Electronic Supplementary Information for

Two-way communication between ex vivo tissues on a microfluidic chip: application to tumor-lymph node interaction

Sangjo Shim[a], Maura C. Belanger[a], Alexandra R. Harris[c,d], Jennifer M. Munson[b,d,e], and Rebecca R. Pompano[a,b,*]

Contents:

I. CAD file for device design:

The file (Autocad .dwg extension) contains designs for the top, middle and lower layers of the chip. It is provided separately.

II. CAD file for acrylic overlay:

The file (Autocad .dwg extension) contains the design for the laser-etched PMMA layer that was placed atop the chip to prevent distortion when clamped. It is provided separately.

III. SUPPLEMENTAL METHODS

SU-8 master and PDMS layer fabrication

SU-8 3050 (MicroChem Corp, Westborough MA, USA) was spun to 100-µm thickness onto a clean, bare 3 inch silicon wafer at 500 RPM for 10 seconds with an acceleration of 100 RPM/second, and then 1200 RPM for 30 seconds with an acceleration of 300 RPM/second. To make the desired 200-µm-thick masters, SU-8 3050 was spun on twice, with a 10-µm layer of SU-8 3010 (spun on at 2,000 RPM) in between to increase adhesion.

To make the top and lower PDMS layers of the device, PDMS base and curing agent (Sylgard 184 Silicone Encapsulant, Dow Corning, Midland MI, USA) were mixed at a 10:1 mass ratio, poured on the SU-8 master and cured overnight at 60 °C. The PDMS membrane (1-mm thickness) was prepared by mixing base with curing agent at a 7:1 mass ratio, which reduced the PDMS viscosity and minimized bubbles. This mixture was sandwiched between the SU-8 master and a clean glass slide, both previously treated with Tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane (Cat#: SIT8174.0, Gelest, INC, Morrisville, PA, USA). The culture wells (3 mm in diameter) in the PDMS membrane, reservoirs (5 mm in diameter) in the top layer, and tubing ports (0.75 mm in diameter) in the top and the lower layer were punched using biopsy punches before assembling layers.

Darcy permeability used in COMSOL Multiphysics Model

Limited experimental data is available for the permeability of soft tissues. Reports for tumor tissues vary by several orders of magnitude depending on the type of tumor and how the measurement is made, spanning at least 10^{-18} – 10^{-14} m^2.[1,2] No experimental data has been reported for lymph node tissue. We previously estimated lymph node permeability by empirically fitting a Comsol model to an experiment in which a labelled solution was pulsed into through a lymph node slice.[3] The experimental behavior was best fit by a permeability of 3×10^{-12} m^2. This should be considered a rough approximation, but it is within the range of values measured for brain and other organs,[4] and similar to results obtained by simulations of flow through the lymph node. Therefore, in this work the permeability was set up to 3×10^{-12} m^2.

Test of efficacy of BSA blocking

To test the extent to which BSA blocking mitigated surface adsorption of proteins inside the microdevice, we prepared two groups of chips. The first group was prefilled with 1% BSA in 1x PBS and incubated for 30 min at room temperature, as described in the Methods in the main text. As a negative control, we prefilled one group of chips with 1x PBS (no BSA). The devices were assembled without a tissue slice and clamped together. This test used unidirectional, non-recirculating flow through one inlet, culture well, and reservoir, as in Figure 4 in the main text. A solution of 1.25 ng/mL neutravidin-rhodamine was perfused continuously through the chip at 0.5 µL/min for 2 hours, after which the end point-supernatant of 60 uL volume in the reservoirs. To quantify the amount of neutravidin-rhodamine remaining in the supernatant, absorbance was read

at 550 nm on a Clariostar plate reader (BMG LabTech) and compared to a calibration curve of neutravidin-rhodamine in 1x PBS. BSA blocking significantly improved the recovery of protein from an average of 20 to 76 % (Figure S1).

