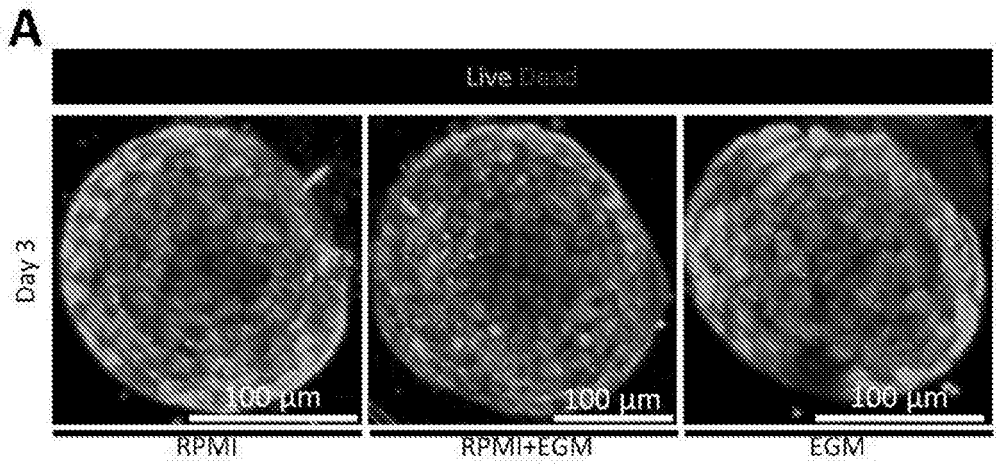
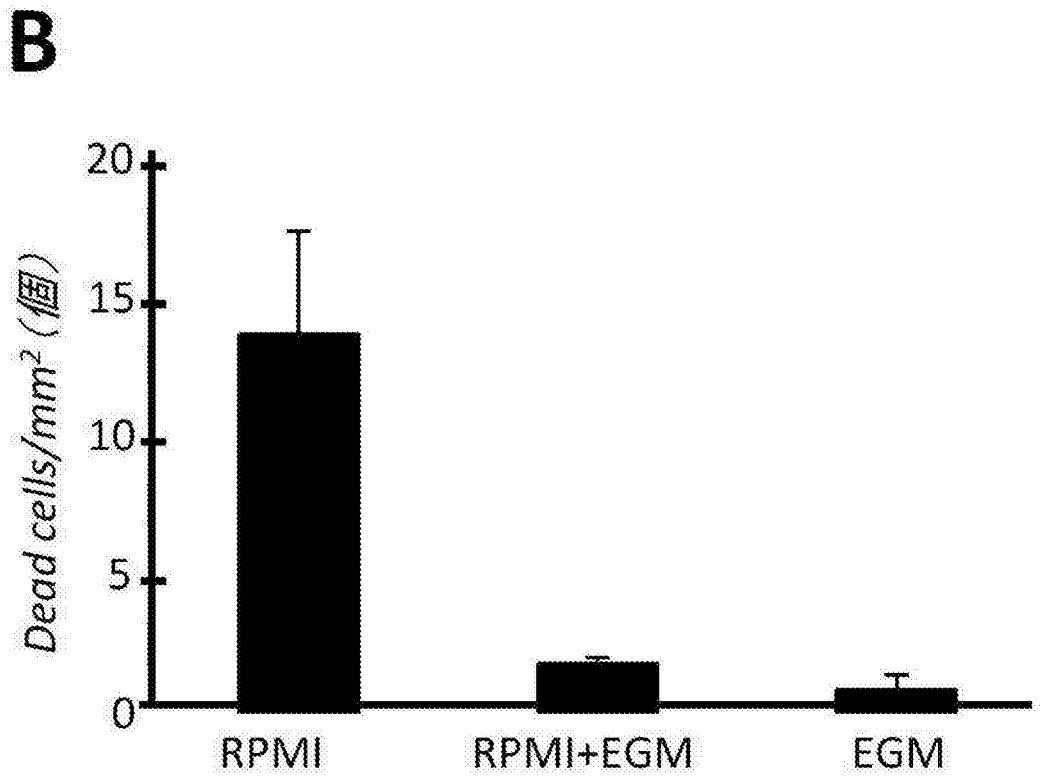
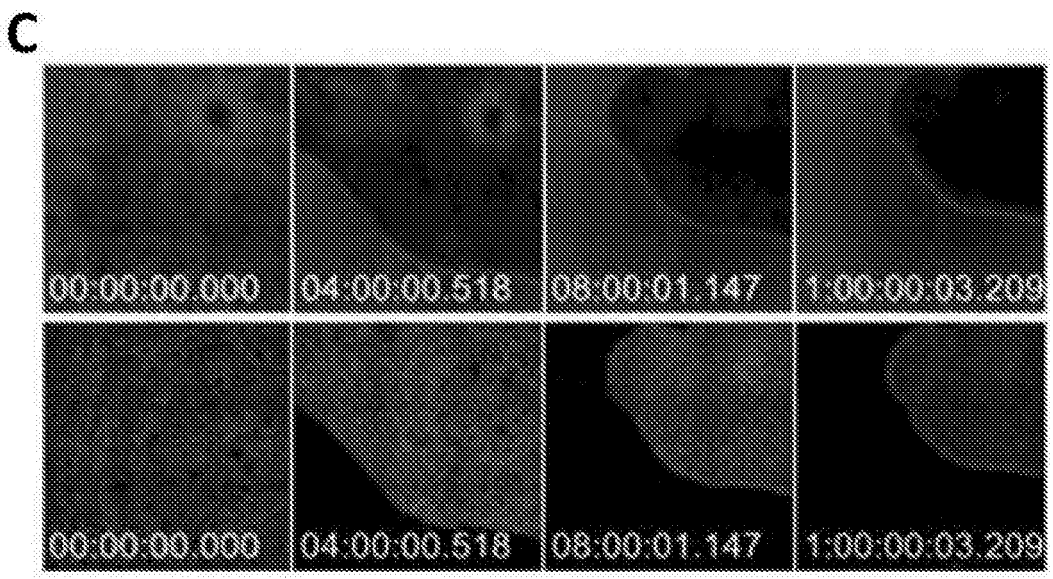
[図1A]


A
Live Dead
Day 3
100 μm
100 μm
100 μm
RPMI
RPMI+EGM
EGM

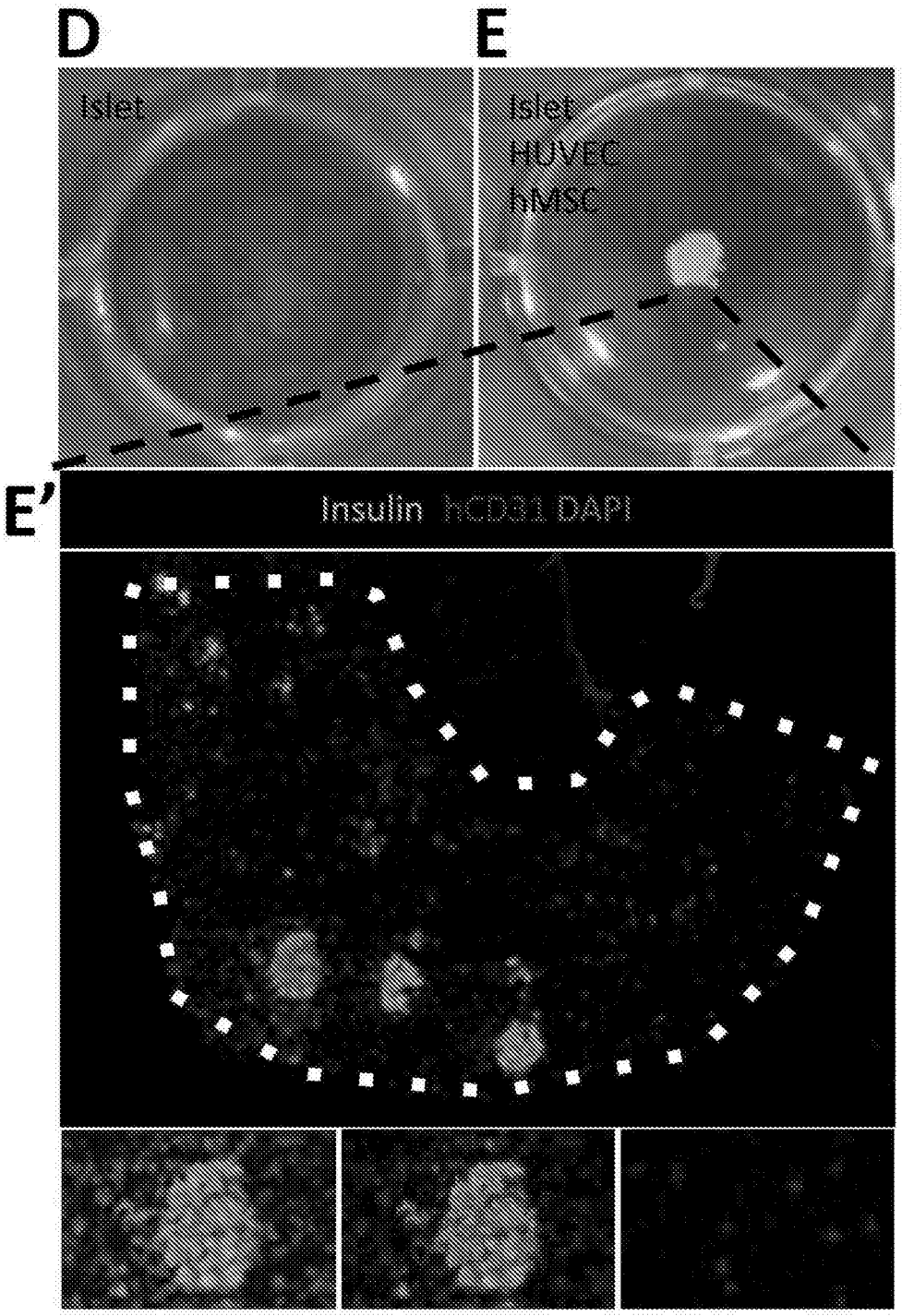
[図1B]


B
20
15
10
5
0
Dead cells/mm² (個)
RPMI
RPMI+EGM
EGM

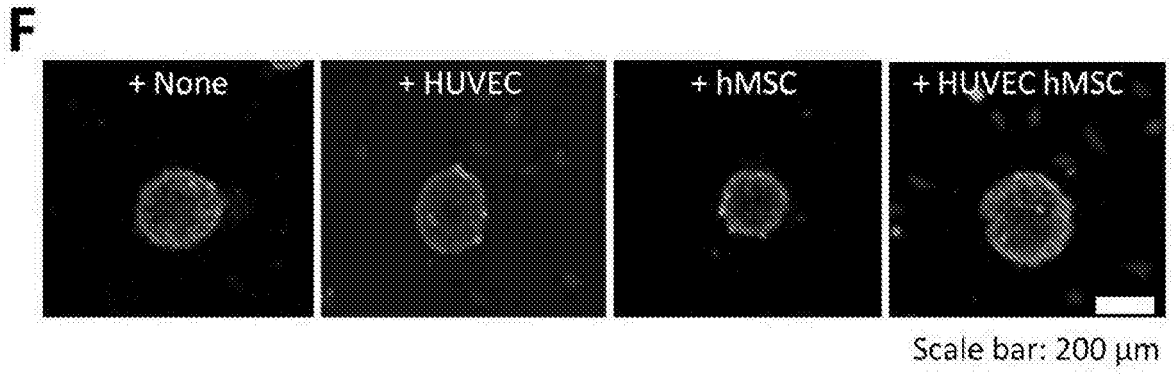
[図1C]


