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Supplementary Materials for

In vivo–mimicking microfluidic perfusion culture of pancreatic islet spheroids

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Supplementary Materials

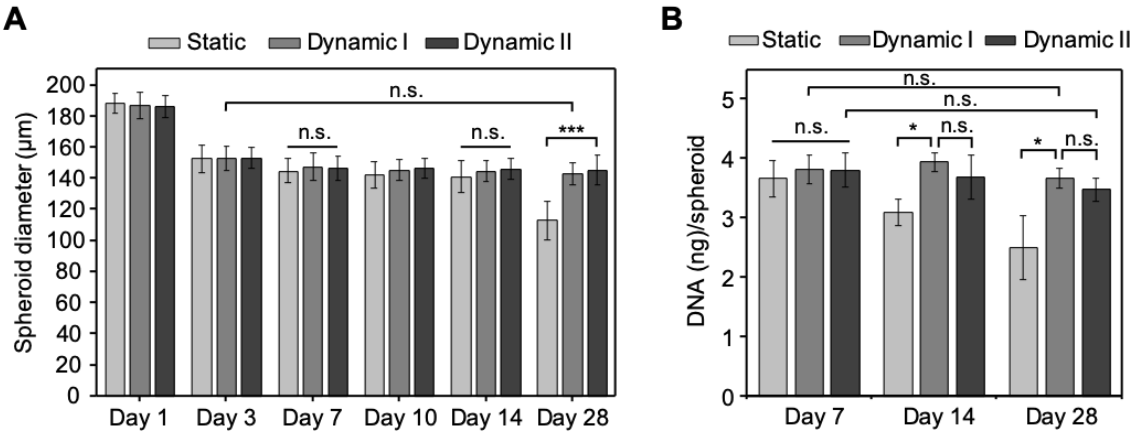


Fig. S1. Size distribution and DNA analysis of pancreatic islet spheroids over 4 weeks of culture. (**A**) Islet spheroids cultured in dynamic conditions maintain a uniform size for 28 days within flow-chips. The data are expressed as the mean ± S.D. (n = 50; ***, $p < 0.001$). (**B**) DNA content measurement of islet spheroids show decreased cell numbers in static condition over time. The data are expressed as the mean ± S.D. (n = 5; *, $p < 0.05$).

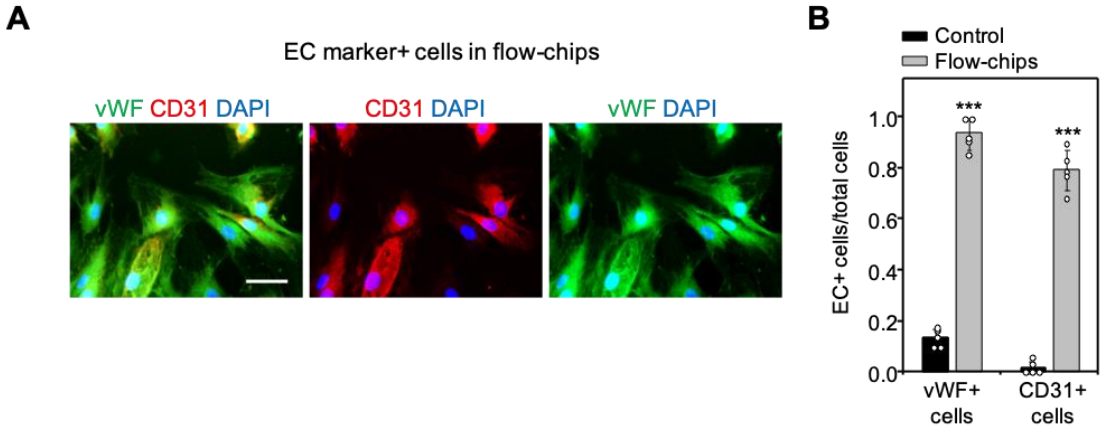


Fig. S2. Immunofluorescent detection of iECs on flow chips. iECs expressed common endothelial markers (vWF; green, CD31; red, DAPI; blue) (**A**). The ratio of vWF+ or CD31+ cells to total cells was determined by immunostaining and compared to the negative control (L929, fibroblast cell line) (**B**). Scale bars, 50 μm. The data are expressed as the mean ± S.D. (n = 5; ***, $p < 0.001$ vs control).

