Supplementary Information

Microbead-based biomimetic synthetic neighbors enhance survival and function of rat pancreatic β-cells

Wei Li,[a] Samuel Lee,[b] Minglin Ma,[a, f] Soo Min Kim,[b] Patrick Guye,[c] James Pancoast,[b] Daniel G Anderson,[a, d, e] Ron Weiss,[c] Richard T Lee,[b] and Paula T Hammond [a, d, *]

[a] Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, 02139
[b] Harvard Stem Cell Institute and Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA 02139
[c] Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, 02139
[d] David H. Koch Institute of Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, 02139
[e] Harvard-MIT Division of Health Science and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139
[f] Current address: Biological and Environmental Engineering, Cornell University, Ithaca, NY, 14850
[*] Corresponding author

Materials.

Fusion proteins including EphA5–Fc, and ephrinA5–Fc were obtained from R&D Systems. 2-Iminothiolane-HCl (Traut's reagent) was purchased from Thermo Fisher Scientific. LIVE/DEAD® viability/cytotoxicity kit was obtained from Invitrogen. Cell culture media and reagents were obtained from Invitrogen unless otherwise noted. All other chemicals were purchased from Sigma-Aldrich.

Methods:

qPCR RT analysis of β-cell specific genes.

Total RNA was isolated using microRNA extraction kit (PureLink® RNA Micro Scale Kit, Life Technologies) and reverse transcribed to obtain cDNA (SuperScript reverse transcriptase; Invitrogen). Quantitative RT-PCR with SYBR green detection was performed using specific primers[1] (Mafa (F)5′-CTT CAG CAA GGA GGA GGT CAT C-3′, (R)5′-GCG TAG CCG CGG TTC TT-3′, Ins2 (F)5′-TCT TCT ACA CAC CCA TGT CCC-3′ (R)5′-GGT GCA GCA CTG ATC CAC-3′, Pdx1 (F)5′-GAC ACA TCA AAA TCT GGT TCC AAA-3′, (R)5′-TCC CGC TAC TAC GTT TCT TAT CTT C-3′). Samples were normalized to a control gene (S25, (F)5′-GTG GTC CAC ACT ACT CTC

TGA GTT TC-3′, (R)5′-GAC TTT CCG GCA TCC TTC TTC-3′), and the comparative threshold cycle method used to calculate gene expression levels.

Glucose stimulated insulin secretion assay.

To determine glucose-responsive insulin secreted from β-cells cultured on Non-coating (at day 1 as positive control, day 7 and day 21) and Bead+EphA/EphrinA+DCM (at day 7 and day 21), cells were preincubated in the Krebs Ringer HEPES (KRBH) buffer solution at 37°C for 45 min.[1] The KRBH buffer consisted of 118 mM sodium chloride, 4.7 mM potassium chloride, 1.1 mM potassium dihydrogen phosphate, 25 mM sodium hydrogen carbonate, 3.4 mM calcium chloride, 2.5 mM magnesium sulfate, 10 mM HEPES (all from Sigma), 2 mg/mL bovine serum albumin (Fisher Scientific), and 2.5 mM glucose. Then the cells were incubated in KRBH solutions containing 2.5 mM for 1 hour and cell media was collected; sequentially, the cells were incubated in KRBH solutions containing 25 mM glucose for 1 hour and cell media was collected. Samples were stored at -80°C. All samples are analyzed at the same time using a rat insulin ELISA kit (Mercodia Inc.) The amount of insulin secretion in 25 mM glucose containing KRBH was normalized to insulin secreted in 2.5 mM glucose containing KRBH and expressed as insulin secretion index.

Lentivirus generation and plasmid construction.