C
00:00:00.000
04:00:00.518
08:00:01.147
1:00:00:03.209
00:00:00.000
04:00:00.518
08:00:01.147
1:00:00:03.209

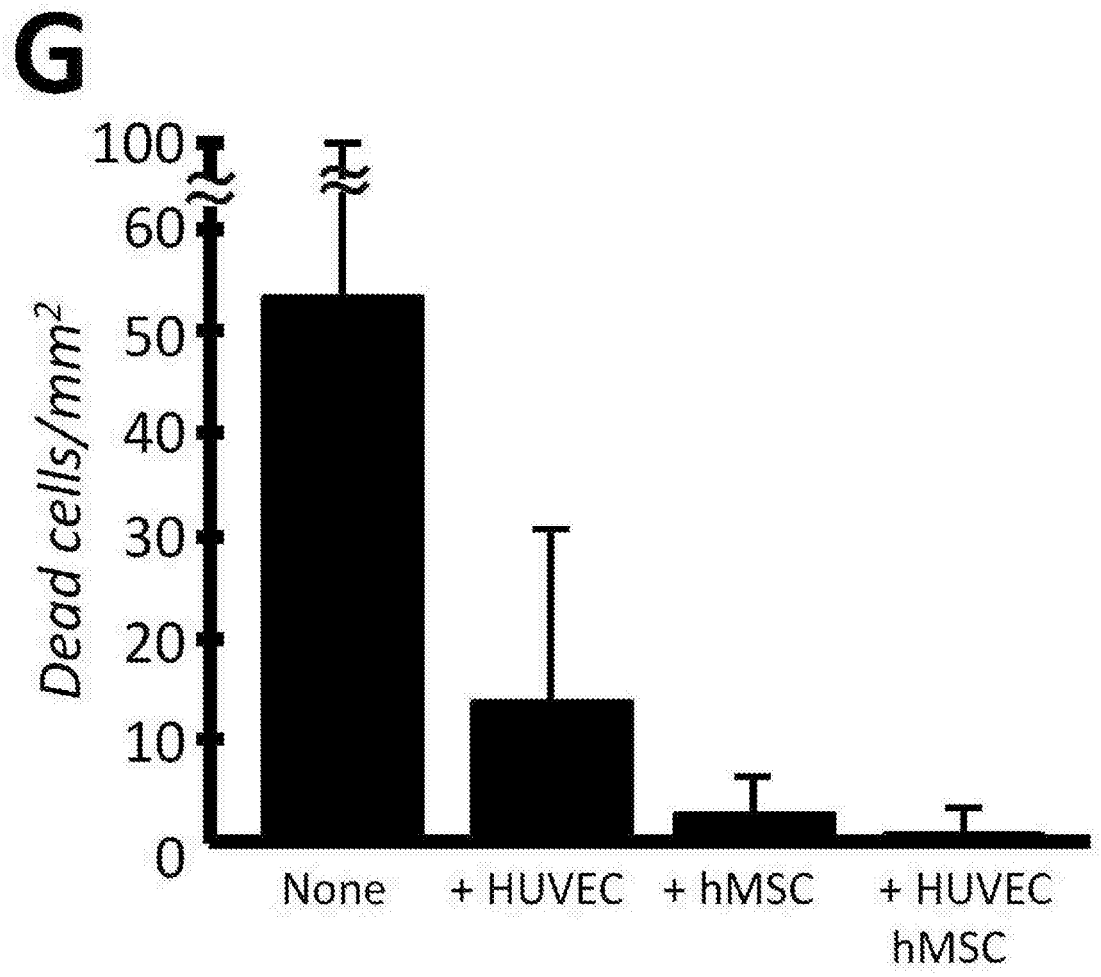
[図1DE]


D
E
Islet
Islet
HUVEC
hMSC
E'
Insulin hCD31 DAPI

[図1F]

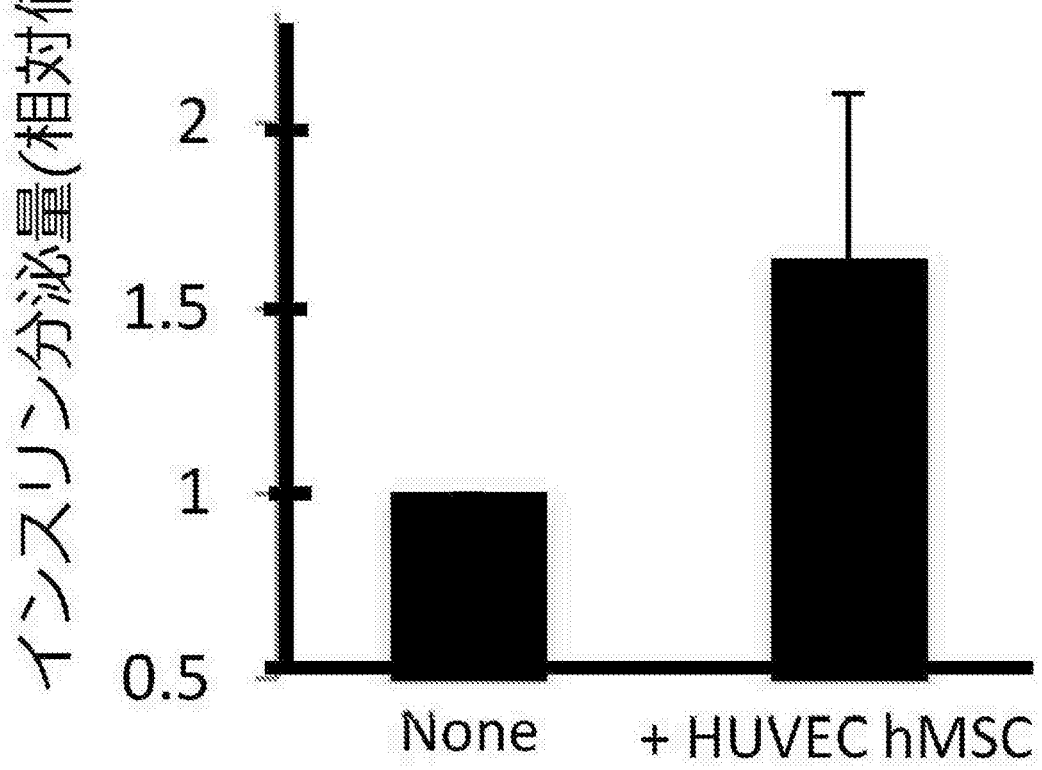

F
+ None
+ HUVEC
+ hMSC
+ HUVEC hMSC
Scale bar: 200 μm

[図1G]

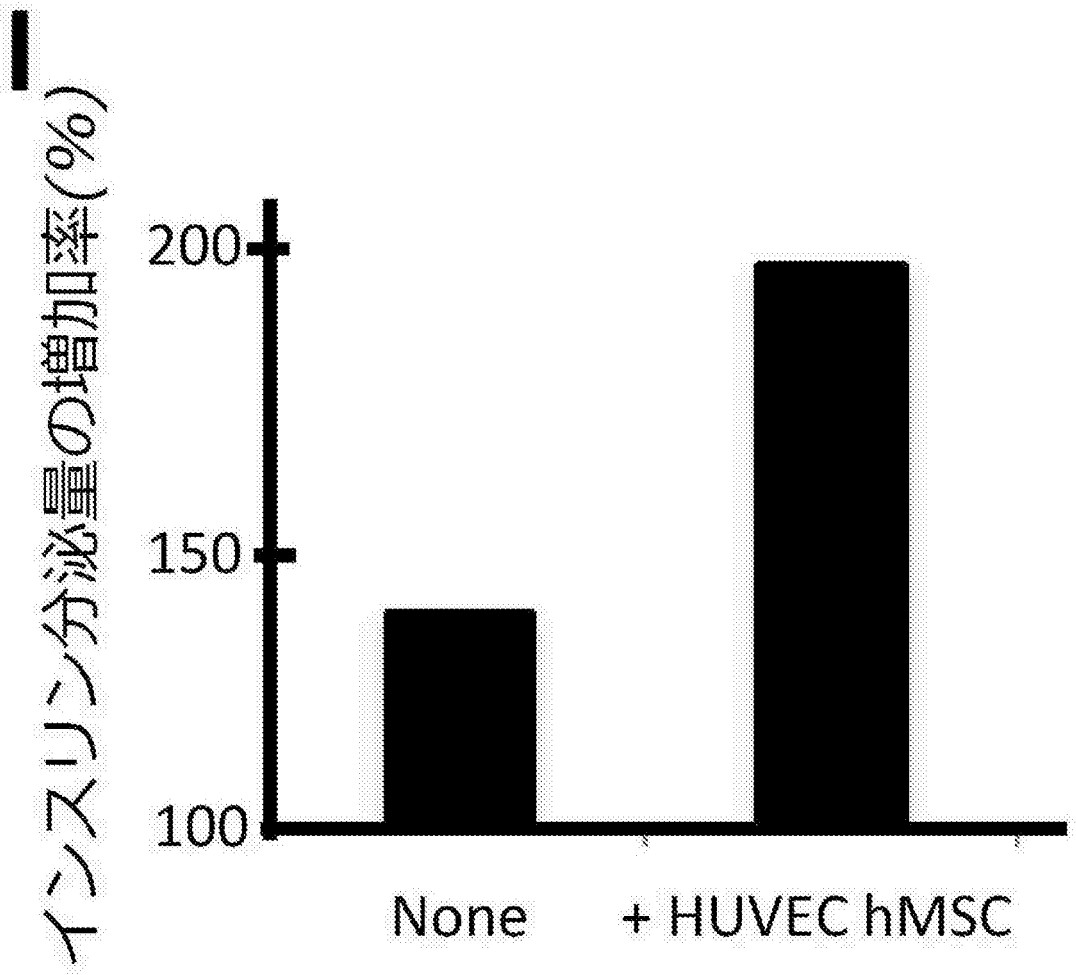

G
100
60
50
40
30
20
10
0
Dead cells/mm2
None
+ HUVEC
+ hMSC
+ HUVEC
hMSC

[図1H]

H



[図1I]



[図1J-1]

J

遺伝子名	共培養で発現増強する遺伝子(2倍以上)	GeneSymbol	GenbankAccession
angiopoietin 2	86404010298	ANGPT2	NM_001147
chemokine (C-C motif) ligand 14	12485228642	CCL14	NM_032963
von Willebrand factor	9723989921	VWF	NM_000552
spondin 2, extracellular matrix protein	5173085781	SPON2	AK124606///NM_012445
interleukin 1 receptor-like 1	2871116401	IL1RL1	AB029084///NM_016232
insulin-like growth factor binding protein 3	1732005234	IGFBP3	NM_001013398
platelet/endothelial cell adhesion molecule	1200911766	PECAM1	NM_000442
MFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	737585210.5	MFNG	NM_002405
decorin	62323314.06	DCN	NM_133507
desert hedgehog	44521819.03	DHH	NM_021044
thrombomodulin	13577375.84	THBD	NM_000361
jagged 2	9178351.802	JAG2	NM_002226
apelin	9075185.72	APLN	NM_017413
aggrecan	6258112.989	ACAN	NM_013227///NM_001135
retinol binding protein 4, plasma	5501722.736	RBP4	NM_006744
collagen, type VI, alpha 3	5342235.144	COL6A3	NM_004369
interleukin 33	4920010.165	IL33	NM_033439
receptor (G protein-coupled) activity modifying protein 1	4884056.732	RAMP1	NM_005855
tumor necrosis factor (ligand) superfamily, member 10	4362487.545	TNFSF10	NM_003810
interleukin 11	3248955.777	IL11	NM_000641
matrix Gla protein	3002928.557	MGP	NM_001190839///NM_000
endothelin 1	2616534.708	EDN1	NM_001955
collagen, type III, alpha 1	2391112.666	COL3A1	NM_000090
pro-melanin-concentrating hormone	2339702.828	PMCH	NM_002674
ephrin-A1	2101511.157	EFNA1	NM_004428
glypican 5	569311.688	GPC5	NM_004466
chemokine (C-X-C motif) ligand 11	509934.3879	CXCL11	NM_005409
collagen, type VI, alpha 2	408334.8057	COL6A2	NM_058174///NM_058175
contactin associated protein-like 4	319100.5847	CNTNAP4	NM_033401///NM_138994
chitinase 3-like 1 (cartilage glycoprotein-39)	281018.1044	CHI3L1	NM_001276
wingless-type MMTV integration site family, member 5A	263620.0876	WNT5A	NM_003392
selectin P (granule membrane protein 140kDa, antigen CD62)	216688.0693	SELP	NM_003005
stimulator of chondrogenesis 1	185152.0394	SCRG1	NM_007281
protease, serine, 2 (trypsin 2)	128606.6424	PRSS2	NM_002770
CD40 molecule, TNF receptor superfamily member 5	127056.3281	CD40	NM_001250
matrix metallopeptidase 7 (matrilysin, uterine)	97987.22531	MMP7	NM_002423
placental growth factor	72232.24283	PGF	NM_002632///AK023843
lymphotoxin beta (TNF superfamily, member 3)	68001.8926	LTB	NM_002341
gremlin 2	65322.89144	GREM2	NM_022469
slit homolog 3 (Drosophila)	62653.46582	SLIT3	NM_003062
chemokine (C-C motif) ligand 23	54595.494	CCL23	NM_005064
periostin, osteoblast specific factor	50897.9268	POSTN	NM_006475
glia maturation factor, gamma	49956.96545	GMFG	NM_004877
bone morphogenetic protein 6	47659.98363	BMP6	NM_001718
fibroblast growth factor 16	46350.68961	FGF16	NM_003868
EGF-like-domain, multiple 7	41728.96374	EGFL7	NM_201446
vasohibin 1	35824.32614	VASH1	NM_014909
colony stimulating factor 3 (granulocyte)	35820.32319	CSF3	NM_000759
fibroblast growth factor 1 (acidic)	16579.67068	FGF1	NR_026696///NM_000800
bone morphogenetic protein 4	14963.88993	BMP4	NM_001202
interleukin 1, beta	10718.08014	IL1B	NM_000576
interleukin 8	8641.750034	IL8	NM_000584
cholesteryl ester transfer protein, plasma	7269.850838	CETP	NM_000078
gremlin 1	6841.392387	GREM1	NM_013372
complement factor I	6413.055523	CFI	NM_000204
v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	6212.681649	KIT	NM_001093772///NM_000
endothelial cell-specific molecule 1	5393.88395	ESM1	NM_007036
leukemia inhibitory factor (cholinergic differentiation factor)	4950.039677	LIF	NM_002309
tubulin, alpha 8	3636.752952	TUBA8	NM_018943
cysteine-rich secretory protein LCCL domain containing 2	3117.885771	CRISPLD2	NM_031476
epithelial mitogen homolog (mouse)	3080.182152	EPGN	NM_001013442
alpha-2-macroglobulin	2091.158733	A2M	NM_000014
chemokine (C-X-C motif) ligand 2	2041.672598	CXCL2	NM_002089
family with sequence similarity 3, member D	1982.131667	FAM3D	NM_138805
WNT1 inducible signaling pathway protein 3	1893.120682	WISP3	NM_198239
bone morphogenetic protein 3	1775.228248	BMP3	NM_001201
insulin-like growth factor binding protein 6	1705.390738	IGFBP6	NM_002178
laminin, alpha 4	1701.743498	LAMA4	NM_001105206///NM_001
RAB3D, member RAS oncogene family	1695.082184	RAB3D	NM_004283
sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	1663.817163	SEMA3C	NM_006379
dedicator of cytokinesis 4	1642.331414	DOCK4	NM_014705
selectin E	1307.998473	SELE	NM_000450
solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	1192.381145	SLC6A3	NM_001044
threonine synthase-like 2 (S. cerevisiae)	1098.132133	THNSL2	NM_018271///NM185274
nerve growth factor (beta polypeptide)	1079.932805	NGF	NM_002506
proprotein convertase subtilisin/kexin type 1	994.7088227	PCSK1	NM_000439
microseminoprotein, beta-	878.5637296	MSMB	NM_002443
cytokine-like 1	863.3098578	CYTL1	NM_018659
interleukin 22 receptor, alpha 2	747.8728319	IL22RA2	NM_181310///NM_052962
tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	613.9723312	TFPI	NM_001032281
tumor necrosis factor (ligand) superfamily, member 18	437.1089511	TNFSF18	NM_005092
urocortin 2	415.0944245	UCN2	NM_033199
progastricsin (pepsinogen C)	403.8345759	PGC	NM_001166424///NM_002
neuregulin 3	336.9189511	NRG3	NM_001010848
chemokine (C-C motif) ligand 25	312.2950065	CCL25	NM_005624
sushi-repeat containing protein, X-linked 2	303.6426689	SRPX2	NM_014467
ADAM metallopeptidase with thrombospondin type 1 motif, 4	303.5576625	ADAMTS4	BC030812
selectin P ligand	293.1140863	SELPLG	NM_001206609
lysyl oxidase	272.618485	LOX	NM_002317
fibroblast growth factor 5	259.9950921	FGF5	NM_004464///NM_033143
chemokine (C-C motif) ligand 16	238.9045109	CCL16	NM_004590
dedicator of cytokinesis 5	221.0027104	DOCK5	NM_024940
apolipoprotein D	185.1239881	APOD	NM_001647
dedicator of cytokinesis 9	142.42849	DOCK9	NM_001130048///AK09075