ELISA

Capture IFN-γ antibody (Clone: XMG1.2, Biolegend, Cat# 505827, San Diego CA, USA) was coated onto a 96-well half-area plate (Corning, Fisher Scientific) at a concentration of 1 μg/mL overnight at 4 °C. Wells were then washed with ELISA wash buffer (0.05% Tween-20 in 1x PBS) and blocked with ELISA block buffer (0.05% Tween-20, 1% BSA, in 1x PBS) 1 hour at room temperature then washed. IFN-γ (Murine IFN-γ, Peprotech, Cat#315-05-100UG, NJ, USA) standard curves were prepared from 2000-31.25 pg/mL in a 1:1 v/v mixture of ELISA block buffer and complete media. Supernatant samples were diluted 1:1 v/v in ELISA block buffer. Samples were loaded onto the plate in duplicate and incubated at room temperature 2 hours, then washed. Biotinylated IFN-γ detection antibody (Clone: R4-6A2, Biolegend, Cat# 505704, San Diego CA, USA) was added at a concentration of 0.5 μg/mL and incubated at room temperature 2 hours, then washed. Avidin HRP (eBiosciences, Cat# 18-4100-51, USA) was added at a 1:100 dilution and incubated at room temperature for 30 minutes, then washed. TMB substrate was added (BD Biosciences) and allowed to react approximately 5 minutes and then stopped with 1 M H2S04. Absorbance at 450 nm was read on a Clariostar plate reader (BMG LabTech).

Viability test

Naïve lymph nodes and tumor tissues were sliced and then cultured on- or off-chip for 24 hours at 37 °C, 5% CO_2. Off-chip control slices were cultured in individual wells of a 12-well plate in 500 μL complete RPMI. On-chip slices were cultured on-chip with recirculating media at 2 μL/min. Slices were then transferred to fresh culture wells and stained with 2 μM Calcein AM viability dye (Cat#: BD 564061, BD Pharmingen TM, San Jose CA, USA) in 1x PBS for 20 min at room temperature, then rinsed 3 times with 2% fetal bovine serum in PBS. Some off-chip slices were left unstained as a negative control. Other off-chip slices were treated with 70% ethanol for 10 min and rinsed in sterile 1x PBS 3 times prior to viability staining, to serve as a killed control. The slices were imaged by Zeiss AxioZoom Macroscope, using a GFP filter set (Zeiss filter set #38, excitation wavelength: 470/40, emission wavelength: 525/50 in nm). Captured images were saved in Tiff file extension and analyzed in ImageJ.

Image analysis

All images were exported as 16-bit Tiff files and imported into ImageJ (1.52e).

Mean Gray Value for Calcium AM viability imaging: Images from the Calcein channel were imported into ImageJ. The outlines of tissue slices were traced using the wand tool, and the mean grey value (MGV, i.e. average intensity per pixel) of this region was calculated. To calculate

background, a portion of the non-tissue area was selected and measured in the same way. The reported MGV was calculated by subtracting background MGV from tissue MGV.

Imaging tumor cell regions: Images from the dsRED channel were imported into ImageJ. We determined a threshold intensity that exceeded most auto-fluorescence in naïve tissues, and used this value to set a minimum threshold for region selection in all tissue slices (Image\Adjust\Threshold). Air bubbles and dust outside of the tissue were excluded from analysis. The integrated intensity (mean x area) of the selected region was calculated and reported.

IV. SUPPLEMENTAL FIGURES

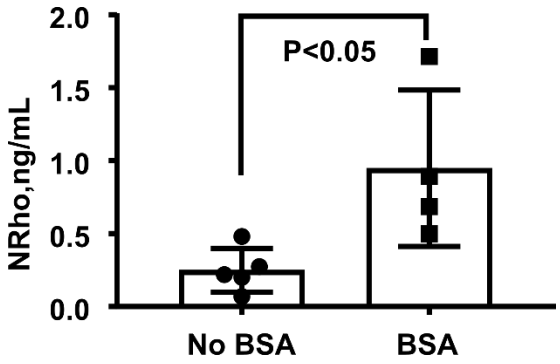

FIGURE S1. Test of the efficacy of BSA blocking in preventing loss of proteins as they flowed through a single culture well and reservoir at ng/mL concentrations. "No BSA" and "BSA" indicates chips pre-coated with 1x PBS or 1% BSA solution. 1.25 ng/mL neutravidin-rhodamine (NRho) was perfused through the chip for 2 hr, and the recovered concentration in the supernatant was determined by the absorbance of the fluorophore. BSA blocking significantly improved the recovery of protein from an average of 20 to 76 %. Each dot represents one chip. Analyzed by one way-ANOVA.

