Supporting information

VEGFR2-Specific Ligands Based on Affibody Molecules Demonstrate Agonistic Effects when Tetrameric in Soluble Form or Immobilized via Spider Silk

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Materials and Methods

Circular dichroism

Secondary structure and thermal stability was analyzed using a Chirascan^{TM} circular dichroism spectrometer (Applied Photophysics, Surrey, UK). Proteins were diluted to 0.3 mg/mL in PBS and the secondary structure was analyzed by measuring ellipticity in the range 250-195 nm at 25°.

VEGFR2 binding analysis of soluble affibody proteins using SPR

VEGFR2 binding studies on tetra-ZVEGFR2-L1-S was performed using a Biacore T200 instrument. Briefly, HSA (Sigma-Aldrich) was immobilized on a CM5 sensor chip and the kinetics of the binding of the affibody molecules were analyzed by injection of tetra-$Z_{VEGFR2\text{-}L1\text{-}S}$, immediately followed by injection of a concentration series of recombinant human VEGFR2-ECD (Sino Biological Inc., (His Tag) product number: 10012-H08H). The experiment was performed in duplicates using freshly prepared reagents.

VEGFR2 phosphorylation study of cells cultured on di-$Z_{VEGFR2\text{-}S}$-silk and tetra-$Z_{VEGFR2\text{-}L1\text{-}S}$-silk coatings

The same coating procedure and setup as in 2.8 was used for cell phosphorylation assays except the cells were incubated in starvation media for 24h. N = 2 was run with parallel replicated n =2.

Viability of cells cultured on di-$Z_{VEGFR2\text{-}S}$-silk and tetra-$Z_{VEGFR2\text{-}L1\text{-}S}$-silk coatings

The same coating procedure and setup as in 2.9 was used for cell viability assays except the cell growth was monitored at day 2, 4 and 7. N = 1 was run with parallel replicates n = 3.

Results

Circular dichroism spectroscopy

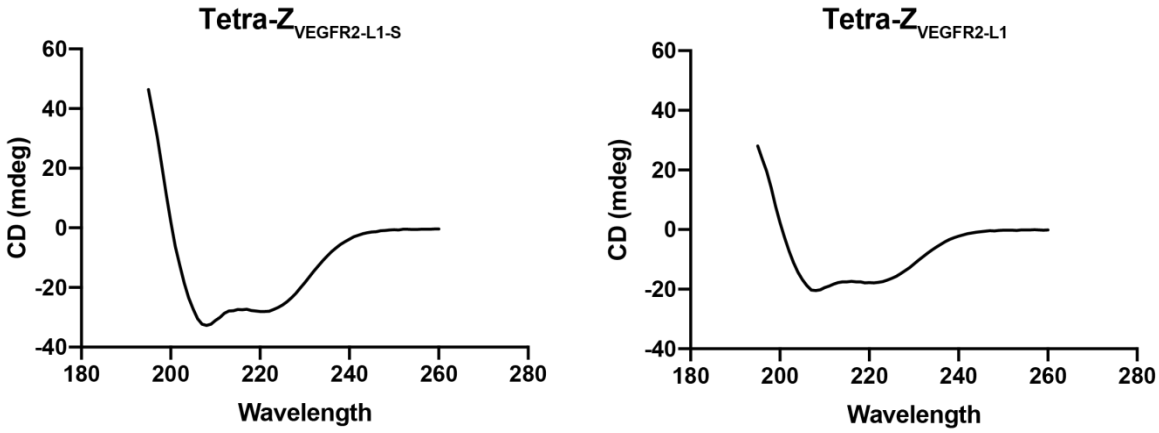

Figure S1. CD spectra for tetra-$Z_{VEGFR2\text{-}L1\text{-}S}$ and tetra-$Z_{VEGFR2\text{-}L1}$.

VEGFR2 binding analysis of soluble affibody proteins using SPR

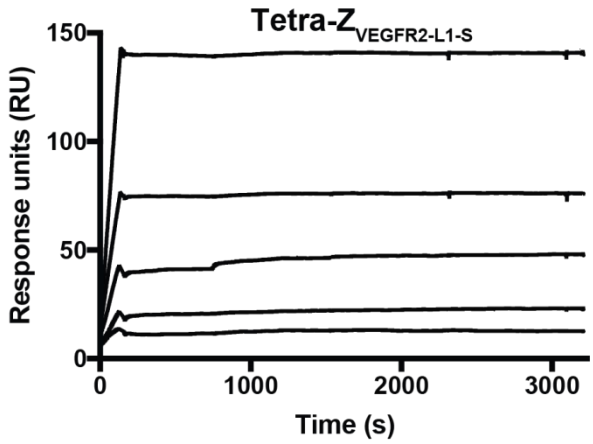

Figure S2. Representative sensorgrams from SPR analysis of tetra-$Z_{VEGFR2\text{-}L1\text{-}S}$ binding to human VEGFR2-ECD. A capture assay was performed by immobilizing HSA and capturing tetra-$Z_{VEGFR2\text{-}L1\text{-}S}$ via the high affinity ABD-HSA interaction. Recombinant human VEGFR2-ECD was then injected as a concentration series (50, 25, 12.5, 6.25, 3.125 nM).

Overview of di-$Z_{VEGFR2\text{-}L1\text{-}S}$-silk and tetra-$Z_{VEGFR2\text{-}L1\text{-}S}$-silk fusion proteins

Figure S3. Schematic overview of di-ZVEGFR2-L1-S-silk and tetra-ZVEGFR2-L1-S-silk.