[図1J-2]

J(continued)

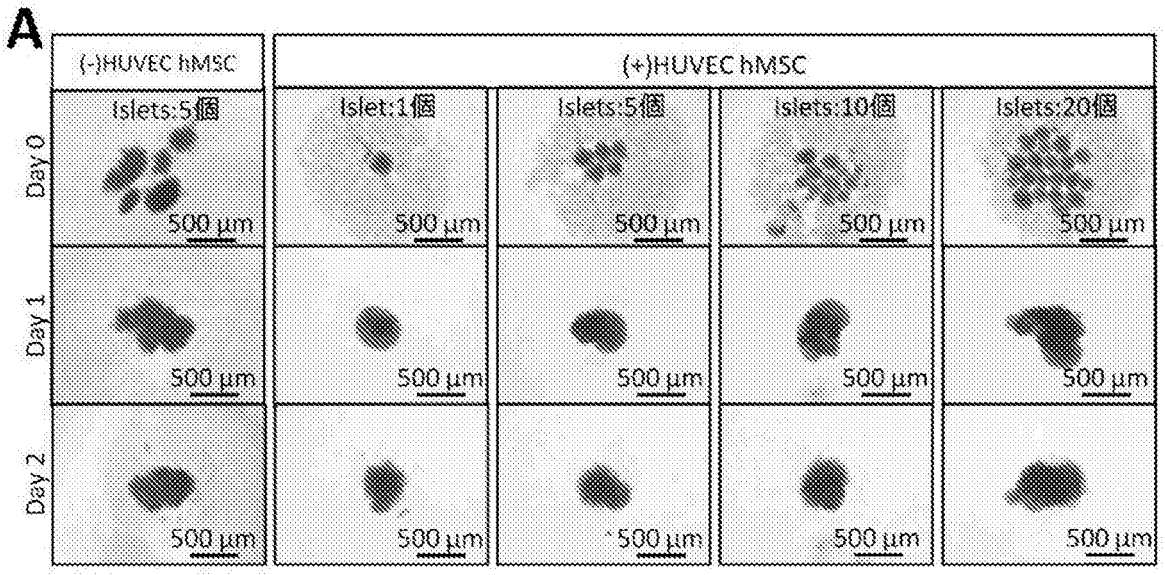
interleukin 15 receptor, alpha	111.072268	IL15RA	NM_002189///NM_172200
elastin microfibril interfacer 1	100.1885866	EMILIN1	NM_007046
c-fos induced growth factor (vascular endothelial growth factor D)	95.29124914	FIGF	NM_004469
follistatin-like 3 (secreted glycoprotein)	88.64529712	FSTL3	NM_005860
interleukin 17B	84.81534637	IL17B	NM_014443
serpin peptidase inhibitor, clade B (ovalbumin), member 2	79.84232039	SERPINB2	NM_002575
purinergic receptor P2X, ligand-gated ion channel, 7	74.58845991	P2RX7	NM_002562
neuregulin 2	71.43796095	NRG2	NM_013982
chemokine (C-C motif) ligand 4	70.57004592	CCL4	NM_002984
interleukin 1, alpha	70.32970569	IL1A	NM_000575
insulin-like growth factor binding protein 1	70.02643222	IGFBP1	NM_000596
CD97 molecule	60.1517449	CD97	NM_078481
chemokine (C-C motif) receptor 3	58.8548287	CCR3	NM_001837
brevican	58.37805213	BCAN	NM_198427
dedicator of cytokinesis 6	55.97183175	DOCK6	NM_020812
C-type lectin domain family 3, member B	55.52562299	CLEC3B	NM_003278
interleukin 21	53.40043564	IL21	NM_021803
sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F	47.83718329	SEMA3F	NM_004186
metastasis suppressor 1	45.71291541	MTSS1	NM_014751
coagulation factor XII (Hageman factor)	45.13489375	F12	NM_000505
interleukin 18 binding protein	44.57019149	IL18BP	NM_173042
laminin, alpha 2	41.08181103	LAMA2	NM_000426
mutS homolog 5 (E. coli)	41.03878268	MSH5	NM_002441///NM_025259
chemokine (C-C motif) ligand 3-like 3	39.8024495	CCL3L3	NM_001001437
prostaglandin D2 synthase 21kDa (brain)	38.61488629	PTGDS	CR610092///NM_000954
interleukin 6 receptor	36.36562031	IL6R	NM_000565
CKLF-like MARVEL transmembrane domain containing 1	30.52048198	CMTM1	NM_052999
nephroblastoma overexpressed gene	30.24886415	NOV	NM_002514
R-spondin 3	28.01382427	RSPO3	NM_032784
gastrokine 1	25.73552072	GKN1	NM_019617
wingless-type MMTV integration site family, member 4	25.56496485	WNT4	NM_030761
angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	23.35608784	AGT	NM_000029
advanced glycosylation end product-specific receptor	22.24914723	AGER	NM_001206954///NM_001
dedicator of cytokinesis 2	22.01202412	DOCK2	NM_004946
keratinocyte growth factor-like protein 1	21.08057189	KGFLP1	NR_003674
neuron-derived neurotrophic factor	15.36653325	NDNF	NM_024574
ovostatin 2	15.23938793	OVOS2	AL831947///XM_00312021
angiopoietin-like 4	15.25881921	ANGPTL4	NM_139314
UL16 binding protein 2	15.12748044	ULBP2	NM_025217
epidermal growth factor receptor	14.03965384	EGFR	NM_201282///NM_201283/
solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 2	13.68148347	SLC9A3R2	NM_004785
proteinase 3	12.6490517	PRTN3	NM_002777
inhibin, beta B	12.03648231	INHBB	NM_002193
ADAMTS-like 4	11.13213335	ADAMTSL4	NM_025008///NM_019032
inhibin, beta A	9.449823565	INHBA	NM_002192
tubulin, alpha 3c	8.918192656	TUBA3C	NM_006001
matrix metallopeptidase 21	8.866300776	MMP21	NM_147191
nucleoporin 43kDa	8.257302406	NUP43	NM_198887
tubulin, alpha 1a	7.612527947	TUBA1A	NM_006009
C-type lectin domain family 11, member A	7.595562932	CLEC11A	NM_002975
multimerin 2	7.518596479	MMRN2	XR_111094
collagen, type V, alpha 1	7.198248298	COL5A1	AK057231///NM_000093
interleukin 27	6.957908288	IL27	NM_145659
transforming growth factor, beta 1	6.719797719	TGFB1	NM_000660
growth differentiation factor 6	6.076175371	GDF6	NM_001001557
nucleoporin 188kDa	5.928274161	NUP188	NM_015354
renin	5.735120404	REN	NM_000537
ficolin (collagen/fibrinogen domain containing) 3 (Hakata antigen)	5.579305278	FCN3	AK309540///NM_003665
jagged 1	5.463098369	JAG1	NM_000214
phospholipase A2 receptor 1, 180kDa	5.433942018	PLA2R1	NM_007366
surfactant protein C	5.410387555	SFTPC	NM_003018
nucleoporin like 1	5.398132498	NUPL1	CR605945
oxidative stress induced growth inhibitor 1	5.382915594	OSGIN1	NM_013370
purinergic receptor P2X, ligand-gated ion channel, 4	5.285294847	P2RX4	NM_002560
four jointed box 1 (Drosophila)	5.128219904	FJX1	NM_014344
CD109 molecule	4.935494815	CD109	NM_133493
tumor necrosis factor (ligand) superfamily, member 9	4.914547082	TNFSF9	NM_003811
caveolin 1, caveolae protein, 22kDa	4.847462968	CAV1	NM_001753
anti-Mullerian hormone	4.635563131	AMH	NM_000479
dickkopf homolog 1 (Xenopus laevis)	4.595810382	DKK1	NM_012242
thyroglobulin	4.588026923	TG	NM_003235
follistatin-like 1	4.335395276	FSTL1	NM_007085
chemokine-like factor	4.24138953	CKLF	NM_181641///NM_016951
interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	4.203997093	IL12A	NM_000882
serglycin	4.200193514	SRGN	NM_002727
	4.100725577		
laminin, beta 1	3.9929475	LAMB1	BC044633///NM_002291
guanine nucleotide binding protein-like 1	3.921002327	GNL1	NM_005275
endoglin	3.90037622	ENG	NM_000118
bridging integrator 1	3.878636643	BIN1	NM_139348
collagen, type VIII, alpha 1	3.852157185	COL8A1	NM_001850
dedicator of cytokinesis 10	3.83181559	DOCK10	NM_014689
mitochondrial trans-2-enoyl-CoA reductase	3.75840537	MECR	NM_001024732
poliovirus receptor	3.720182967	PVR	NM_006505
secreted LY6/PLAUR domain containing 1	3.711362369	SLURP1	NM_020427
growth differentiation factor 5	3.513555354	GDF5	NM_000557
meteorin, glial cell differentiation regulator	3.474899111	METRN	NM_024042
epiregulin	3.378501764	EREG	NM_001432
rabeprilin, RAB GTPase binding effector protein 2	3.352418279	RABEP2	NM_024816
neuregulin 1	3.336921299	NRG1	NM_004495
transforming growth factor, beta 3	3.336107888	TGFB3	NM_003239
brain-derived neurotrophic factor	3.188190769	BDNF	NM_170735
thiosulfate sulfortransferase (rhodanese)	3.162499897	TST	NM_003312
glucosidase, beta, acid	3.152702564	GBA	NM_001005741
lymphotoxin alpha (TNF superfamily, member 1)	3.041885667	LTA	NM_000595
tubulin, beta 8	3.032914448	TUBB8	NM_177987

[図1J-3]