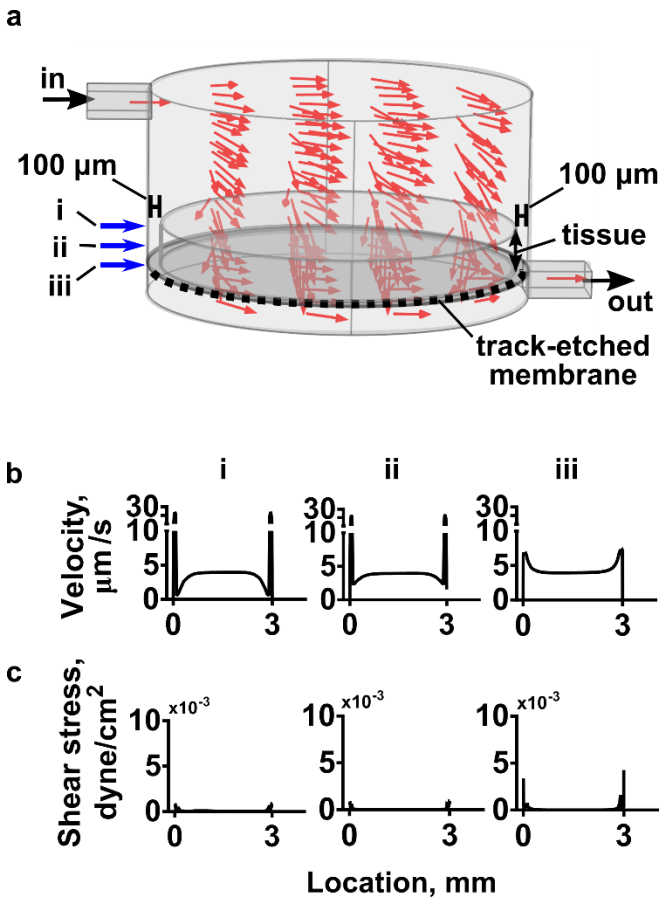

FIGURE S2. COMSOL Multiphysics 3D simulation of fluid flow through a tissue-loaded culture well with a gap of 100 µm between the edge of the slice and wall of the 3-mm well. The same parameters were used as for the simulation in Figure 3 of the main text. (a) Red arrows show the predicted flow direction and magnitude at each location in the well, indicating that flow passes transversely through the slice. (b)Velocity magnitude and (c) shear stress were measured along three scan lines, placed at the top (i), middle (ii), and bottom (iii) of the tissue slice, as indicated by blue arrows in panel (a).

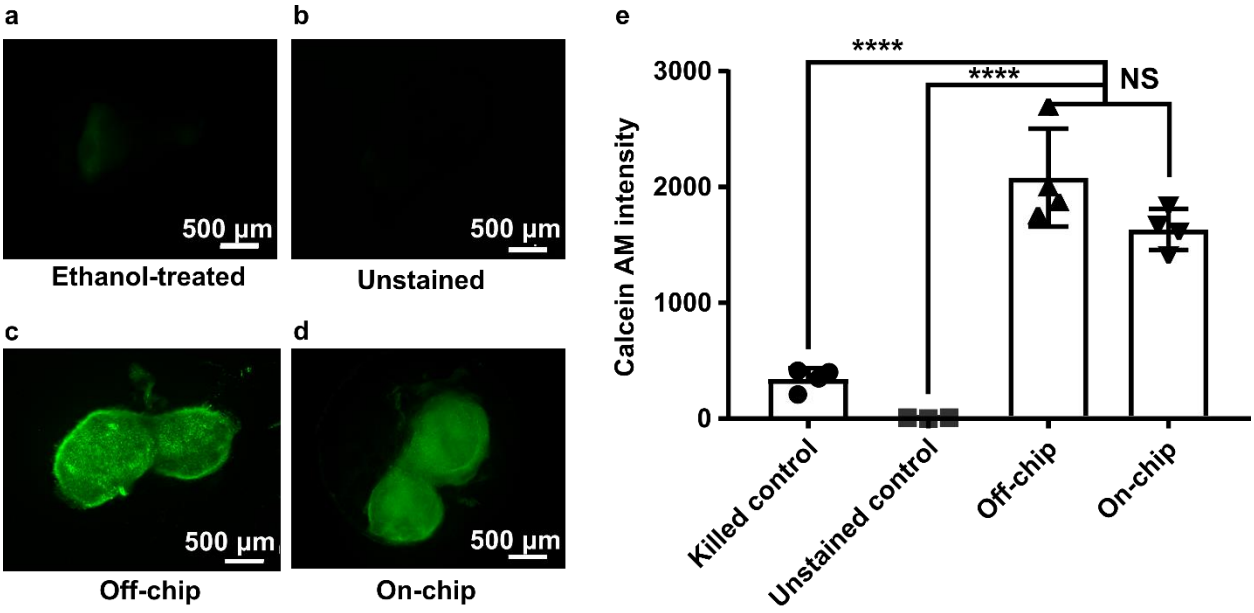

FIGURE S3. Mouse lymph node viability after on-chip culture was assessed by Calcein AM staining. All tests were performed in a 37 °C incubator for 24 hours. Microscopic images of (a) ethanol-treated killed control, (b) unstained control, (c) off-chip control, cultured in a standard well plate, and (d) on-chip culture with flow at 2 µL/min. (e) Quantification of Calcein AM fluorescence (background-subtracted mean grey value). NS indicates no significant difference, and * and ** indicate p-value < 0.05 and < 0.01, respectively, by one-way ANOVA. N = 3–4 slices. Each dot indicates one tissue slice.