The lentiviral vectors used in this study are based on the published method.[2] For generating and packaging lentiviral particles we used pCMV-VSV-G and pCMV-dR8.2. [3] The construction of pLV_Dest-R4R2 has been described elsewhere. [4] The insulin promoter reporter construct (pENTR_L1_hIns(3x)_L2) was constructed in multiple cloning steps based on the design of pGL3.hINS-363-3×. [5] In a first step, a 406 bp fragment was PCR-amplified from from human genomic DNA (Bioline, Taunton MA, USA) using oligos oPG222 (CCG GAA TTC GGA TCC ACG CTC GAG CTG AAA GCT TCA GCA GCG CAA AGA GCC CCG CCC TGC) and oPG221 (CCG GAA TTC GAG GGC TGC TGG GCC CCCG), digested with EcoRI, ligated into pENTR_L4R1 (Life Technologies) and checked for directionality, yielding pENTR_L4_hIns(1x)_R1. In a second step a 358 bp fragment was PCR-amplified from pENTR_L4_hIns(1x)_R1 using oligos oPG224 (GAT CCG CTC GAG CAG CAG CGC AAA GAG CCC CGC CCT GC) and oPG225 (GCA TCC CAA GCT TGT CTC AGA GCC CAT CTC CCC TACC), digested with XhoI/HindIII and ligated into XhoI/HindIII-cut pENTR_L4_hIns(1x)_R1, yielding pENTR_L4_hIns(2x)_R1. In a third step a 354 bp fragment was PCR-amplified from pENTR_L4_hIns(1x)_R1 using oligos oPG226 (CGC GGA TCC CAG CAG CGC AAA GAG CCC CGC CCT GC) and oPG227 (GAT CCG CTC GAG GTC TCA GAG CCC ATC TCC CCT ACC), digested with BamHI/XhoI and ligated into BamHI/XhoI-cut pENTR_L4_hIns(2x)_R1, yielding pENTR_L4_hIns(3x)_R1. pENTR_L1_mKate-2A-Puro_L2 was constructed by PCR-amplifying mKate (Evrogen, Moscow, Russia) with oPG130 (CTT AGG ATC CGG TAC CGG TCG CCA CCA TGG TGT CTA AGG GCG AAG AGC TG) and oPG131 (CGT CGC CGC AGG TCA GCA GGC TGC CGC GGC CCT CGG AAT TAA GTT TGT GCC CCA GTT TGC) and the Puromycin resistance gene with oPG132 (CTG ACC

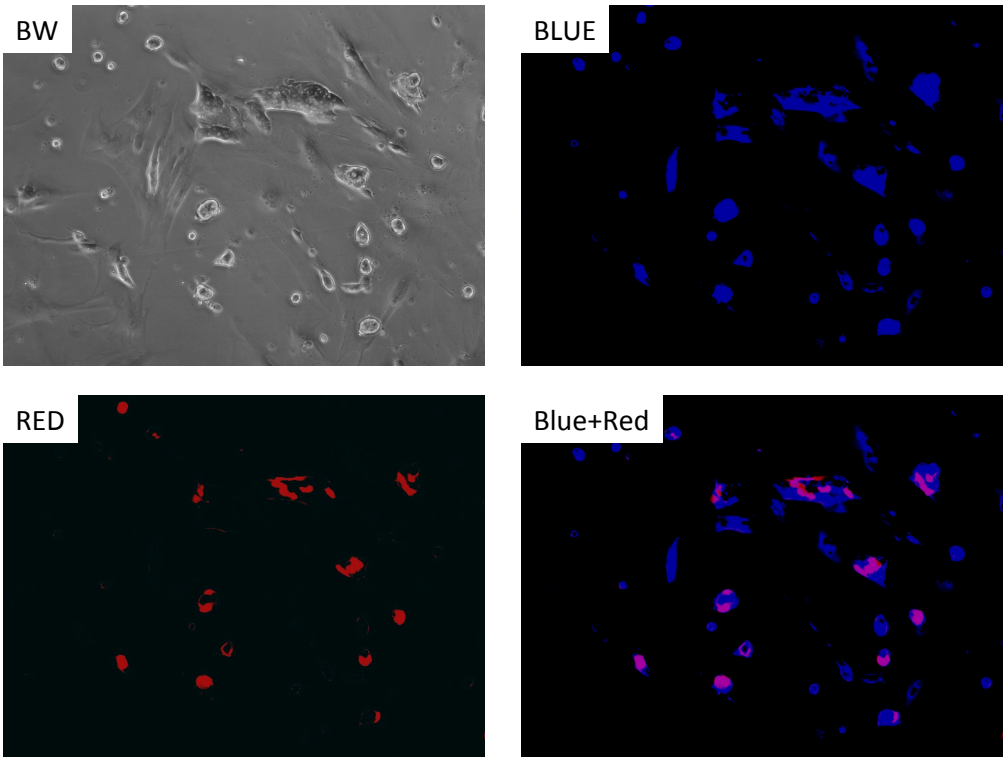
TGC GGC GAC GTG GAG GAA AAC CCC GGC CCC ATG ACC GAG TAC AAG CCC ACG GTG) and oPG133 (GGT AGG TAG AAT TCT CAG GCA CCG GGC TTG CGG GTC ATG) separately. In a second PCR fusing the two fragments together by Gene splicing by overlap extension [6] using oPG134 (GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT AGG ATC CGG TAC CGG TCGC) and oPG135 (GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA GGT AGA ATT CTC AGG CAC). This PCR product was recombined into pDONRP1P2 using the BP reaction (Life Technologies). pENTR_L1_EBFP2_L2 was constructed by PCR-amplifying EBFP2 (Evrogen) with the oligo oPG1160 (GGG GAC AAG TTT GTA CAA AAA AGC AGG CTG ACC GCC ACC ATG GTG AGC AAG GG) and pPG1161 (GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA TTA CTT GTAC AGC TCG TCC ATG CCGA) and recombining this PCR product into pDONRP1P2 using the BP reaction (Life Technologies). The lentiviral vector pLV_hIns(3x)_mKate-2A-Puro was generated by recombining equimolar amounts of pENTR_L4_hIns(3x)_R1, pENTR_L1_mKate-2A-Puro_L2 and pLV_Dest-R4R2 using the LR recombinase (Life Technologies). The lentiviral vector pLV_hEF1a_EBFP2 was generated by recombining equimolar amounts of pENTR_L4_hEF1a_R1,[7] pENTR_L1_EBFP2_L2 and pLV_Dest-R4R2 using the LR recombinase (Life Technologies).