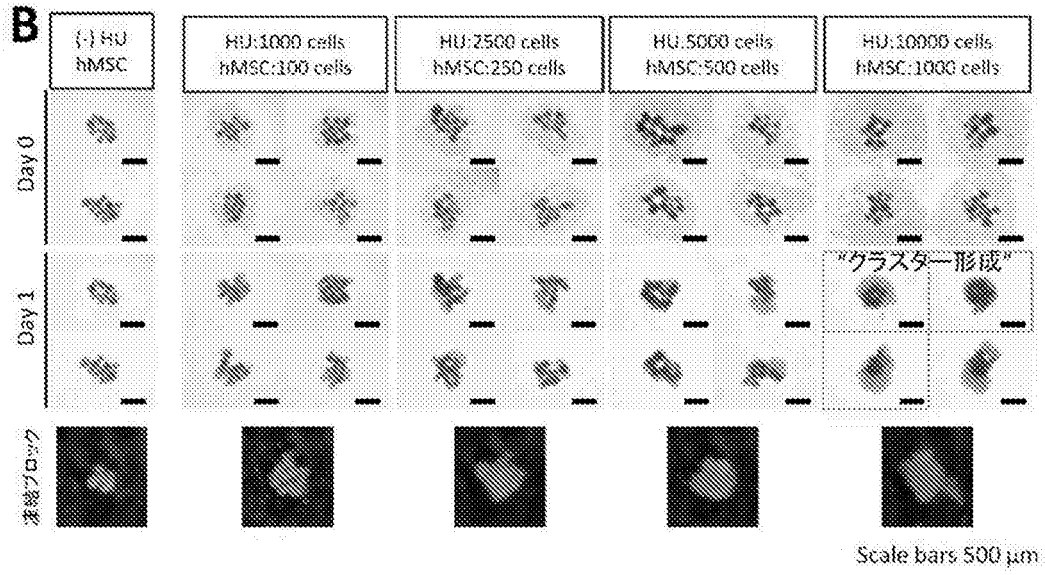
J(continued)

eukaryotic translation initiation factor 5A2	2.992937171	EIF5A2	NM_020390
ectonucleoside triphosphate diphosphohydrolase 6 (putative)	2.917940581	ENTPD6	NM_001247
interferon, alpha 4	2.896271023	IFNA4	NM_021068
interleukin 6 signal transducer (gp130, oncostatin M receptor)	2.704125478	IL6ST	NM_001190981///NM_002
EGF containing fibulin-like extracellular matrix protein 1	2.661340849	EFEMP1	NM_001039348
tachykinin 4 (hemokinin)	2.611744667	TAC4	NM_170685
tumor necrosis factor (ligand) superfamily, member 13b	2.600079337	TNFSF13B	NM_006573
collagen, type XXV, alpha 1	2.547336978	COL25A1	NM_198721
chemokine (C-C motif) ligand 1	2.498823606	CCL1	NM_002981
interleukin 17D	2.437773771	IL17D	NM_138284
Janus kinase 2	2.422259121	JAK2	NM_004972
wingless-type MMTV integration site family, member 10B	2.415007034	WNT10B	NM_003394
interleukin 6 (interferon, beta 2)	2.393918715	IL6	NM_000600///AK298077
cerebral dopamine neurotrophic factor	2.385407608	CDNF	NM_001029954
ADAM metallopeptidase domain 9	2.381497336	ADAM9	NM_003816
interleukin 16	2.340589002	IL16	NM_004513///NM_001172
slit homolog 2 (Drosophila)	2.325355194	SLIT2	NM_004787
proopiomelanocortin	2.307688809	POMC	NM_001035256
eukaryotic translation initiation factor 5A-like 1	2.269680153	EIF5AL1	NM_001099692
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	2.201240932	SMARCD3	NM_003078
contactin associated protein-like 3	2.157960545	CNTNAP3	AK024257///NM_033655
MHC class I polypeptide-related sequence A	2.072583804	MICA	NM_000247
annexin A1	2.033587436	ANXA1	NM_000700
tumor necrosis factor receptor superfamily, member 4	2.024829129	TNFRSF4	NM_003327

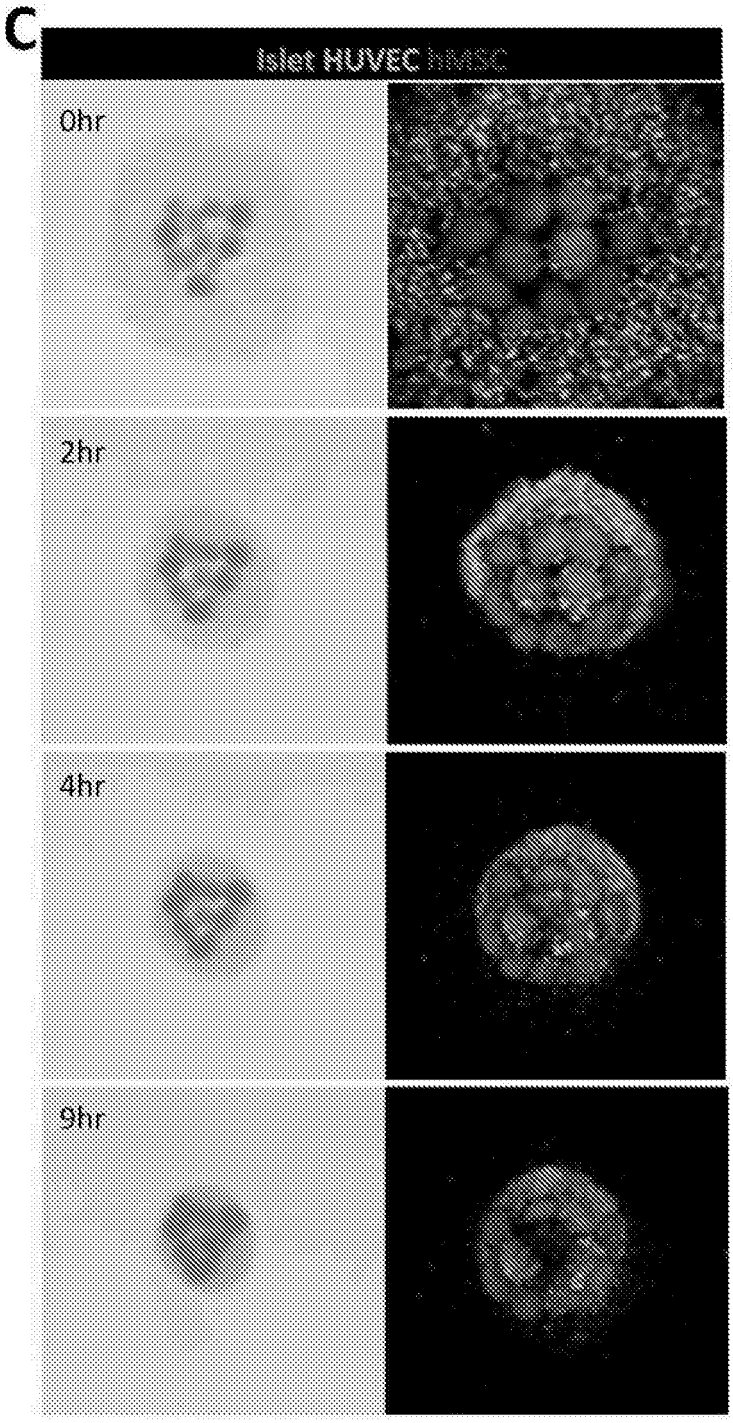
[図2A]


A
(-)HUVEC hMSC
(+)HUVEC hMSC
Islets:5個
Islet:1個
Islets:5個
Islets:10個
Islets:20個
Day 0
Day 1
Day 2
500 μm

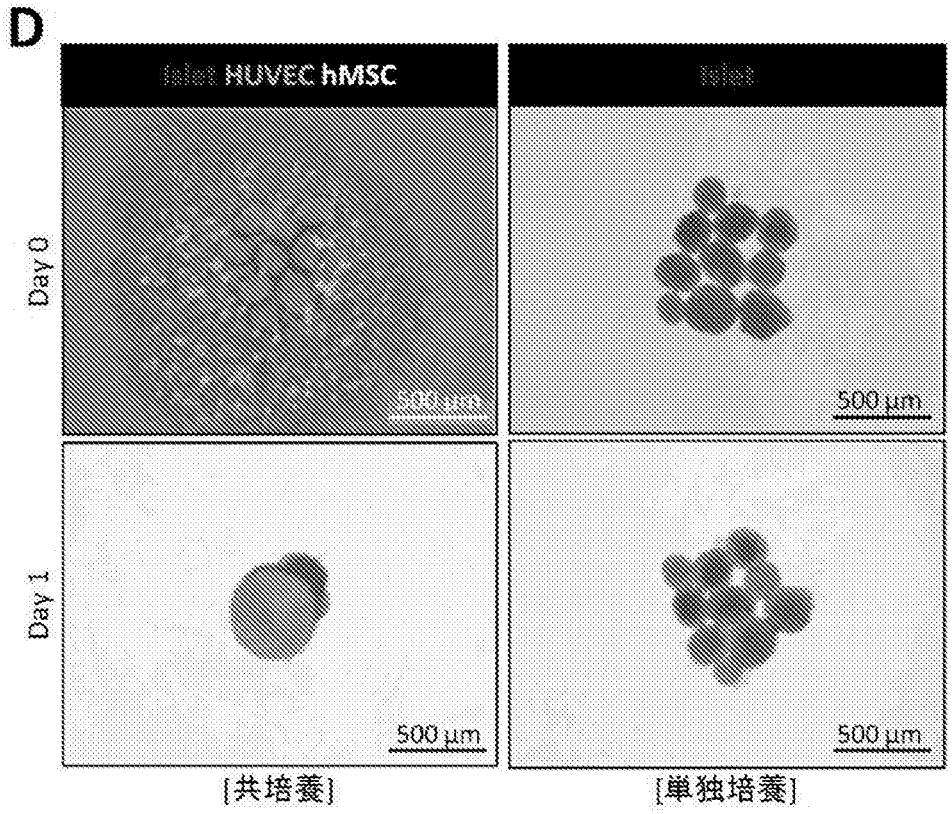
[図2B]


B
(-) HU hMSC
HU:1000 cells hMSC:100 cells
HU:2500 cells hMSC:250 cells
HU:5000 cells hMSC:500 cells
HU:10000 cells hMSC:1000 cells
Day 0
Day 1
"クラスター形成"
連続ブロック
Scale bars 500 μm

[図2C]

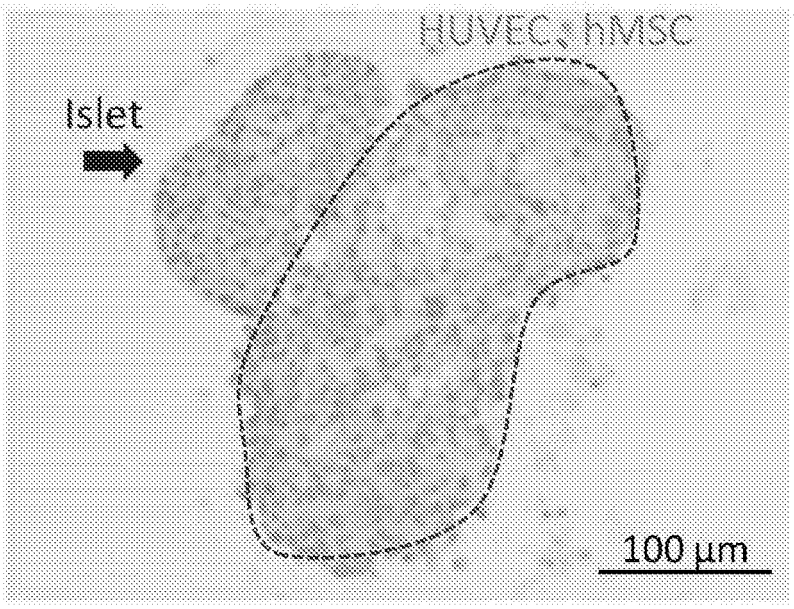


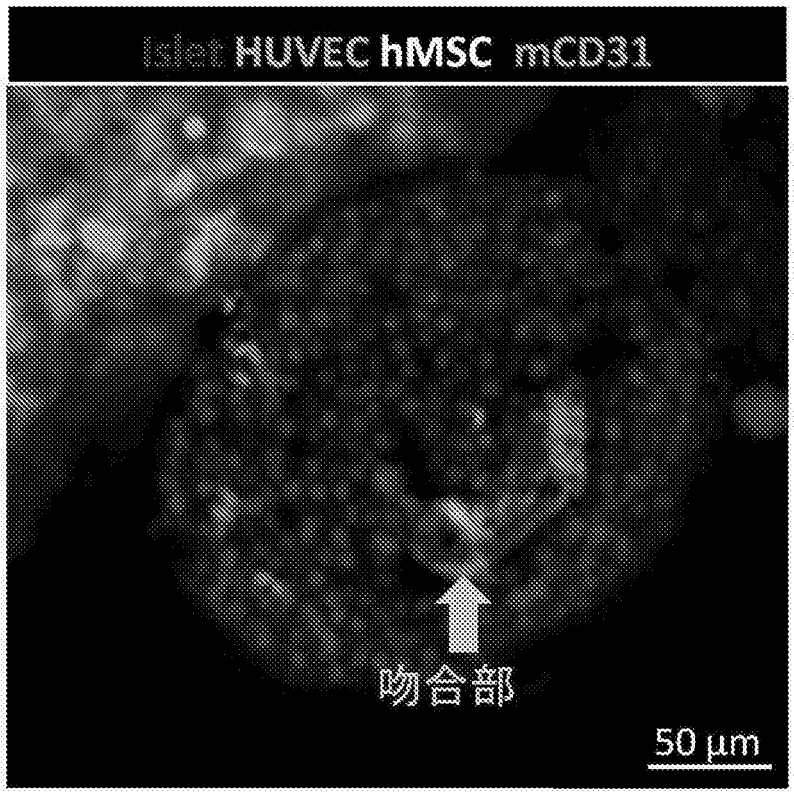
[図2D]


D
Islet HUVEC hMSC
Islet
Day 0
Day 1
500 μm
500 μm
500 μm
500 μm
[共培養]
[単独培養]