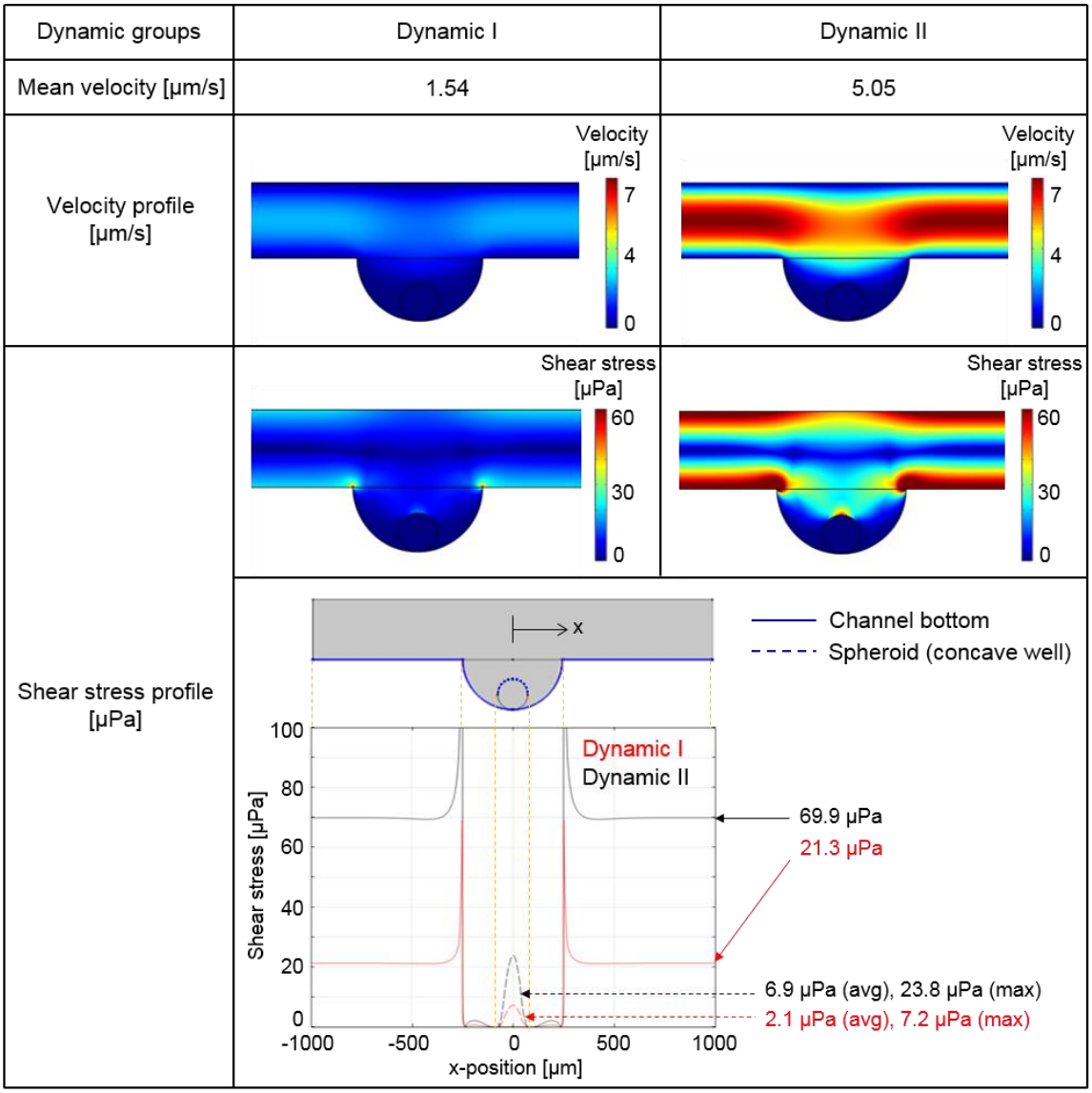


Fig. S3. Simulation of flow velocity and shear stress on channel bottom and spheroid surface. The Navier-Stokes equations for incompressible fluids were solved using COMSOL software to obtain a flow field within the microchip for dynamic I and II conditions. For the velocity and shear stress profiles, constant flow rate (dynamic I and II) at the inlet and no-slip boundary condition at the walls were assigned. Calculated mean velocities driven by 0.05 and 0.2 M PEG were 1.54 and 5.05 