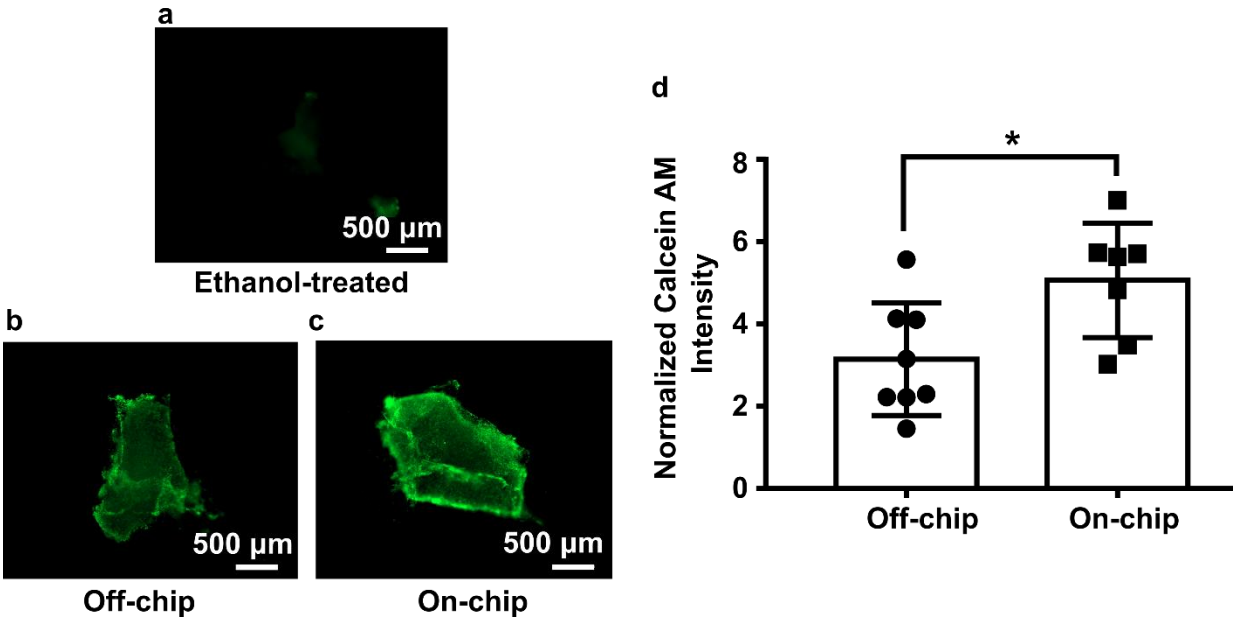

FIGURE S4. Tumor slice viability after on-chip culture assessed by Calcein AM staining. All tests were performed in a 37 °C incubator for 24 hours. Microscopic fluorescent images of (a) ethanol-treated killed control, (b) off-chip control, cultured in a standard well plate, and (C) culture on a chip, with flow at 2 µL/min. (d) Quantification of Normalized Calcein AM fluorescence; Calcein AM MGV was devided by Killed control MGV. * indicates p-value < 0.05 by one-way ANOVA. N = 7-8 slices. Each dot indicates one tissue slice.