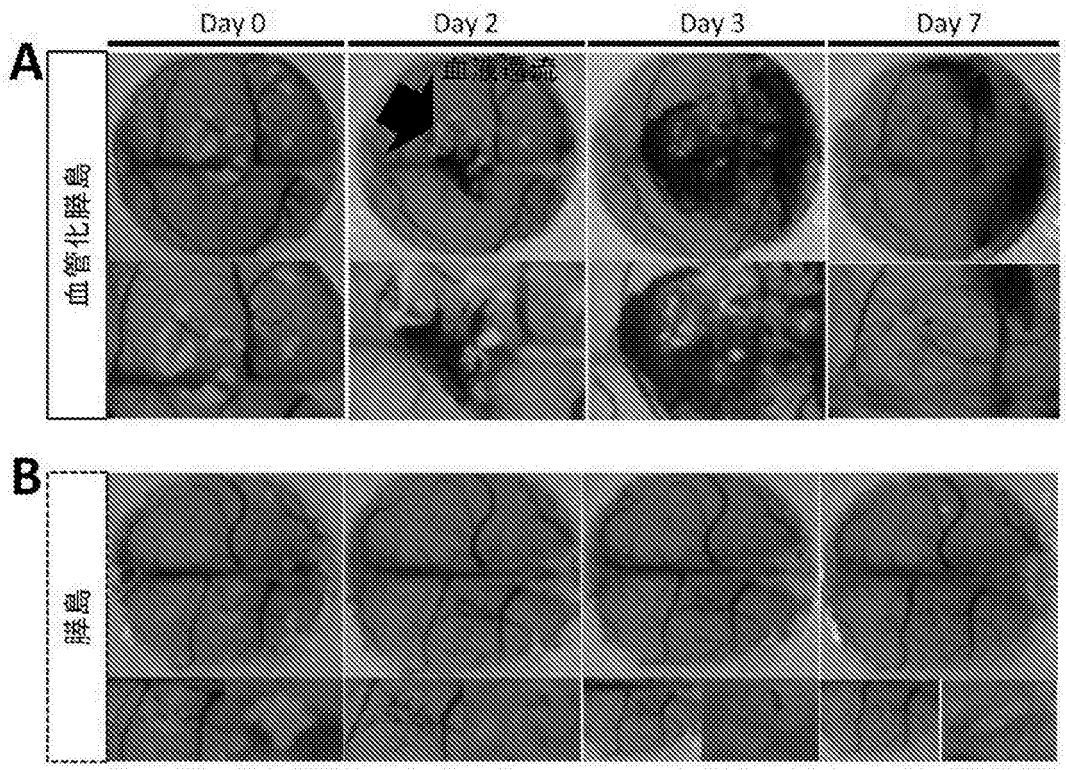
[図2E]

E

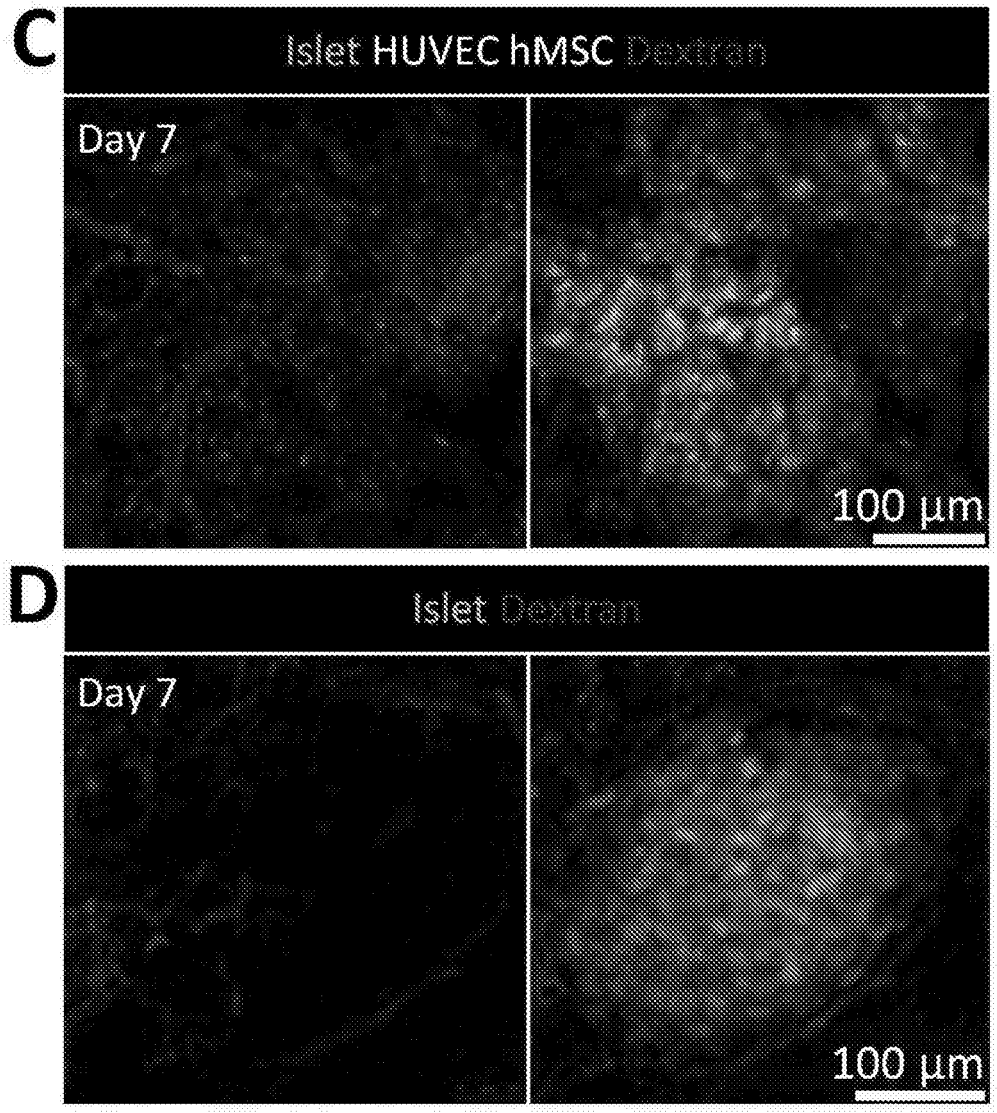




[図3AB]

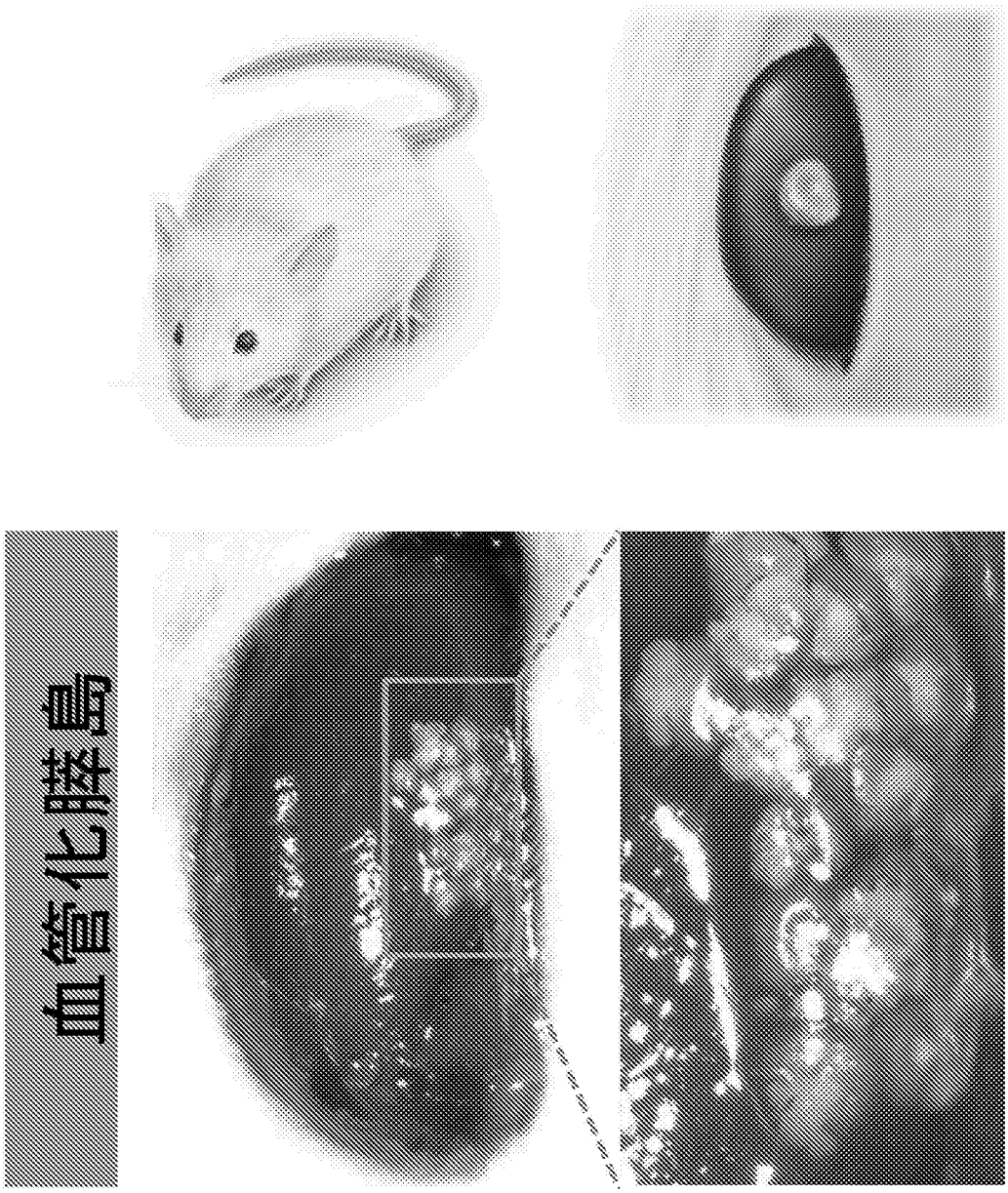


[図3CD]

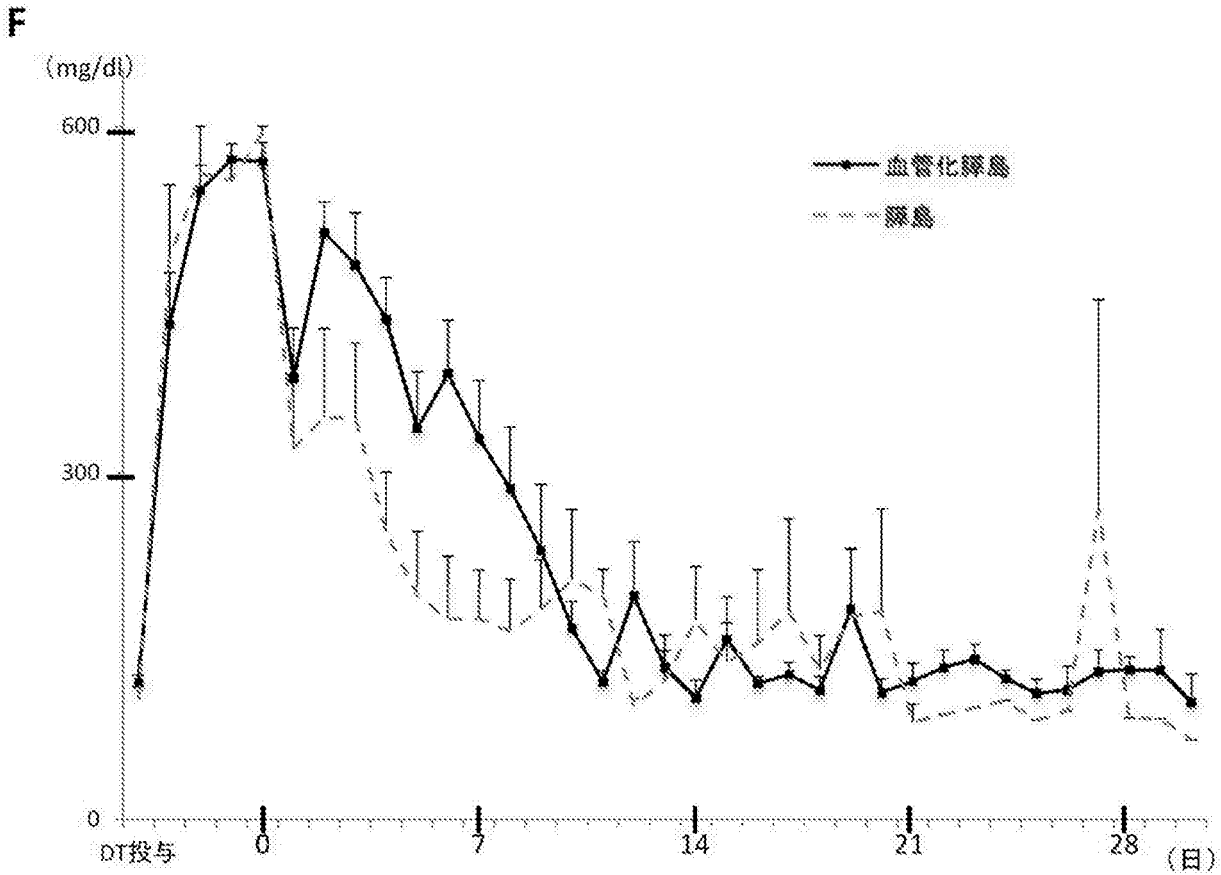

C
Islet HUVEC hMSC Dextran
Day 7
100 μm
D
Islet Dextran
Day 7
100 μm

[図3E]