μm/s, respectively. Wall shear stress depended linearly on flow rate. Shear stresses at the bottom of the channel where iECs spread were estimated to be 21.3 and 69.9 μPa for dynamic I and II, respectively. With regard to shear stresses applied to spheroid surface inside the well, average levels were 2.1 and 6.9 μPa for dynamic I and II, respectively, where maximum values calculated on top of spheroid were 7.2 and 23.8 μPa, respectively.

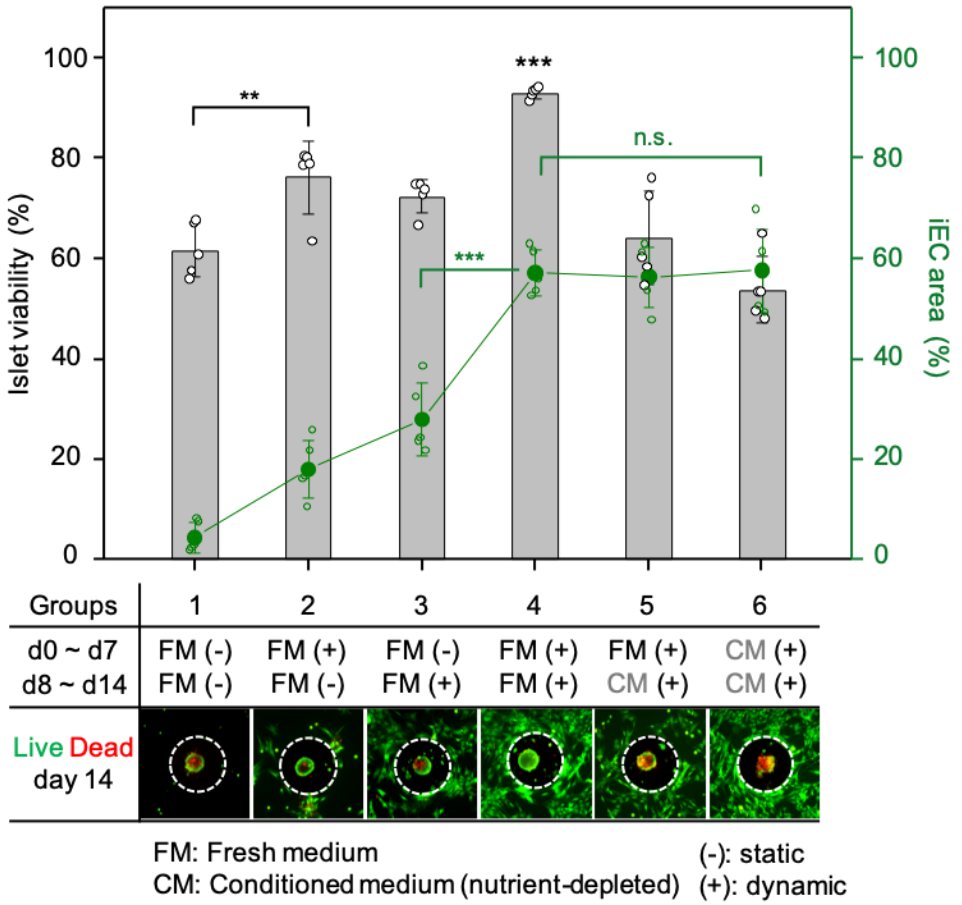
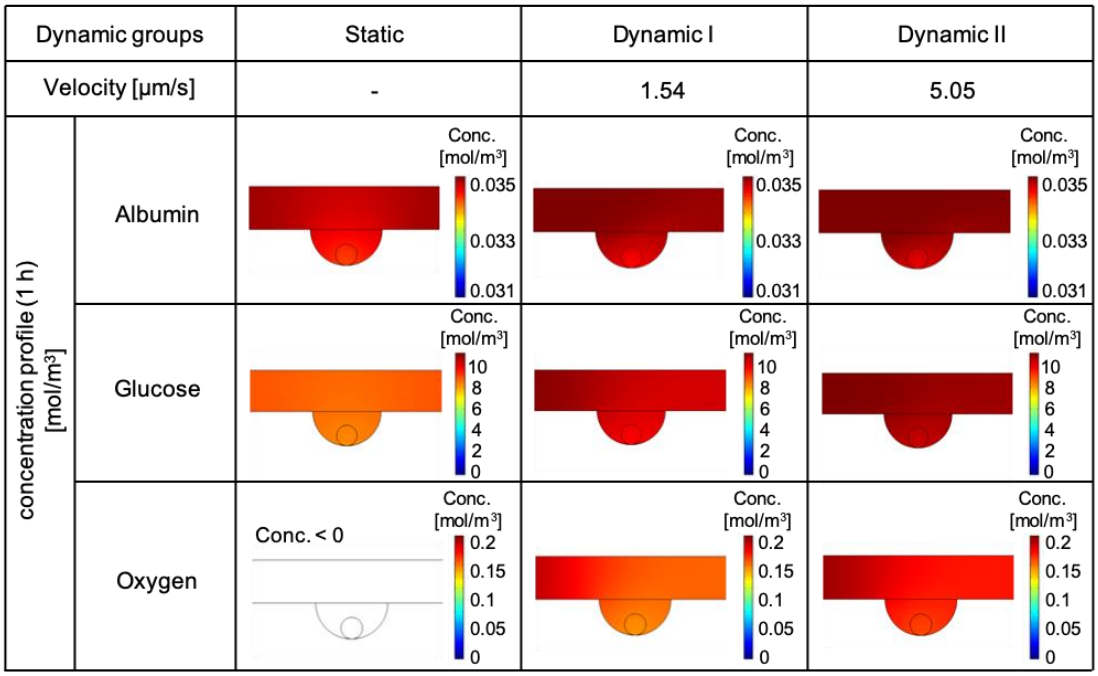


