Supplementary Information

Microwell Devices with Finger-like Channels for Long-Term Imaging of HIV-1 Expression Kinetics in Primary Human Lymphocytes

Brandon Razooky[a,e,f,h], Edgar Gutierrez[b,h], Valeri H. Terry[e], Celsa A. Spina[c,d], Alex Groisman[b]*, Leor Weinberger[a,e,g]*

[a]Department of Chemistry and Biochemistry and [b]Department of Physics, [c]Department of Pathology, [d]VA San Diego Healthcare System, San Diego, CA 92161, [e]Gladstone Institute of Virology and Immunology, [f]Biophysics Graduate Group, University of California, San Francisco, CA 94158, and [g]Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94158

[h] equal contribution

* to whom correspondence should be addressed: leor.weinberger@gladstone.ucsf.edu and agroisman@ucsd.edu

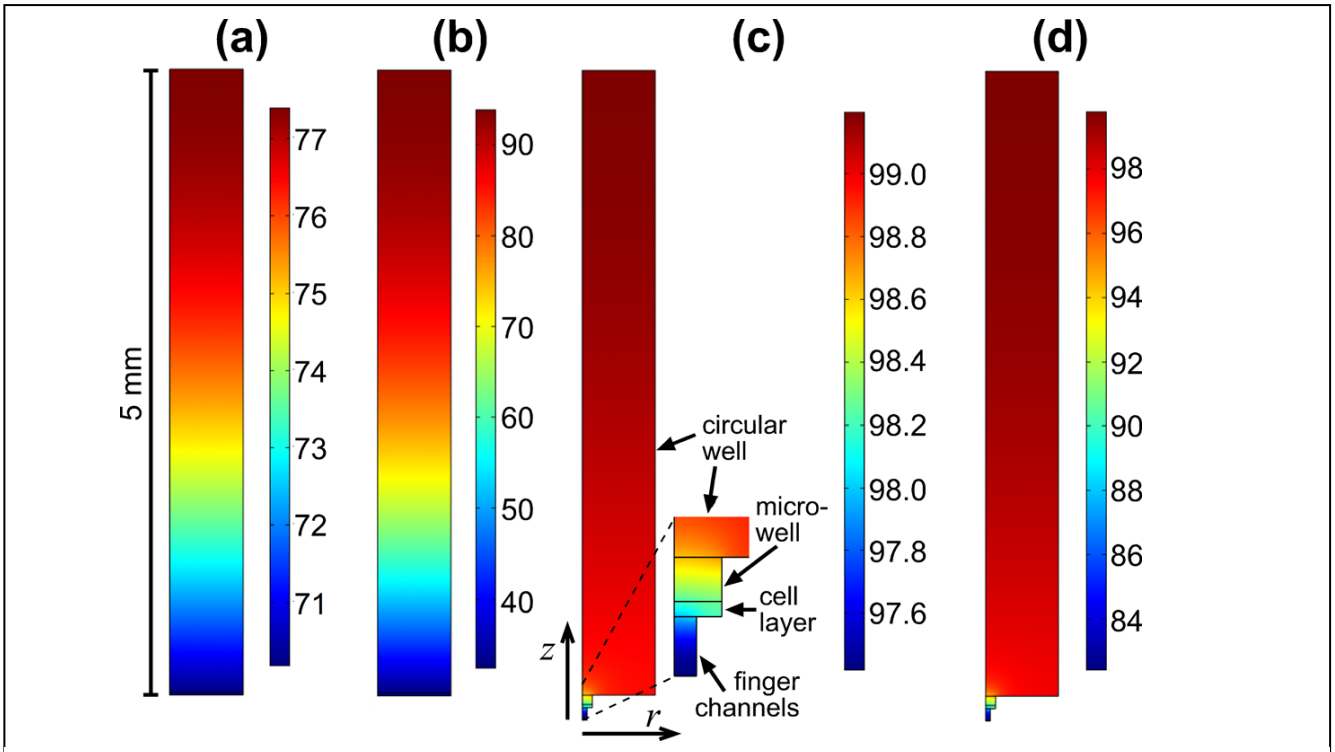

Figure S1. Results of numerical simulations on the diffusion and consumption of nutrients by cell culture at the bottom of a dish and in the microfabricated device. The color-coded plots show nutrient distributions after 24 hours of cell culture under a 5-mm-deep layer of medium with an initial nutrient concentration of 100%. The legends, with concentrations in percents, are shown to the right of the corresponding distributions. (a) and (b) are two-dimensional simulations of cell culture at the bottom of a dish (~0.6-mm-wide fragment is shown). Cell culture is represented by a 25-μm-thick layer at the bottom of the computational domain with a uniform reaction rate of -0.0579%/sec, corresponding to consumption of 25% of the nutrient within 24 hours. The coefficient of diffusion of the nutrient is D_1 = 500 μm^2/sec in (a) and D_2 = 50 μm^2/sec in (b). (c) and (d) are simulations of cell culture in the microfabricated device. A circular well of the device is represented by a 5-mm-tall, 1.17-mm diameter cylinder; a microwell is represented by a 100-μm tall, 160-μm-diameter cylinder; the finger-like channels are represented by a 100 μm tall, 75 μm in diameter cylinder. The three-dimensional configuration of the device is reduced to a two-dimensional axisymmetric computational domain, along the radial, r, and vertical, z, axes of the three-cylinder structure. Cells at the bottom of the microwell of the device are represented by a 25 μm thick layer with a uniform reaction rate of -0.0579%/sec at the bottom of the 160-μm diameter cylinder; cells in the finger-like channels are represented by the same uniform reaction rate all over the 75-μm diameter cylinder, corresponding to four layers of cells with the same density as in (a) and (b). The coefficient of diffusion of the nutrient is 500 μm^2/sec in (c) and 50 μm^2/sec in (d).

Numerical simulations of consumption and diffusion of nutrients in the microwell device

To test for variations in medium conditions in the microfabricated device, we numerically simulated the diffusion of nutrients in the device and their consumption by cultured cells. The goal of the simulation was to compare the medium conditions around cells in a finger-like channel of the device to cells at the bottom of a conventional Petri culture dish. The thickness of the medium layer was set at 5 mm for both the device and the dish. For the purpose of comparison, we chose a dish with a monolayer cell culture at the bottom, with a density of 1 cell per 64 μm^2 (8×8 μm), and set the nutrient consumption rate at such a level that the monolayer culture consumes 25% of the initial content