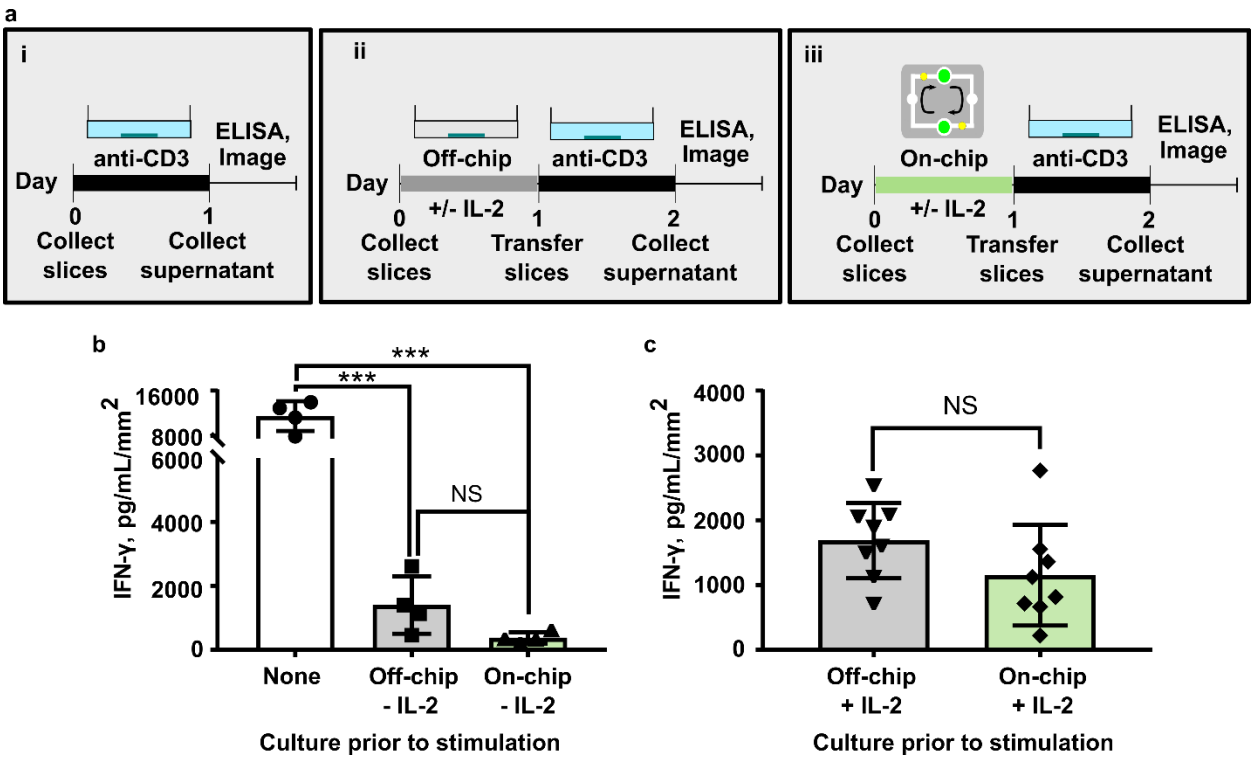

FIGURE S5. Immune functionality of naïve lymph node slices was reduced by overnight culture off-chip or on-chip, and partially rescued by adding the growth factor IL-2 to the media. (a) Lymph node slices were collected from naïve Balb/c mice, and stimulated secretion of IFN-γ was tested after three different culture conditions: (i) Freshly collected slices were immediately incubated overnight with anti-CD3, (ii) Slices were cultured overnight off-chip with or without IL-2 (+/- IL-2), followed by incubation overnight with anti-CD3, or (iii) Slices were cultured overnight on-chip in pairs, with or without IL-2, followed by incubation overnight with anti-CD3. (b) Stimulated IFN-γ responses in the absence of IL-2. Fresh tissues (white bar) responded significantly more strongly than tissues that were cultured overnight off-chip or on-chip prior to stimulation. Furthermore, culture on-chip appeared to further weaken the response compared to culture-off chip, although the difference was not significant. (c) When IL-2 was included in the media during overnight culture, IFN-γ responses to subsequent stimulation were similar between tissues cultured off-chip and off-chip. In b and c, IFN-γ concentration in the supernatant was normalized to the area of the tissue slice (mm^2). Each dot indicates one lymph node slice. Each data set (b and c) was analyzed by one-way ANOVA. NS indicates no significant difference, and *** indicates p-value < 0.001.

REFERENCES

1. S. Ramanujan, A. Pluen, T. D. McKee, E. B. Brown, Y. Boucher and R. K. Jain, *Biophysical Journal*, 2002, **83**, 1650–1660.
2. A. Fahlgren, L. Johansson, U. Edlund and P. Aspenberg, *Acta of bioengineering and biomechanics*, 2012, **14**, 47–51.
3. A. E. Ross, M. C. Belanger, J. F. Woodroof and R. R. Pompano, *Analyst*, 2017, **142**, 649–659.
4. L. J. Cooper, J. P. Heppell, G. F. Clough, B. Ganapathisubramani and T. Roose, *Bulletin of Mathematical Biology*, 2016, **78**, 52–71.