Fig. S4. Interstitial flow effect on expansion of iECs cultured in conditioned media. Islet spheroids were cultured in fresh medium (FM) or conditioned medium (CM) with (+) or without (-) flow in microfluidic chips. The following 6 groups of culture conditions were employed in this study: 1) static condition in FM for 14 days, 2) dynamic condition for 7 days was followed by static condition for 7 days both in FM, 3) static condition for 7 days was followed by dynamic condition for 7 days both in FM, 4) dynamic condition in FM for 14 days, 5) dynamic condition in FM for 7 days, followed by CM for 7 days, and 6) dynamic condition in CM for 14 days. Islet viability in each condition was assessed by using live/dead assay and shown in confocal images (bottom). The graph shows the quantification of results from live/dead assay (gray columns) and iEC area on flat channels (green line) on day 14. The iEC area was quantified by measuring the area of calcein-AM stained live cells on flat channels using the ImageJ software. For CM preparation, islet spheroids were cultured in the chip, and after 24 h incubation with FM, islet-cultured medium was collected from the outlet of the chip. Retrieved medium was centrifuged and filtered through a 0.22 μm-filter. CM was prepared by mixing 1.5:1 ratio of culture medium and fresh medium. The data are expressed as the mean ± S.D. (n = 5; **, $p < 0.01$, ***, $p < 0.001$ vs other groups).