Lentiviral infection of β-cells

For lentiviral particle production, we used the 2nd generation lentiviral packaging system. Briefly, co-transfection of the vector encoding the mRNA to be packaged with pCMV-dR8.2 and pVSV-G into HEK293FT cells (Invitrogen) induced production of lentiviral particles. Supernatant was harvested at day 2 and day 3 post-transfection, pooled and concentrated using 100kDa centrifugal filer unit (Amicon Ultra-15, Millipore). Lentiviral particle stocks were kept at -80 °C.

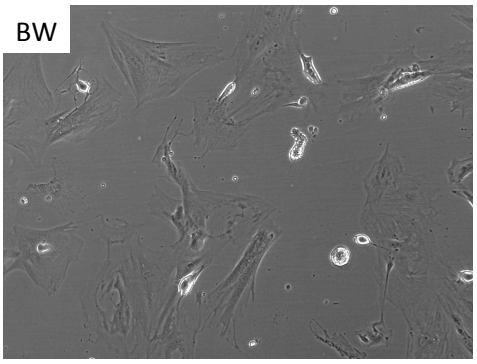
Microscope images in different fluorescent channels for Figure 8 .

After infection

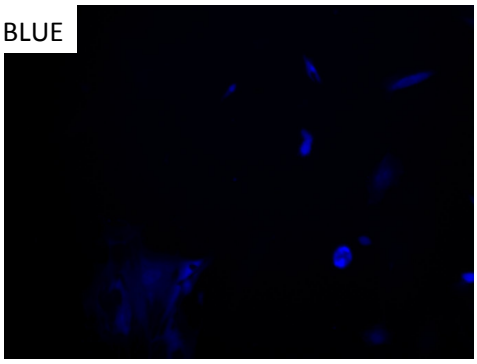


2D Non-coating
day7

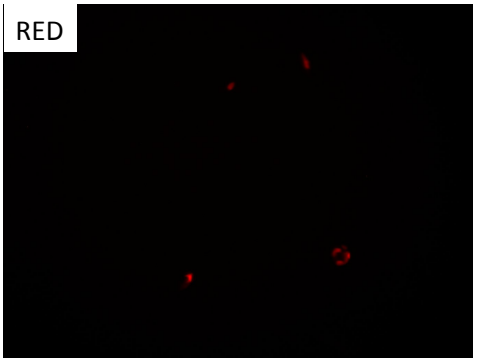
BW



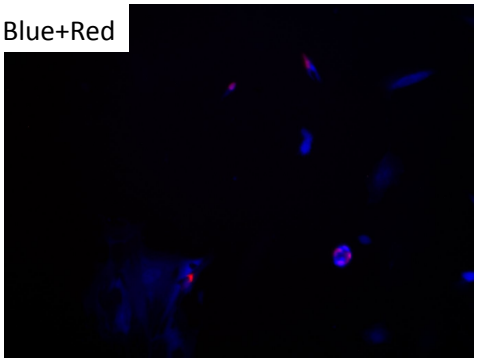
BLUE



RED

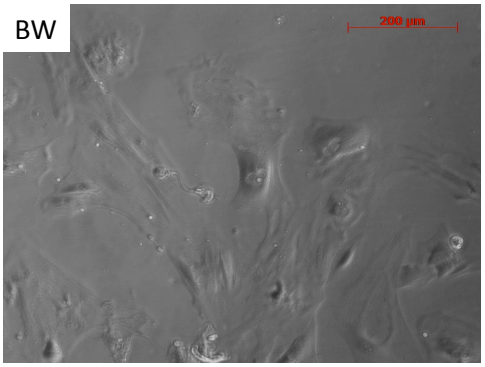


Blue+Red

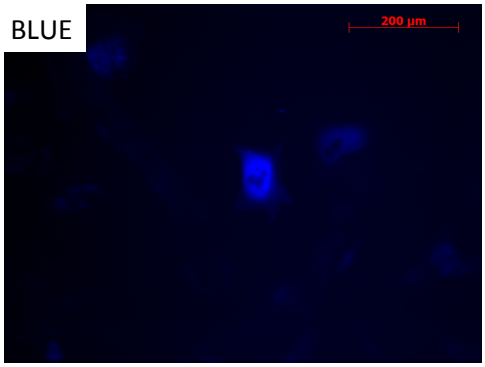


2D Non-coating
day 21

BW



BLUE



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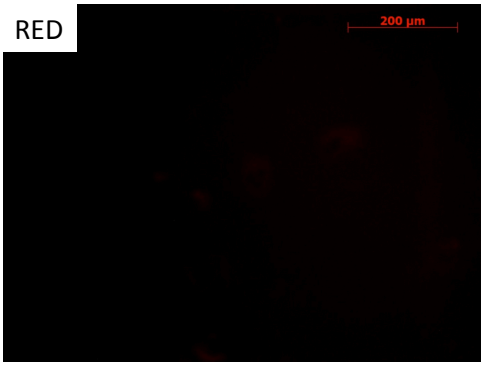
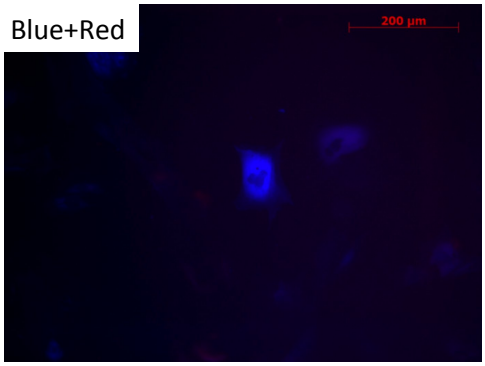
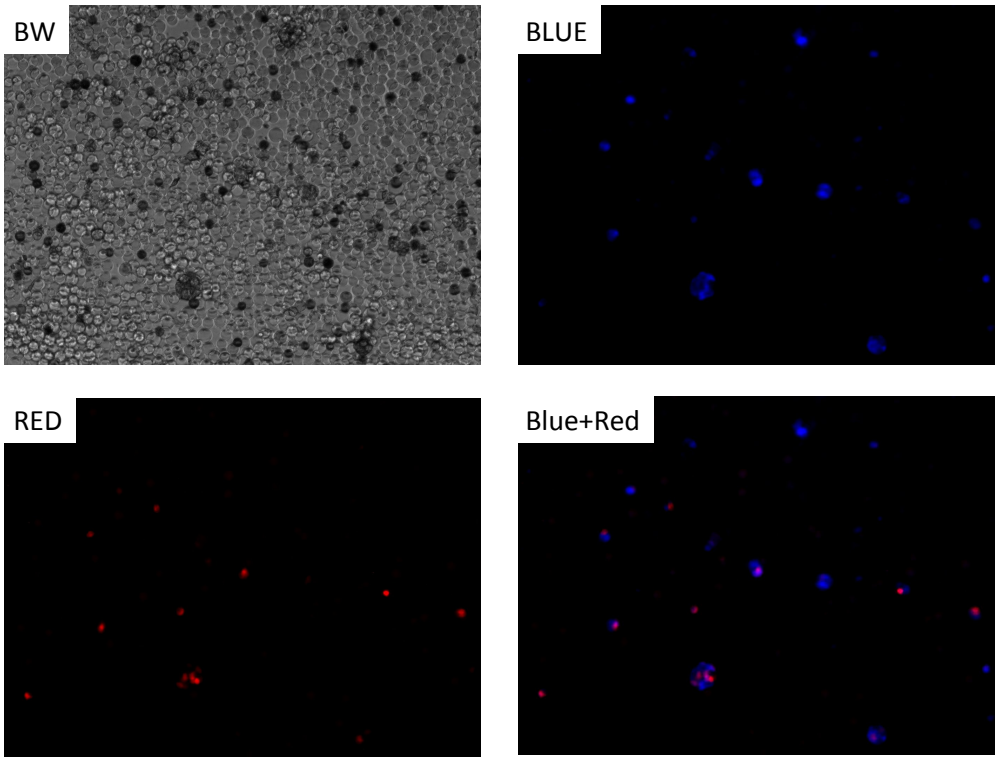