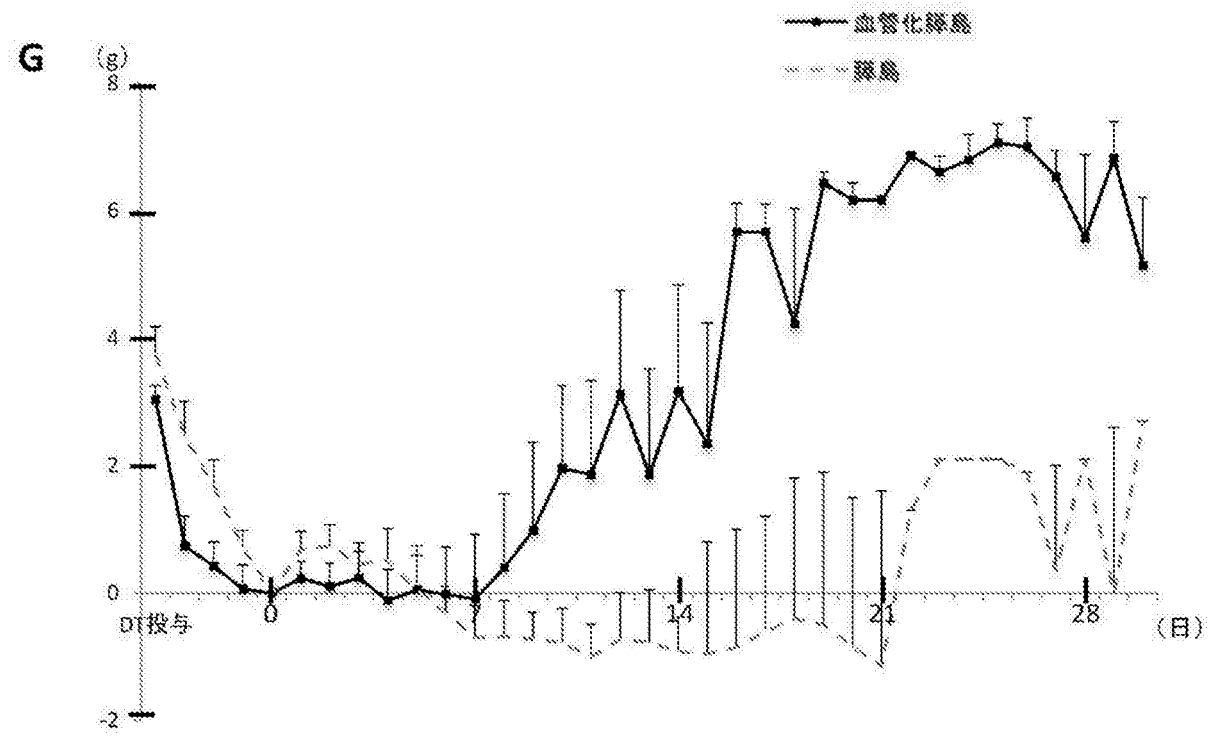
E



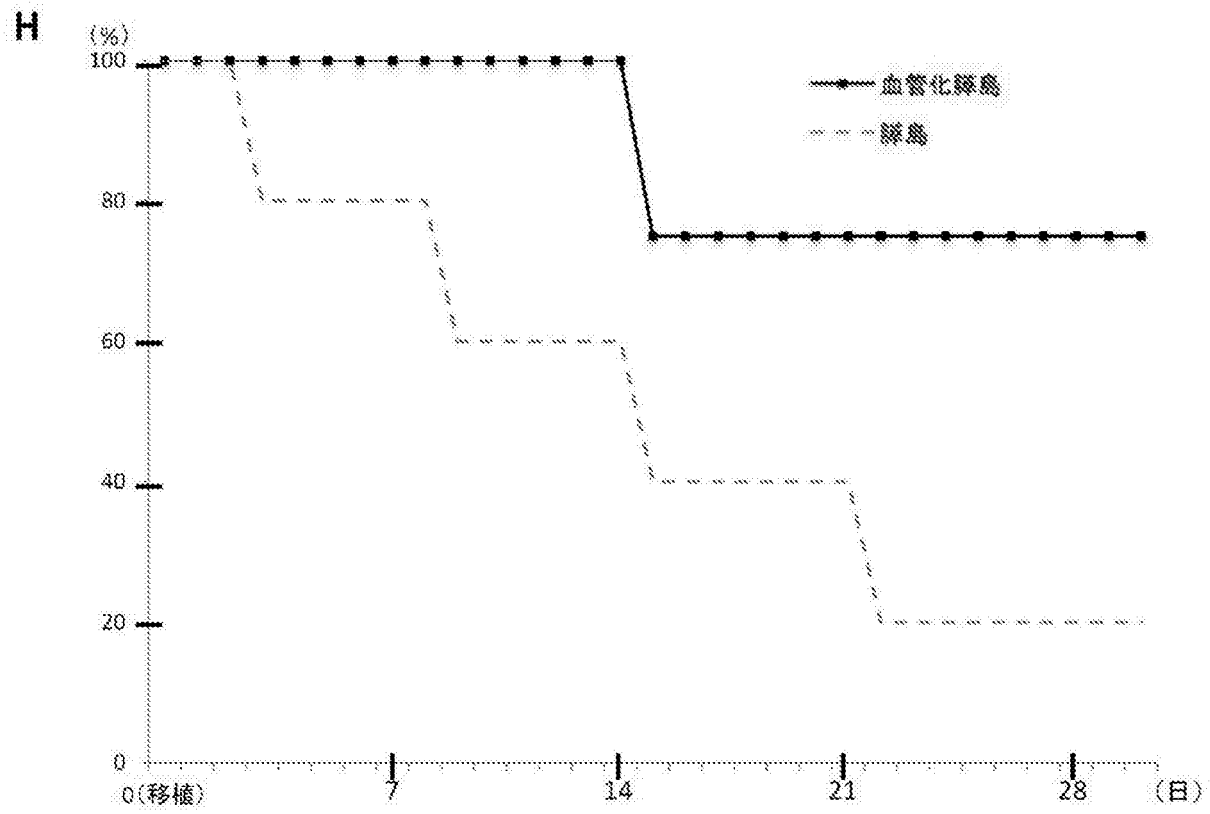
[図3F]


F
(mg/dl)
600
300
0
血管化膵島
膵島
DT投与
0
7
14
21
28
(日)

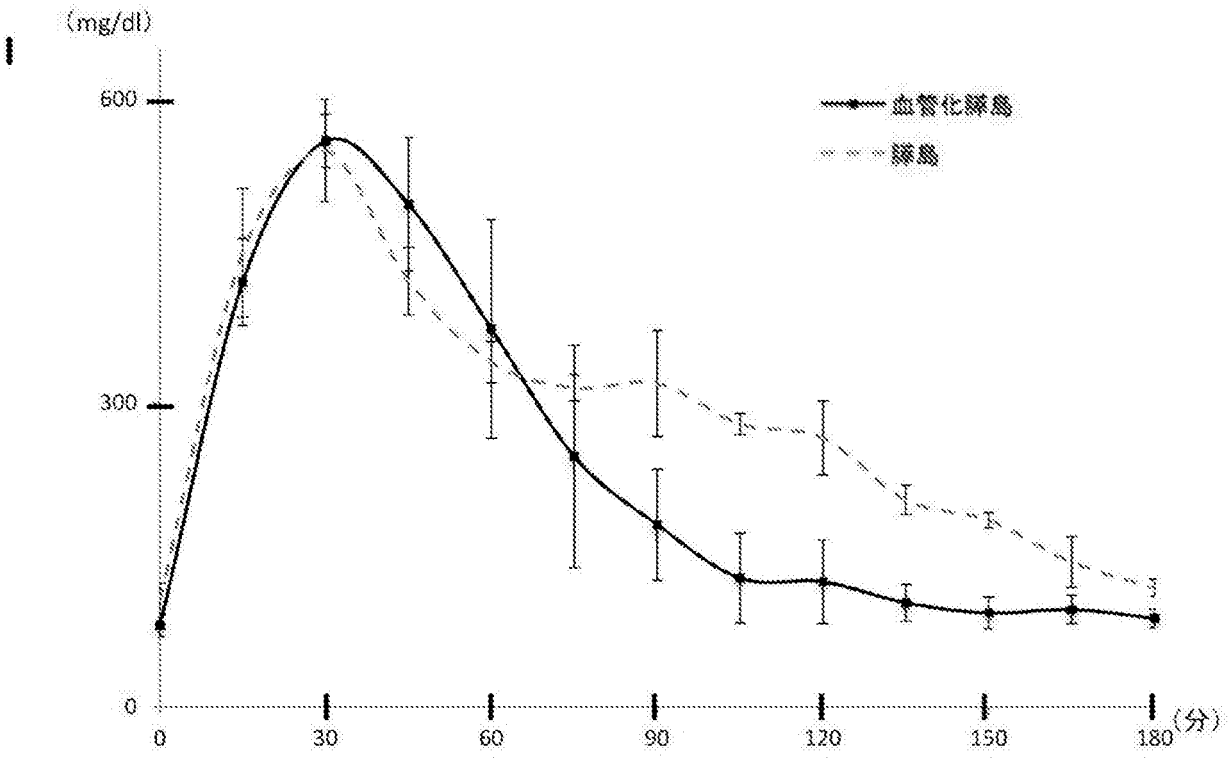
[図3G]


G
(g)
8
6
4
2
0
-2
血管化膵島
膵島
DT投与
0
14
21
28
(日)

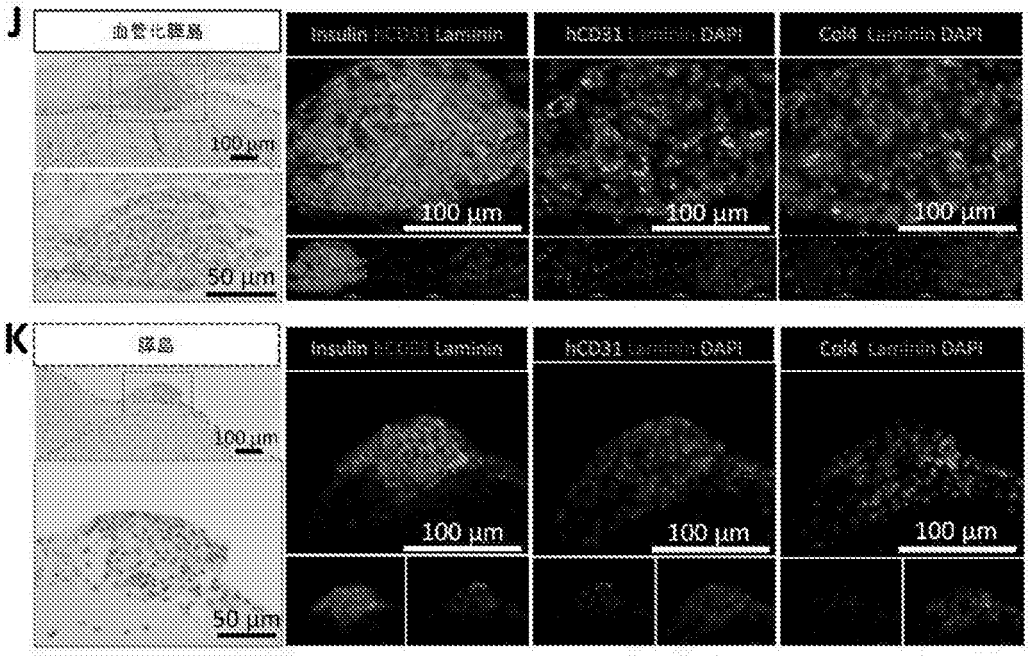
[図3H]


H
(%)
100
80
60
40
20
0
0(移植)
7
14
21
28
(日)
血管化膵島
膵島

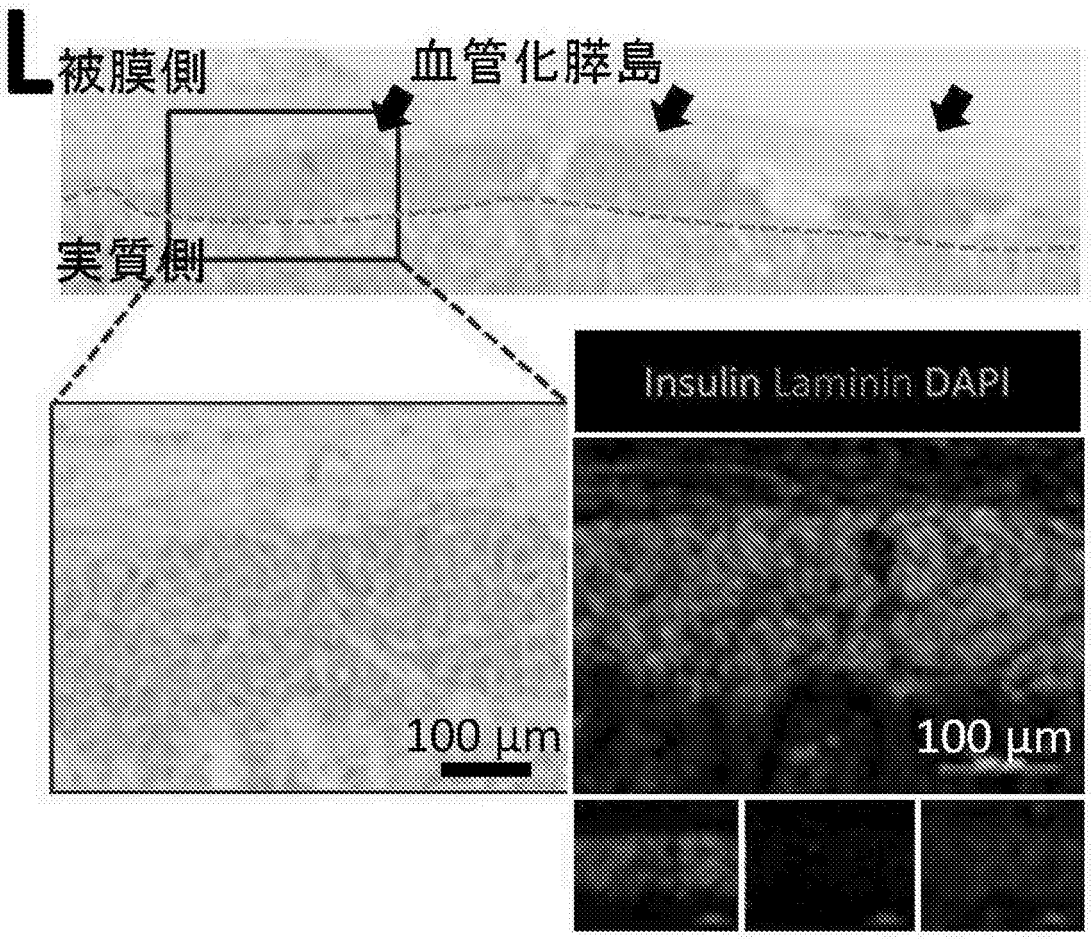
[図3I]