A

Molecules	MW [kDa]	Diffusion coefficient (in medium) [μm²/s]	Initial concentration (in medium) [mol/m³]	Consumption rate [mol/m³/s]	Ref.
Albumin	66	70	0.034	- 2.365 x 10^{-6}	(41,42)
Glucose	0.18	580	11.1	- 0.267	(43)
Oxygen	0.016	3000	0.21	- 0.034	(44)

B



C

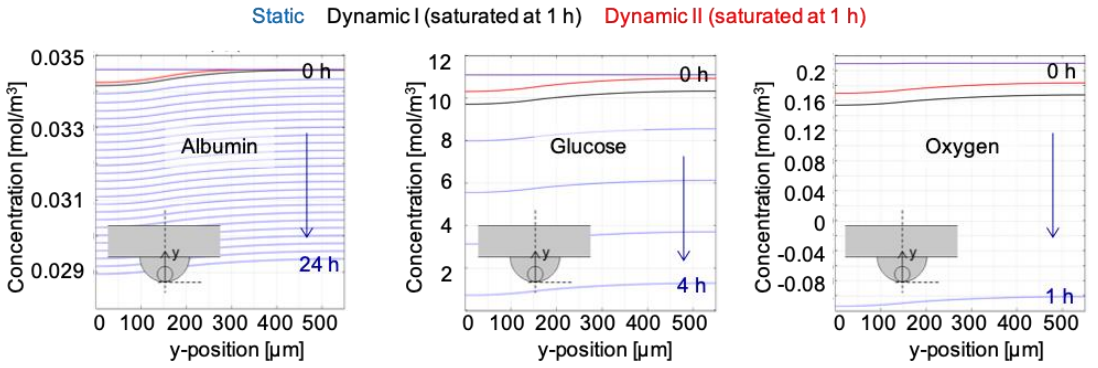


Fig. S5. Simulation of the diffusion and consumption of nutrients introduced into the culture systems. The diffusion equation was solved to obtain the simulation result of molecular transport through a microwell using COMSOL software. A steady-state solution to the convection and diffusion modules was established to investigate the concentration of the sample molecule at each point in the microchannel. (**A**) Parameters used in these calculations describe diffusion coefficient, initial concentration at boundary, and consumption rate by islets for three representative molecules (albumin, glucose and oxygen) in medium. For the calculation of consumption rate by islet spheroids, it was assumed that a spheroid with diameter of 150 μm has

1000 single cells. Related parameters were estimated from the literature *(41-44)*. (**B**) Relative concentration intensity of each molecule after an hour when the concentration level was saturated under dynamic conditions in the microchip. (**C**) Concentration profile of each molecule along the y-axis from the bottom of the microwell over time.

A

	Driven pump	Flow rate [μl/h]	Velocity [μm/s]	Shear stress (channel) [μPa]	Shear stress (well) [μPa]
Dynamic I	Osmotic (0.05 M PEG)	8	1.54	21.3	2.1 (avg), 7.2 (max)
Dynamic II	Osmotic (0.2 M PEG)	25	5.05	69.9	6.9 (avg), 23.8 (max)
Fast flow	Syringe pump	200	38.6	534	59.4 (avg), 188 (max)

