Supporting Information

Bioinspired human stomach-on-a-chip with in vivo like function and architecture

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Table S1. Genetic profiling of gastric cell lines, MKN74, AGS and NST-20. Autosomal short tandem repeat (STR) profile for all the cell lines used was obtained by co-amplification of 15 STRs and amelogenin. In some cases, allelic imbalance was identified. Low intensity signal, below 20% of the major allele (but above 10%), are marked with (+). A signal below 10% was not called.

	STRs															
Cell Lines	D3S1358	TH01	D21S11	D18S51	Penta E	D5S818	D13S137	D7S820	D16S539	CSF1PO	Penta D	Amel	VWA	D8S1179	TPO	FGA
MKN74	16	6	33.2	12	11-14	11	11	9	9-11	12	9	X	16-20	11-16	8-11	23
AGS	16	6-7	29	13	13-16	9-12	12	10-11	11-13	11-12	9-10	X	16-17	13	11-12	23-24
NST-20	14-15	6	27-31.2	13-16	12-18	10-12	11	8-10	11-12	10-12 (+11)	9-11	XY	16-17	11-13	9	19-20

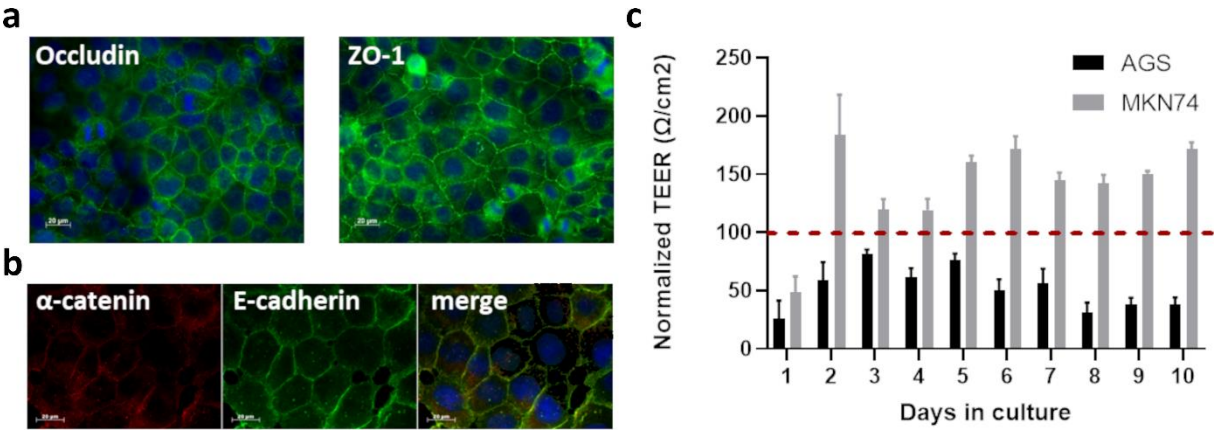

Figure S1. (a) Immunocytochemistry of tight-junction partners, occludin and ZO-1 on MKN74 cells. The proper expression and localization of these two molecules at the cell membrane denoted the correct assembly of the tight-junction complex. (b) Immunocytochemistry of MKN74 cells against adherens-junction partners E-cadherin and α-catenin, evidencing proper assembly of the adherens-junction complex that mediates cell-to-cell contact and adhesion. Immunocytochemistry images acquired on an inverted fluorescence microscope at 63x objective magnification (model: Zeiss axiovert). Scale bars: 20 μm. (c) Transepithelial electric resistance (TEER) of MKN74 cell line measured over a course of 10 days. The AGS gastric cell line was used as a negative control. The red line denotes the threshold normalized TEER value that indicates a fully formed barrier.