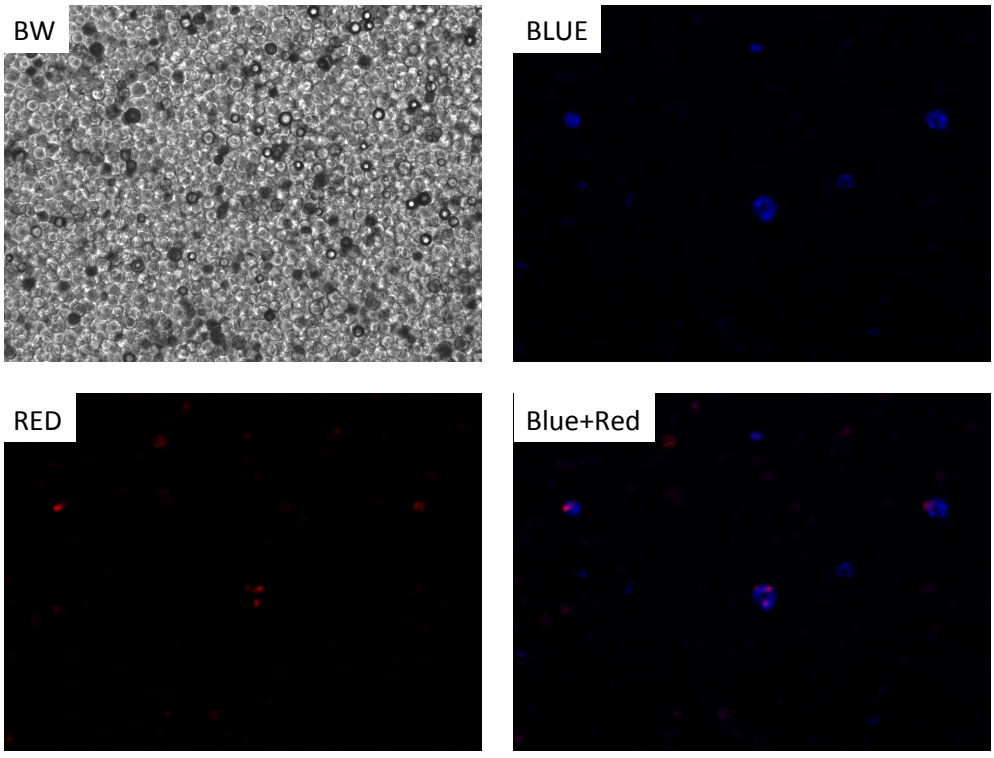
Blue+Red



Beads+EphA/EPHrinA+DCM
day7



Beads+EphA/EPHrinA+DCM
day 21



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