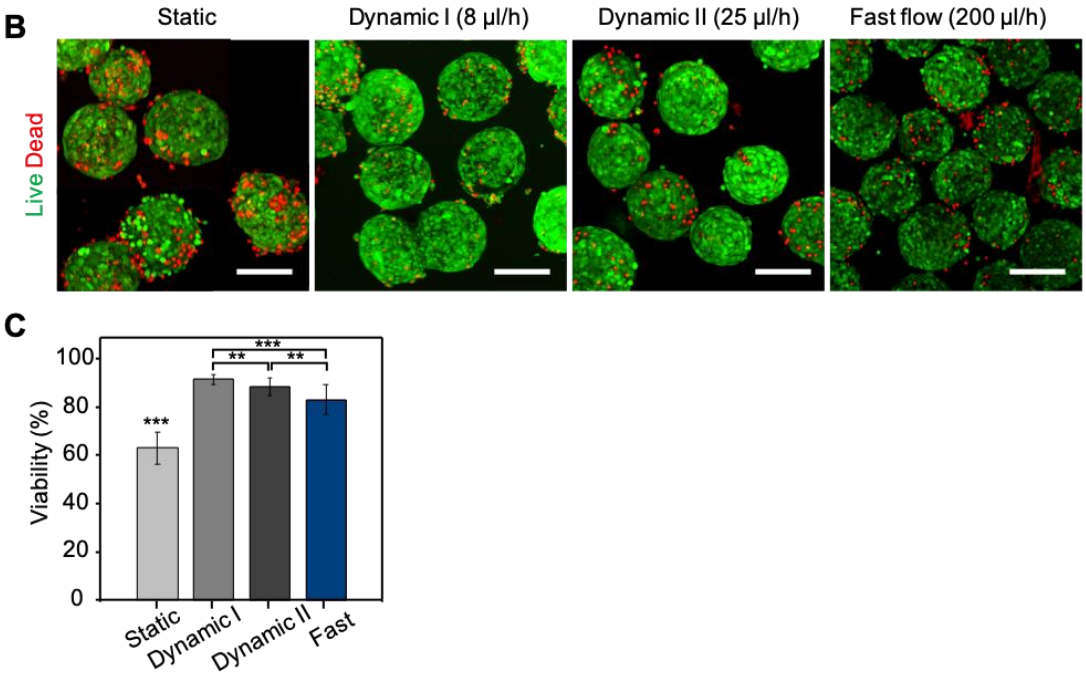
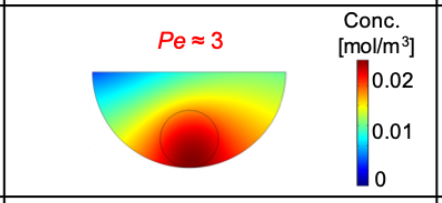
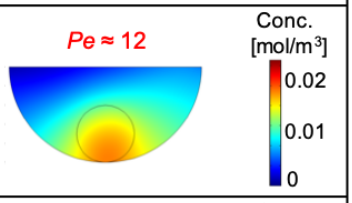
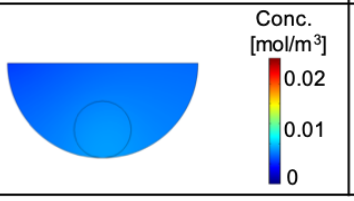
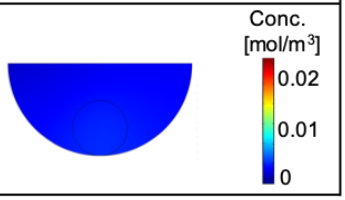


Fig. S6. Fast flow condition beyond interstitial levels resulted in decreased viability of islet spheroids. (**A**) Comparison of dynamic conditions between slow (dynamic I and II) and fast fluid flow in the microfluidic device. Fast fluid flow of 200 μl/h was introduced into the device by connecting a syringe pump to the outlet of the concave chamber. Average velocity and shear stress values were estimated by computational modeling. (**B-C**) Cell viability in islet spheroids cultured for 14 days under static, slow flow (dynamic I and II) and fast flow conditions. (**B**) Confocal images of live/dead assay (live cells; green, dead cells; red). Scale bar, 100 μm. (**C**) Quantification of results from live/dead assay. The data are expressed as the mean ± S.D. (n = 17; **, $p < 0.005$; ***, $p < 0.001$ vs other groups).

A

Dynamic groups		Dynamic I	Dynamic II
Velocity [μm/s]		1.54	5.05
concentration profile [mol/m³]	D1: 150 [μm²/s] (e.g. insulin)		
	D2: 1500 [μm²/s] (e.g. wastes)		

B

Molecules		MW [kDa]	Diffusion coefficient (in medium) [μm²/s]	Peclet number (*Pe*) [*v*L/D]		Ref. of D
				Dynamic I	Dynamic II	
Paracrine factors	VEGF-A	38.2	92	9.21	30.19	(45)
	Insulin	5.8	150	5.65	18.52	(46)
	Glucagon	3.5	254	3.33	10.94	(47)
	Somatostatin	1.6	350	2.42	7.94	(47)
	ATP	0.507	368	2.30	7.55	(48)
	Glutamate	0.147	500	1.69	5.56	(49)
Waste products	Lactate	0.09	1120	0.76	2.48	(50)
	Ammonium	0.018	3000	0.28	0.93	(44)
	CO_2	0.044	2550	0.33	1.09	(51)