of the 5-mm layer of the medium within 24 hours. The cell culture at the bottom of the dish was represented by a 25-μm thick layer (thicker than lymphocyte diameter to avoid unnecessarily high density of the simulation mesh) with a reaction (consumption) rate of -25%×5mm/25 μm/24 hours or -0.0579%/sec. The initial concentration in the dish was set at 100%. The distributions of concentrations in a section of the dish after 24 hours for a small-molecule nutrient, with a diffusion coefficient D_1 = 500 μm^2/sec (similar to that of glucose), and a macromolecule (protein), with a diffusion coefficient D_2 = 50 μm^2/sec (similar to that of albumin), are shown in Fig. S1A and S1B. As expected, the concentrations averaged over the bulk of the dish are 75% for both the small molecule and protein, but both concentration profiles are non-uniform and non-linear. Moreover, the slower diffusion of the protein makes its distribution substantially less uniform, with concentration around the cells culture being as little as 32%, whereas it is ~70% for the small-molecule nutrient.

The simulation of the lymphocyte culture in the microfabricated device was built to incorporate the three-dimensionality of the device architecture, comprising the finger-like channels, microwells, and circular wells, without making the simulation excessively complex or heavy. To this end, a rectangular microwell of the device (100×200 μm in the cross-section) was represented in the simulation by a cylindrical microwell, with the same cross-section area (160 μm in diameter) and the same depth of 100

μm. Because a single 3.3 mm in diameter circular well of the device has eight microwells at the bottom, the circular well was represented in the simulation by a cylinder with 1/8 of the cross-section of the circular well (1.17 mm in diameter) and the same depth of 5 mm. Finally, all finger-like channels connected to the microwell, with an integral cross-section area of 4375 μm^2, were represented by a single cylindrical microwell, with the same cross-section area, corresponding to a 75-μm diameter, and the depth of 100 μm, equal to the length of the finger-like channels. The resulting simulation geometry, consisting of three co-axial cylinders, has the same connectivity and features with the same cross-section areas and lengths as their counterparts in the device, but is substantially easier to analyze and more simple to simulate, because of its axial symmetry. To represent cells remaining at the bottom of the microwell of the device after the finger-like channels are loaded, the 160-μm diameter microwell of the computational domain has a 25 μm tall layer at the bottom with a nutrient consumption (reaction) rate of -0.0579%/sec, the same as for the monolayer of cells at the bottom of the simulated dish (see above). In our experiments, a typical number of lymphocytes in a finger-like channel was ~25. Given the finger-like channel cross-section of 25×25 μm and the 8×8 μm footprint of a lymphocyte in the simulation of a monolayer in a dish, this number of lymphocytes per finger-like channel is equivalent to 2.6 layers of lymphocytes at the bottom of a dish. For our simulation, we set a uniform reaction rate of -0.0579%/sec in the 100-μm deep 75-μm diameter microwell (representing the finger-like channel), corresponding to four layers of lymphocytes or 39 lymphocytes per finger-like channel, that can be considered as a safe upper limit.