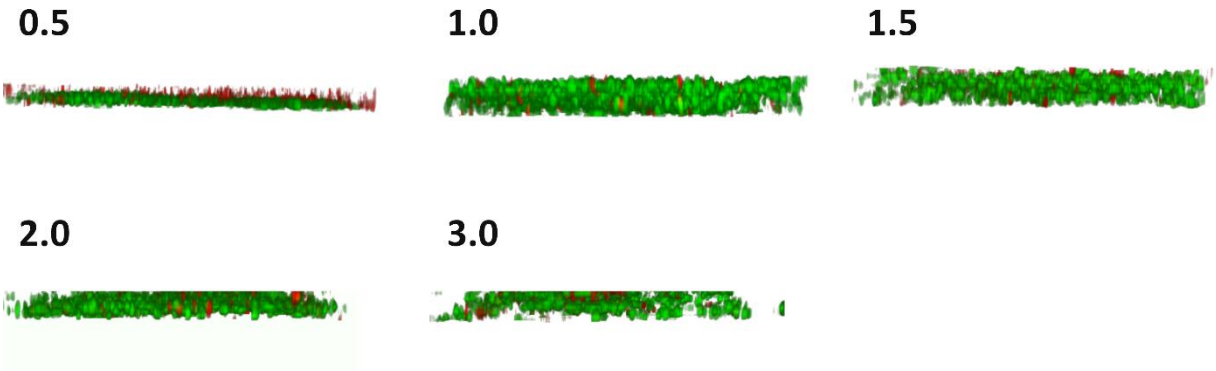

Figure S2. Z-stack orthogonal projections of collagen type I hydrogels embedded with gastric fibroblasts. Numbers indicate collagen type I gel density an are defined as $mg.mL^{-1}$. 3D projections were generated using Fiji's 3D viewer plugin. Live cells are stained with calcein (green) and dead cells are stained with propidium iodide (red). Fibroblasts can be observed growing across the entire thickness of the hydrogel. 3D projections not to scale.

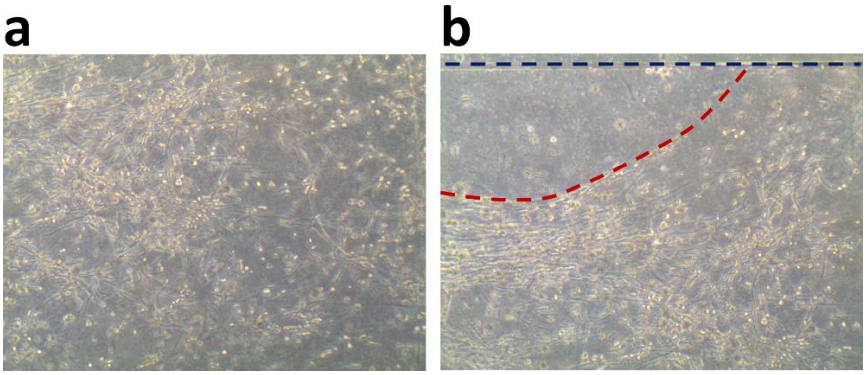

Figure S3. Microphotographs of collagen type-I embedded NST-20 fibroblasts grown on-chip for 5 days, 3 of which under mechanical actuation. a) ECM maintains integrity despite the mechanical distension effected by the microactuator. b) Although very uncommon, due to the high contraction effected by the fibroblasts, we identified instances when the ECM (red line) detaches from the chip wall (blue line).

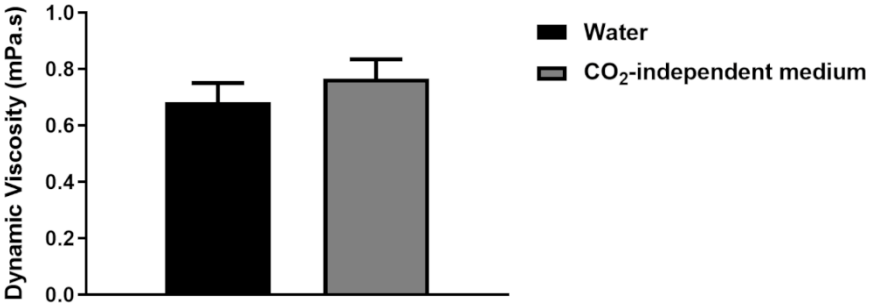

Figure S4. Average dynamic viscosity of CO_2-independent medium used as a buffering system for the stomach-on-a-chip operation. The average dynamic viscosity of the culture medium was determined to calculate the transient shear stress over the cell culture substrate. Graph represents average measurements ± SD (n=4).

Experimental section (supplementary information):

Barrier integrity for gastric cell lines: Transepithelial electric resistance (TEER) was monitored to assess the ability of MKN74 cells to establish a fully functional epithelial barrier. AGS and MKN74 cells were seeded at a density of $0.5x10^5$ cells.mL^{-1} to ensure an even distribution of cells, and approximately 60% confluency, 24 h post-seeding. Cells were seeded on the apical side of a 24-well transwell insert (8 µm pore size; Corning) and allowed to equilibrate for 24 h under normal cell culture conditions. TEER was measured using an EVOM2 voltohmeter (World Precision Instruments) every day up to 10 days and cell culture media was refreshed every other day. Results were plotted as normalized TEER (Ω.cm^{-2}). Barrier integrity was considered effective for measurements above 100 Ω.cm^{-2}.

Calculation of transient shear stress: Transient shear stress imparted by the perfusion regimen, was determined by the following equation:[1]

$\tau = 6.\mu.Q/h^2.w$

where μ is the dynamic viscosity of the culture medium (mPa.s), Q is the volumetric flow rate (cm^3.s^{-1}) and *h* and *w* are the height and width, respectively, of the microchannel (cm). The average dynamic viscosity of the CO_2 independent cell culture medium was determined by rheometry and averaged at 0.77 ± 0.07 mPa.s (Figure S4).

References

[1] Y. Wan, J. Yang, J. Yang, J. Bei,S. Wang, Biomaterials **2003**, 24, 3757.