Fig. S7. Simulation of localized accumulation of secreted soluble factors from islet spheroids under two different dynamic conditions. Simulation of localized accumulation of secreted soluble factors from islet spheroids under two different flow conditions. (**A**) Relative concentration of secreted molecules from an islet spheroid within a microwell for dynamic I and II conditions. For computational modeling, initial concentration of each molecule was set to 0, and the diffusion coefficient was set to D1 (150 $\mu m^2/s$) for secreted protein factors or D2 (1500 $\mu m^2/s$) for waste products; considering both values being assumed in the range of those of representative islet-secreted molecules such as insulin. Secretion rate of each molecule from an islet spheroid was assumed to be 2.1 x $10^{-4} mol/m^3/s$, based on insulin parameter from literature *(46)*. (**B**) Properties of selected soluble factors released from islets. The Peclet number (*Pe*) is a measure of the relative contribution of convection with respect to diffusion. Average *Pe* for representative hormones (insulin, glucagon and somatostatin) in islets was estimated to 3 and 12 in dynamic I and II conditions, respectively. *Pe* numbers were calculated using standard equation (*Pe* = *v*L/D where *v* is velocity scale, L is channel height, and D is diffusivity). Diffusion coefficients used in the calculation were estimated from the literature *(44-51)*.

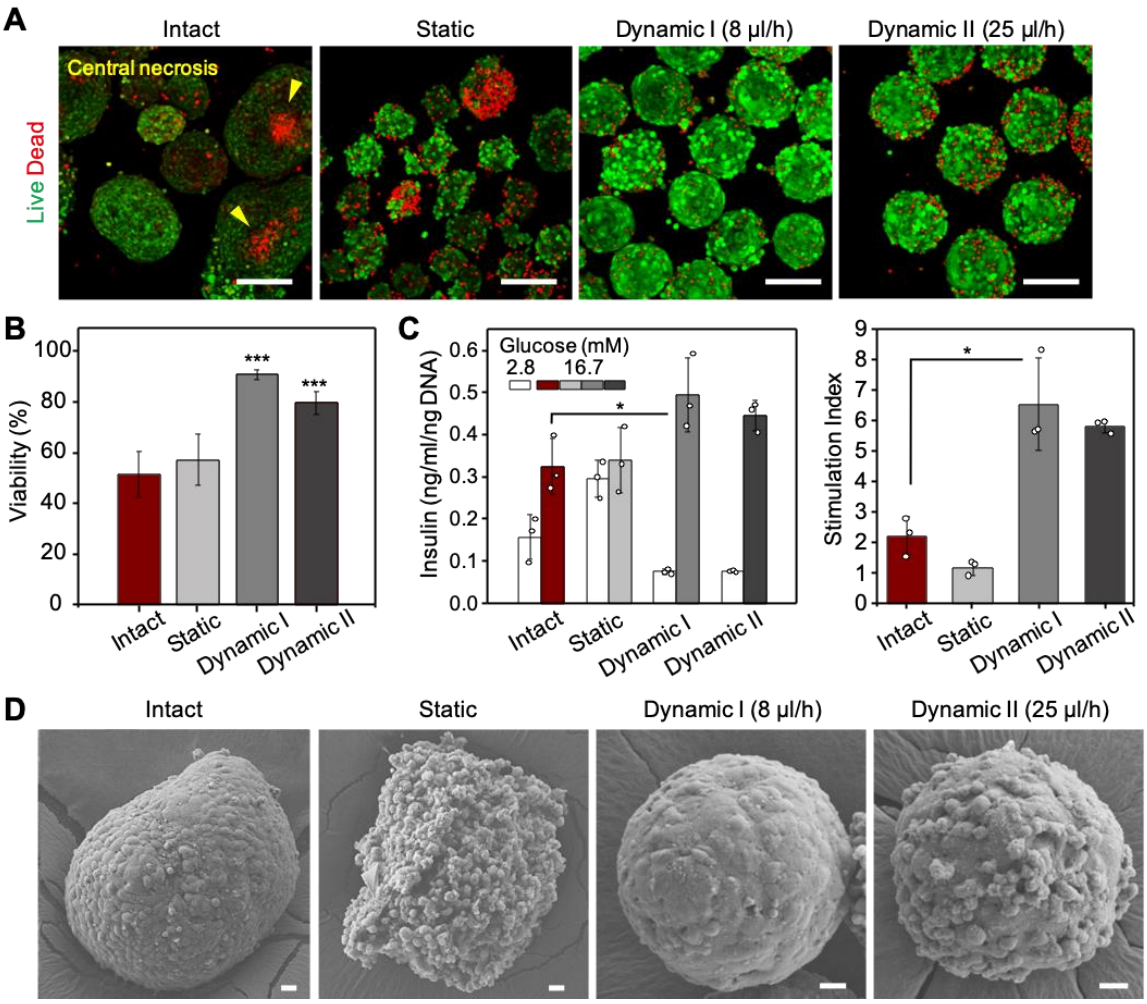


Fig. S8. Long-term culture (4 weeks) of islet spheroids in microfluidic chips. (**A-B**) Cell viabilities in intact islets and islet spheroids cultured under static, dynamic I and II conditions for 28 days. (**A**) Confocal images of live/dead assay (live cells; green, dead cells; red). Central necrosis was shown in large intact islets (arrowheads). Scale bar, 100 