The results of the simulation of diffusion and consumption of a small molecule (D_1 = 500 μm^2/sec) in the model of the microfabricated device (Fig. S1C) indicate substantially more favorable cell culture conditions than in the model of a dish with the cell monolayer (Fig. S1A). Specifically, after 24 hours of culture, the nutrient concentration around cells in the finger-like channel is ~97% (Fig. S1C), as compared to ~70% in the culture-dish model (Fig. S1A). The variation of concentration along the finger-like channel is only ~1.5%, suggesting nearly uniform concentration of small molecules. For a model

protein (D_2 = 50 μm^2/sec), the concentration varies more appreciably, from 88% at the beginning of the model finger-like channel (top of the 75-μm diameter cylinder) to 82% at its end (bottom of the cylinder). Nevertheless, those variations are relatively minor, and the mean concentration (85%) still compares favorably with that in the monolayer culture in a dish (32%). The simulation can be corrected for partial blocking of a finger-like channel by cells in it, resulting in an effective reduction of the diffusion coefficient. Nevertheless, given that the total volume of 25 lymphocytes, ~6,400 μm^2, is ~10 times smaller than the volume of a finger-like channel (62,500 μm^2), the reduction of the effective diffusion is expected to be ~10%, with only a minor effect on the profile of concentration in the finger-like channel.

Overall, the effects of depletion of both small-molecule and macromolecule nutrients are expected to be substantially weaker for cells in the finger-like channels (at 39 cells per channel) than for a cell monolayer at the bottom of a dish. According to our simulations (not shown), average concentration of small-molecule nutrient around cells in the finger-like channel at their typical population level is similar to that in a culture in a dish at a density of 1 cell per 640 μm^2, which is 1/10 of a monolayer. For macromolecules, the ratio is smaller, ~1/4, but still favorable for cells in the finger-like channels of the microwell device. The variation of concentration along the finger-like channel is negligible for small molecules and small for macromolecules. The variation would be larger for a more rapidly metabolized protein (or a lower initial absolute concentration), e.g. between 75 and 65% across a finger-like channel, if a monolayer culture in a dish reduces the concentration of the protein within 24 hrs by 50% (rather than 25%). However, such culture conditions would be somewhat extreme and not directly applicable to the lymphocyte cultures and the media used in our experiments.

The distributions of nutrients in Fig. S1 can be used to estimate distributions of molecules secreted (rather than consumed) by cells. If, in an analogy to the above discussion, we assume that for cells in a dish the average concentration of secreted molecules reaches 25% of some threshold value in 24 hours, distributions of secreted molecules can be obtained from the distributions in Fig. S1 by subtracting these latter distributions from 100%. For small-molecules (metabolites and small signaling

molecules) secreted by cells, this fact implies that their concentration around cells always remains low (~2.5% after 24 hours vs. ~30% for the dish culture; Fig. S1C vs. S1A). For secreted macromolecules with a representative diffusion coefficient of 50 μm^2/sec, the mean concentration around cells in a finger-like channel is still substantially lower than in a dish, ~15% vs ~68%, but the concentration varies along the finger-like channel by as much as a factor of 1.5 (from 18% to 12%). Variations of concentration in the finger-like channel would be even greater for secreted molecules or particles with lower diffusivity, such as HIV viruses, with high concentrations accumulating at the dead end.

If the length of the finger-like channels is increased to 200 μm, while the number of cells in them remains unchanged, the variations of concentrations of small-molecule nutrients along the finger-like channels still remain small, 98% at the beginning vs. 96.8% at the end of the finger-like channel after 24 hours (not shown). Gradients of small-molecule metabolites become substantial, with an ~1.6-fold change from the beginning to the end, but their absolute concentrations are still very low, at 2.6% on average. Gradients of protein nutrients (D_2 = 50 μm^2/sec) are more significant, with ~88% concentration at the beginning and ~76.5% at the end, but their average concentration still compares favorably to that in the dish culture (~30%). For macromolecules with the same diffusivity secreted by cells, the variation of concentration is stronger, from 12% at the entrance to 23.5% at the end, corresponding to an almost two-fold change. But again, the average concentration of macromolecular metabolites compares favorably to that in the dish culture (68%). Further increases in the length of the finger-like channels or the number of cells in them would lead to even stronger gradients of macromolecular compounds that can be detrimental for the functionality of the device.