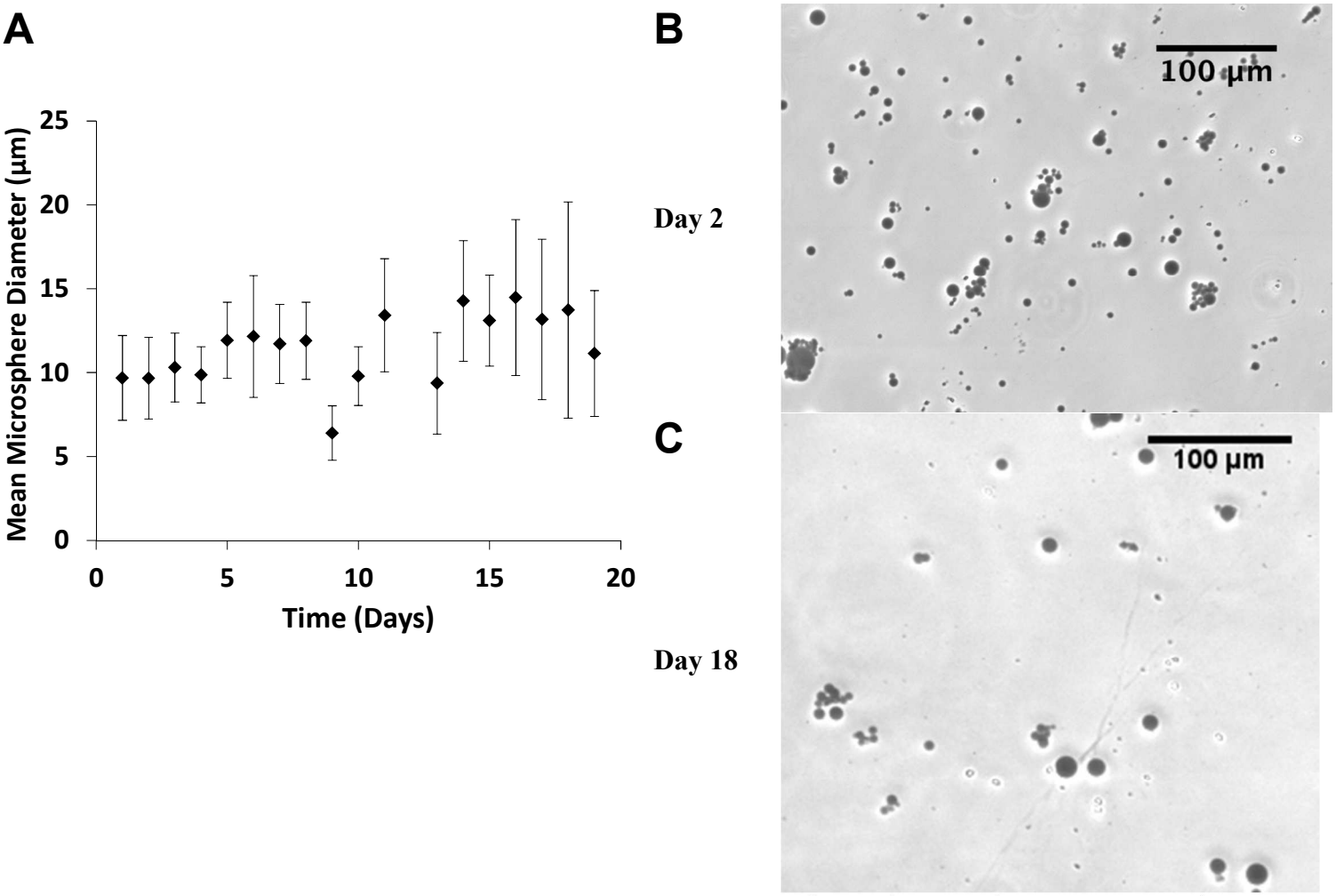

Figure 7S. Microsphere size as a function of time. A: Microspheres size over time was measured as previously described[3]. Blank PEG microspheres were suspended at 1 mg mL^{-1} in PBS pH 7.4 at 37°C, 95% RH. Each day, a small volume of suspended microspheres was isolated and diluted two-fold in Trypan blue. Trypan blue has previously been shown to stain PEG microspheres for enhanced contrast. Microspheres were imaged using a 10X objective on phase contrast (Nikon), and two images were captured at each time point. At least 100 microspheres were used for statistical analysis. Microsphere diameter was measured using ImageJ software. Microsphere diameter did not change over the time course of the experiment (ANOVA p-value > 0.05). Data is presented as mean microsphere diameter (µm) +/- standard error ($SD/\sqrt{n}$). B,C: Example phase contrast images of microspheres at day 2 (B) and day 18 (C). Scale bar = 100µm.

References Cited

(1) Lin, C.-C.; Metters, A. T. *Adv. Drug Deliv. Rev.* **2006**, *58*, 1379–1408.

(2) Sakiyama-Elbert, S. E.; Hubbell, J. A. *J. Control. Release* **2000**, *65*, 389–402.

(3) Parlato, M.; Johnson, A.; Hudalla, G. A.; Murphy, W. L. *Acta Biomater.* **2013**, *9*, 9270–9280.