µm. (**B**) Quantification of live/dead assay results. The data are expressed as the mean ± S.D. (n = 15; ***, $p < 0.001$ vs other groups). (**C**) Glucose-stimulated insulin secretion assays at low (2.8 mM) and high (16.7 mM) glucose, and stimulation index values on day 28. The data are expressed as the mean ± S.D. (n = 3; *, $p < 0.05$, ***, $p < 0.001$). (**D**) SEM images that shows different surface morphology of culture groups on day 28. Scale bars, 10 µm.

Table S1. Primer design for qRT-PCR. (18S rRNA, 18S ribosomal RNA; *Pdx1*, pancreatic and duodenal homeobox 1; *Glut-2*, glucose transporter family 2; *Pecam*, platelet/endothelial cell adhesion molecule; *Tubb3*, tubulin beta 3; *Col4a1*, collagen type IV alpha 1 chain; *Col1a1*, collagen type I alpha 1 chain)

Target gene		Primer sequence	Tm (°C)	Size (bp)	Genbank Accession number
18S rRNA	F	CTGAGAAACGGCTACCACAT	58	115	NR_046237.1
	R	ATTACAGGGCCTCGAAAGAG	58		
Insulin (*Ins1*)	F	GGATCTTCAGACCTTGGCAC	58.26	116	NM_019129.3
	R	GGTGGACTCAGTTGCAGTAG	57.91		
Pdx1	F	AGCAGAACCGGAGGAGAATAAG	56	103	NM_022852.3
	R	AGGCCGGGAGATGTATTTGT	56		
Glut-2	F	GCACATCCTACTTGGCCTATC	55.2	118	NM_012879.2
	R	CTTTGCCCTGACTTCCTCTT	54.8		
Glucagon	F	GATCATTCCCAGCTTCCCAG	58.02	114	NM_012707.2
	R	GGGAGTCCAGGTATTTGCTG	57.96		
Pecam	F	GGAGCAAGACCACCTGTTAG	57.9	146	NM_031591.1
	R	TCTCAGGAGGCTGGATTTGA	58.04		
Tubb3	F	TTGTGTCTGCTACCATGAGC	57.9	119	NM_139254.2
	R	CATGAAGAAATGCAAGCGGG	58.08		
Bcl-2	F	GTGGTGGAGGAACTCTTCAG	58	107	NM_016993.1
	R	GGTGACATCTCCCTGTTGAC	58		
Fas	F	GTACACGTCTTGGGGATTTG	58	111	NM_139194.2
	R	CTGAACGCTACTGGGTTTGT	58		
Bax	F	TCTGGAAGAAGATGGGCTGA	58.04	138	NM_017059.2
	R	TCCCACCCCTCCCAATAATT	57.99		
Collagen IV (*Col4a1*)	F	CCTCTCCCCTCCTGTTGTAT	55.7	126	NM_001135009.1
	R	GAAGCGGGGTGTGTTAGTTA	54.7		
Collagen I (*Col1a1*)	F	TTCTGAAACCCTCCCCTCTT	55.6	121	NM_053304.1
	R	CCACCCCAGGGATAAAAACTG	55.7		