I
(mg/dl)
600
300
0
0
30
60
90
120
150
180
(分)
血管化膵島
膵島

[図3JK]

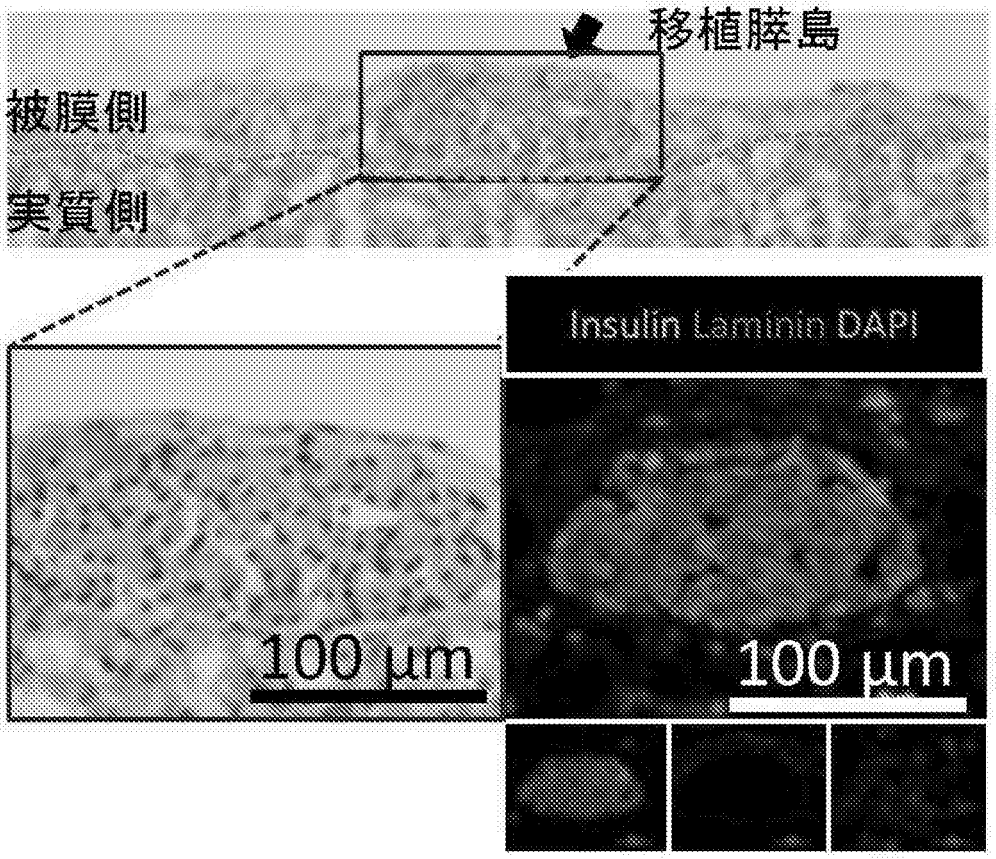

J
血管化膵島
Insulin hCD31 Laminin
hCD31 Laminin DAPI
Col4 Laminin DAPI
100 μm
50 μm
100 μm
100 μm
100 μm
K
膵島
Insulin hCD31 Laminin
hCD31 Laminin DAPI
Col4 Laminin DAPI
100 μm
50 μm
100 μm
100 μm
100 μm

[図3L]

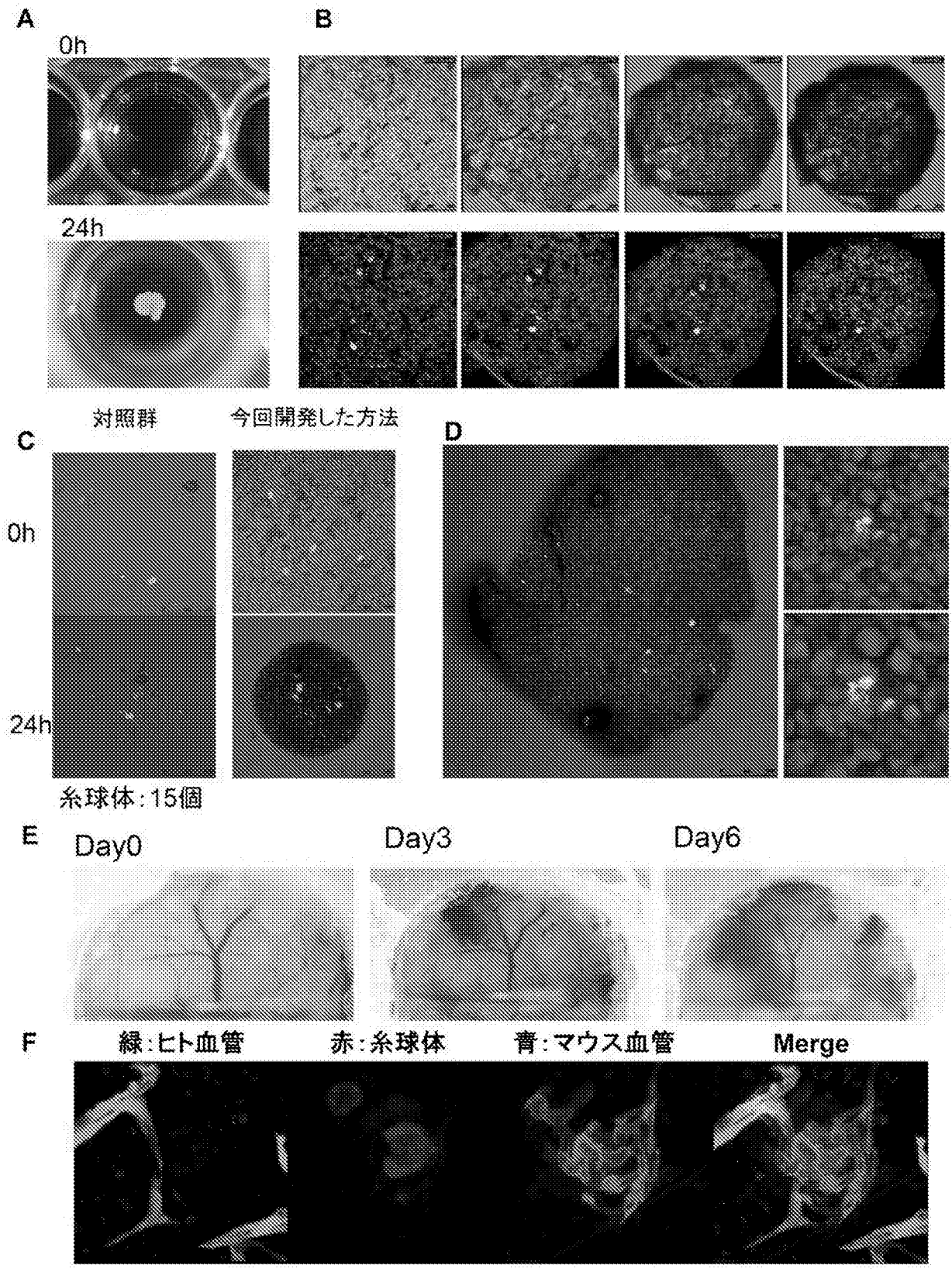

L
被膜側
血管化膵島
実質側
Insulin Laminin DAPI
100 μm
100 μm

[図3M]