Activation of VEGFR2 signaling

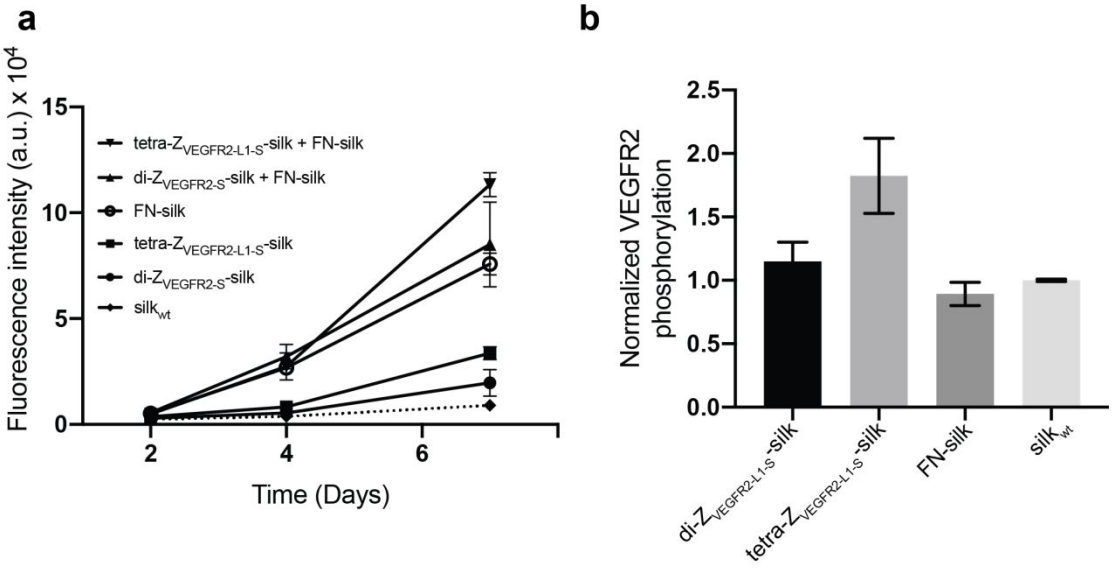

Figure S4. Activation of VEGFR2 signaling in 293/KDR cells by immobilized binders. Data are shown as mean with SD. The wells were first coated with $silk_{wt}$ followed by top coating of either di-$Z_{VEGFR2\text{-}S}$-silk, tetra-$Z_{VEGFR2\text{-}L1\text{-}S}$-silk, di-$Z_{VEGFR2\text{-}S}$-silk + FN-silk, tetra-$Z_{VEGFR2\text{-}L1\text{-}S}$-silk + FN-silk. FN-silk and $silk_{wt}$ coated wells were used as control. All coatings were prepared using equal molar amounts. (a) Cell viability was assessed by Alamar blue at day 2, 4 and 7. N=1 in parallel triplicates. Legends are ordered from highest signal at day 6. (b) After adhesion and incubation in starvation media (24 h), cells were analyzed in terms of VEGFR2 phosphorylation. All obtained signals are normalized to cells cultured on $silk_{wt}$. (Independent experiments N = 2 with parallel replicates n = 2)

Micrographs showing staining of vasculature markers

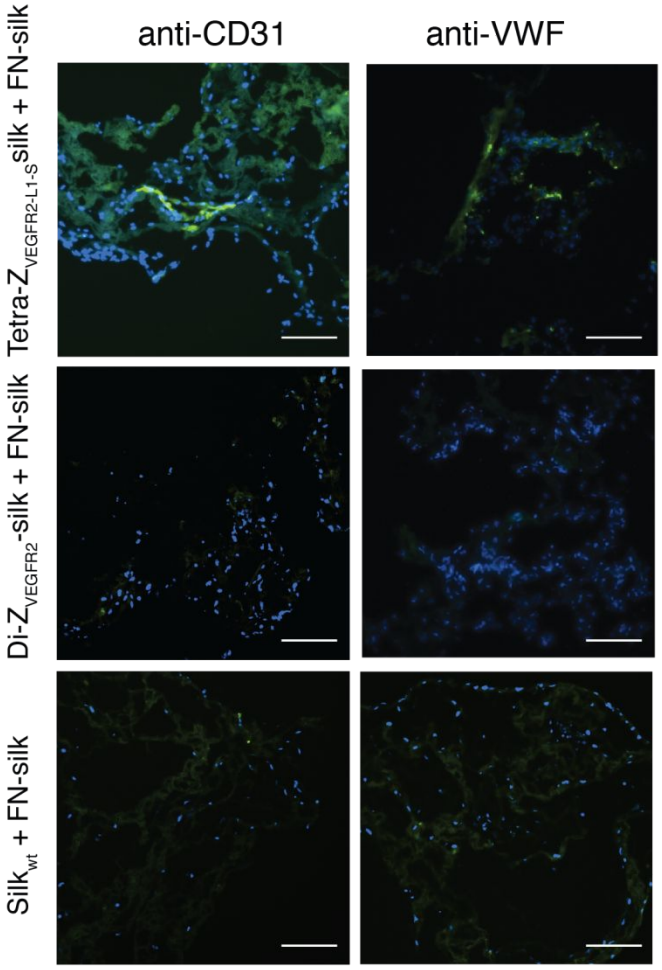

Figure S5. Micrographs showing staining of vasculature markers, CD31 (green) and vWF (green) and cell nuclei staining (blue), within different sectioned scaffolds. (N=2, in duplicates). Scale bar: 100 μm.