Integrin β3 Targeting Biomaterial Preferentially Promotes Secretion of bFGF and Viability of iPSC-Derived Vascular Smooth Muscle Cells

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Materials:

Type I rat tail collagen type-I was purchased from Enzo Life Sciences, USA. Human plasma fibronectin was purchased from Milipore Sigma, USA. 4-arm polyethylene glycol succinimidyl glutarate Mw 10,000 (4S-StarPEG) was purchased from JenKem Technology, USA. Growth factor reduced matrigel was purchased from Corning Life Sciences (Catalog Number: 356231), USA. Trinitrobenzene sulfonic acid (TNBSA) and AlamarBlue reagents were purchased from ThermoFisher Scientific, USA. Rhodamine phalloidin (R415) was purchased from Invitrogen, Thermo Fisher Scientific, USA. Smooth muscle cells culture medium (SmGM-2) was purchased from PromoCell, Germany. All other cell culture reagents were purchased from ThermoFisher Scientific unless otherwise stated.

2,4,6-trinitrobenzene sulfonic acid assay:

2,4,6-Trinitrobenzene Sulfonic Acid (TNBSA) assay was performed to determine the amount of free amines in the scaffolds with and without cross-linking. Briefly, the scaffolds were first made inside Eppendorf tubes following the supplementary table described above. Then the scaffolds were disrupted using crushers, which would turn the scaffold inside a viscous consistency. 250uL of 0.01% TNBSA was added into 500uL of each sample. A 0.2% glutaraldehyde solution was used as a positive control. The resultant mixtures were incubated at 37^{o}C for 2 hours. Glycine titration in sodium bicarbonate (0.1M) pH 8.5 standard curve was established at: 300 µm, 250 µm, 200 µm, 150 µm, 100 µm, 75 µm, 50 µM, 25 µm, and 0 µm. At the conclusion of the incubation, 250 µl of 10% sodium dodecyl sulfate was added. 125 µl of 1M HCI was added to stop the reaction. 100 µl of the final solution was used to test for the degree of

free amine groups in fibronectin infused collagen scaffolds. Plate reader absorbance was set at 335 nm.

Table-S1: Information related to primary antibodies.

Primary Antibody	Dilution	Catalog Number/ Manufacturer
SDF-1α	1:2500 ELISA	MAB350 (R&D)
PDGFAA	1:2500 ELISA	500-P46-100 (PeproTech)
bFGF	1:2500 ELISA	500M38 (PeproTech)
VEGF	1:2500 ELISA; 1:200 IHC	AB-119 (Abcam)
SM-22α	1:300 IF and IHC 1µg/ml FACS	AB-10135 (Abcam)
α -SMA	1:200 IF 1µg/ml FACS	SC-32251 (SantaCruz Biotech)
Calponin	1:200 IF and IHC 1µg/ml FACS	C-2687 (Sigma)
SM-MHC	1:300 IF and IHC 1µg/ml FACS	AB-53219 (Abcam)

Secondary Antibody	Dilution	Catalog Number/ Manufacturer
Anti-Mouse-HRP	1:2500 ELISA	AB6789 (Abcam)
Anti-Rabbit-HRP	1:2500 ELISA	A0545 (Sigma)
Anti-Mouse-Alexafluor488	1:400 IF and IHC	A11029 (ThermoFisher)
Anti-Mouse-Alexafluor568	1:400 IF and IHC	A11031 (ThermoFisher)
Anti-Rabbit-Alexafluor555	1:400 IF and IHC	A31572 (ThermoFisher)
Anti-Goat-Alexafluor488	1:400 IF and IHC	A27012 (ThermoFisher)

Table-S2: Information related to secondary antibodies.

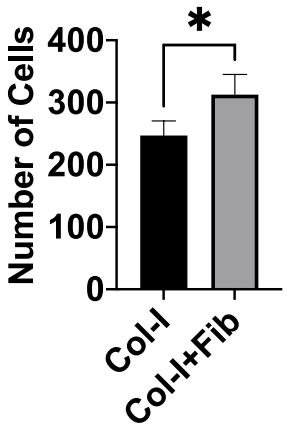

Figure S1: **Characterization of cell viability in the scaffold.** The graph shows the total number of cells (combined number of Calcein-AM positive live cells and EthD-1-stained dead cells). Collagen scaffolds without fibronectin were kept as controls for all the experiments. * denotes statistical significance differences between the different groups (n=3, t-test, *$p<0.05$).

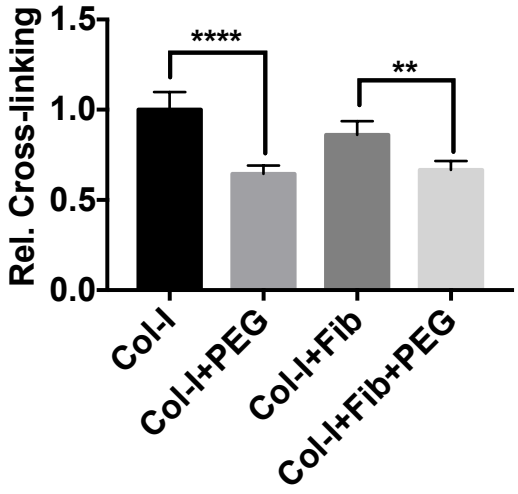

Figure S2: Characterization of 4S-StarPEG cross-linking. TNBSA showing a qualitative analysis of free amine groups in crosslinked collagen sacffolds of 4mg/ml of collagen concentration with and without fibronectin (100 µg/ml). Uncross-linked collagen scaffolds with and without fibronectin were kept as a controls. * denotes statistical significance differences between the different groups (n=4, t-test, **p<0.01, ****p <0.0001).

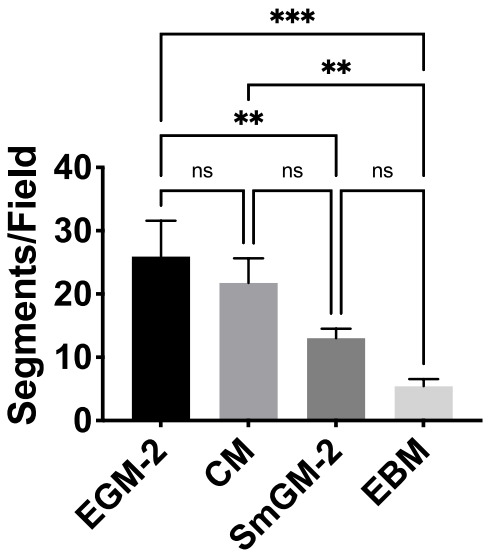

Figure S3: Characterization of in vitro network formation. Graph showing the number of segments/field of the endothelial cell network formed on matrigel. Endothelial cell (EGM-2) and smooth muscle cell (SmGM-2) growth medium, and endothelial cell basal medium (EBM) were used as controls. * denotes statistical significance differences between the different groups (n=3, one-way ANOVA, *p<0.05, **p<0.01, ***p<0.001).