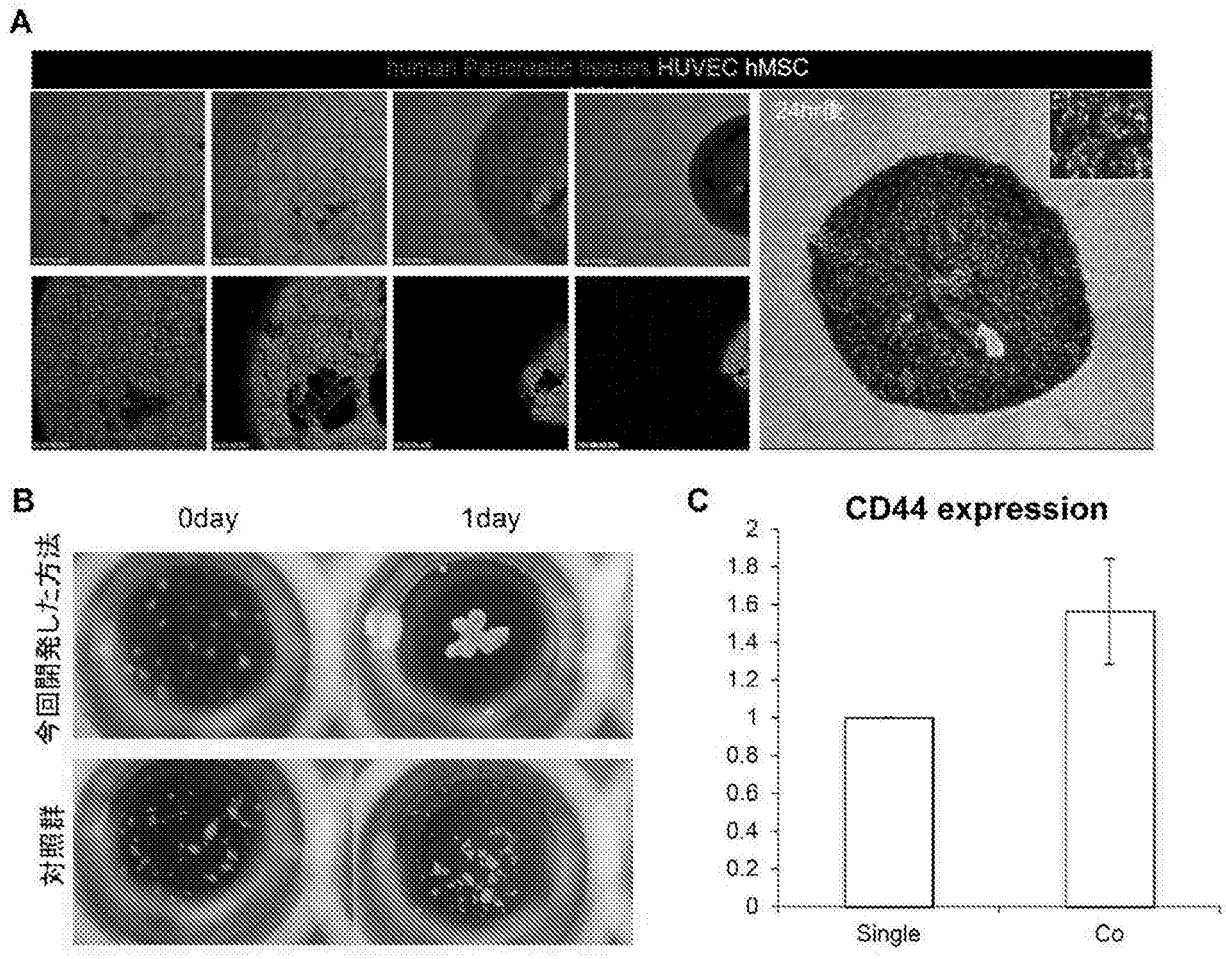
M



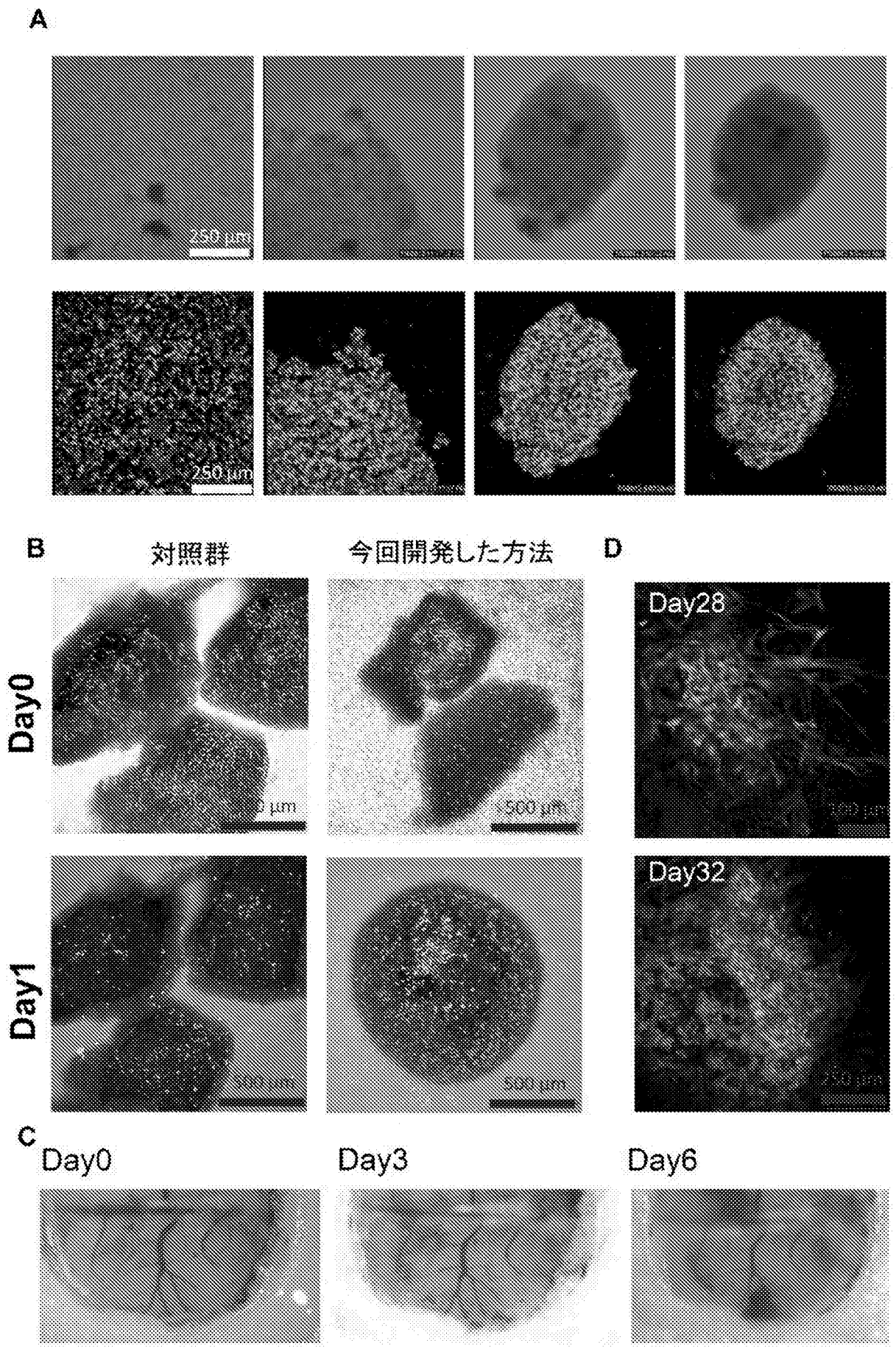
[図4]


A
0h
24h
B
対照群
今回開発した方法
C
0h
24h
糸球体：15個
D
E
Day0
Day3
Day6
F
緑：ヒト血管
赤：糸球体
青：マウス血管
Merge

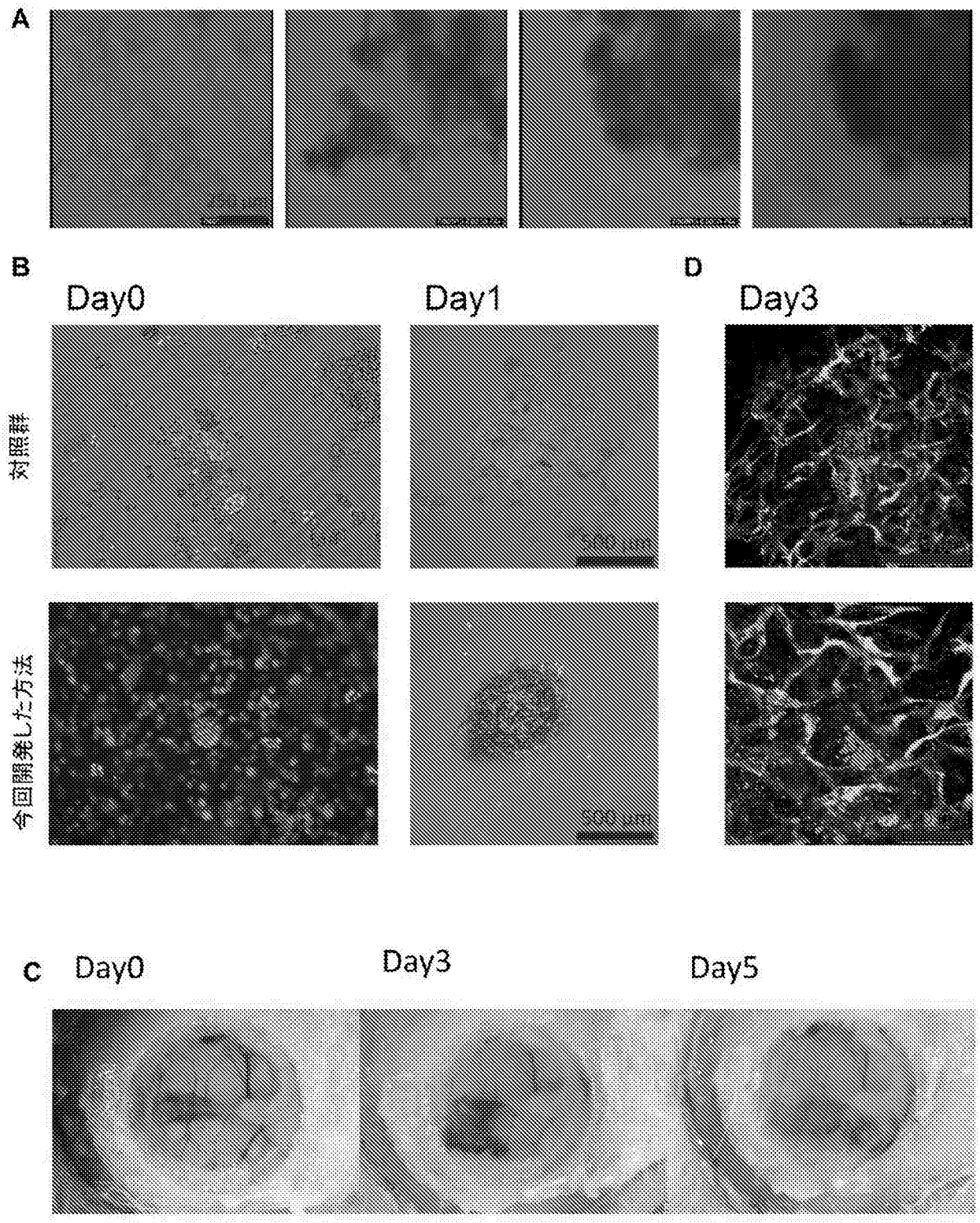
[図5]


A
Human Periodontal tissues HUVEC hMSC
B
0day
1day
今回開発した方法
対照群
C
CD44 expression
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Single
Co

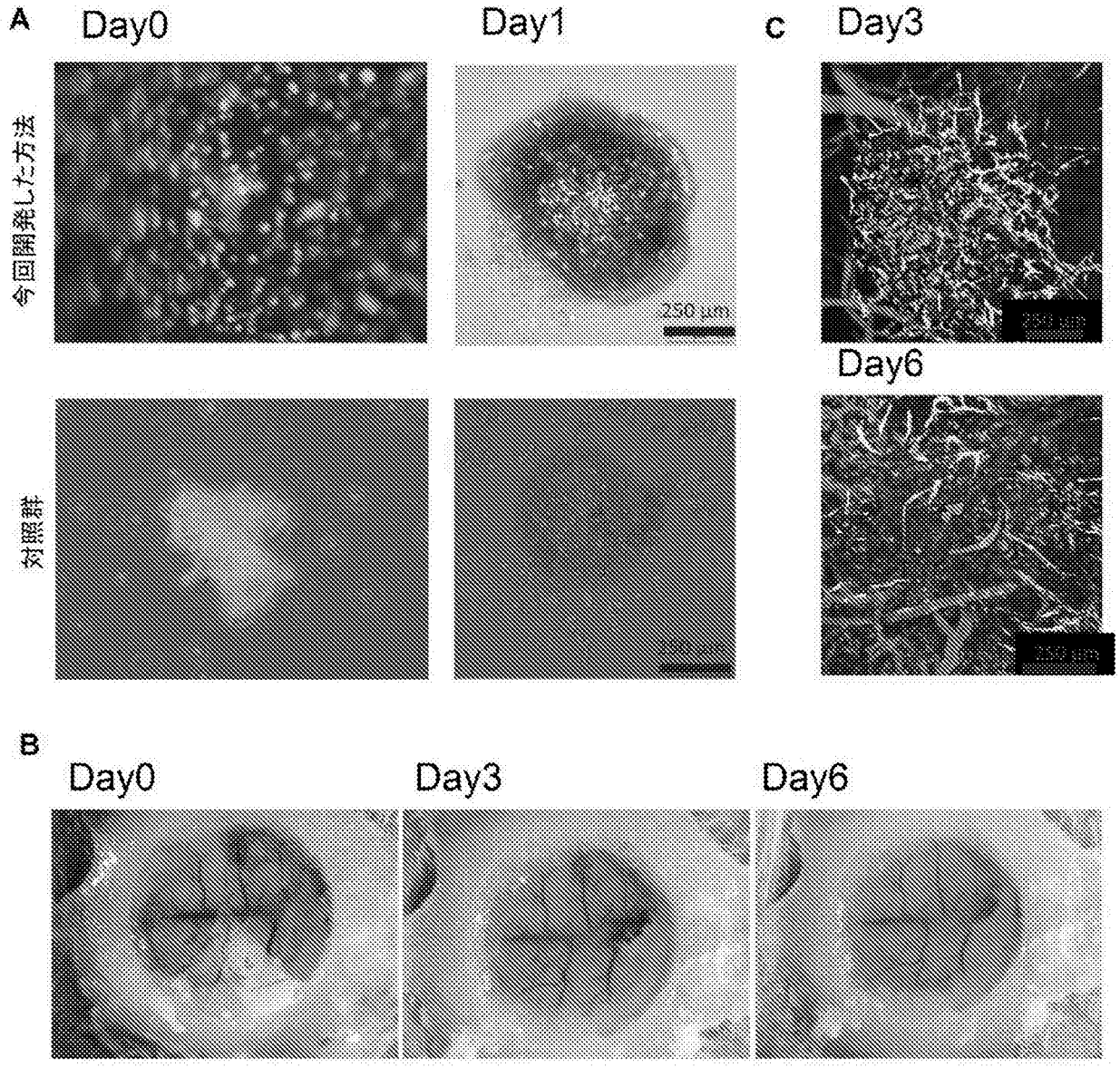
[図6]


A
250 μm
250 μm
B
対照群
今回開発した方法
Day0
Day1
500 μm
500 μm
500 μm
500 μm
D
Day28
Day32
C
Day0
Day3
Day6

[図7]



[図8]

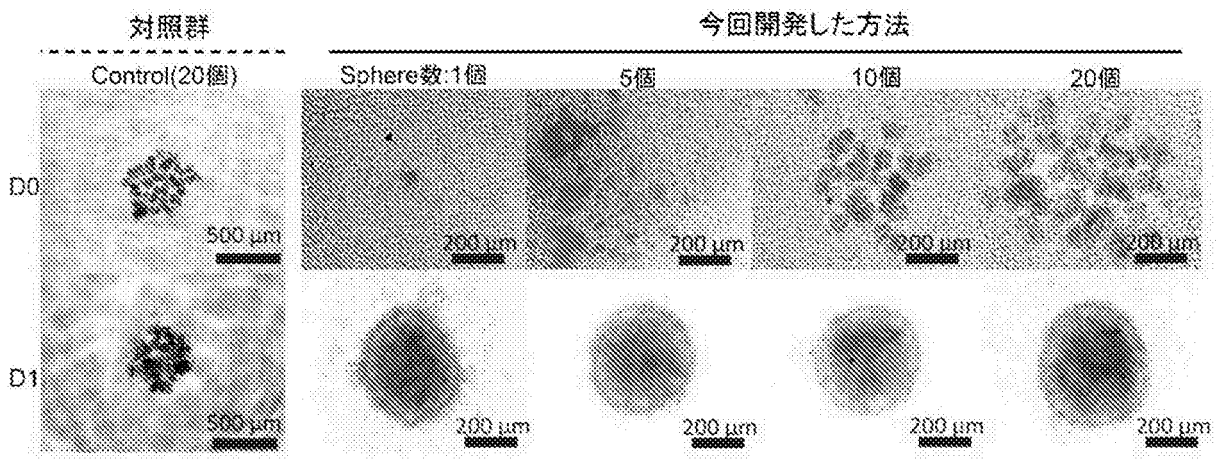

A
Day0
Day1
C
Day3
今回開発した方法
250 μm
Day6
対照群
250 μm
B
Day0
Day3
Day6

[図9]

A

iPS細胞 → 内胚葉細胞への分化誘導 → 小型組織(100-200μmの形成 ⇒ 血管網の付与

B



C

