

ELECTRONIC SUPPLEMENTAL INFORMATION (ESI):

Capillary pumping analysis:

The design and dimensions of the microchannel and posts are key components to ensuring that the fluid remains in the channel and does not wick out through the post and into the absorbent pad (Fig S1. A). As indicated in the figure, 2 locations in the channel have different capillary properties: (1) the microchannel with a rectangular cross-section, and (2) the post with an open interface. The first location, location 1, is designed to have a higher capillary action than the location 2, such that the fluid prefers to flow in into the microchannel (i.e. the differential pressure of the channel dominates the differential pressure of the post). The differential pressure, ΔP , in a capillary system can be calculated with the Laplace law, assuming pseudo-steady state, for a liquid of surface tension γ , and the two radii of curvature in perpendicular directions, r_1 and r_2 , chosen at any location in the interface.

$$\Delta P = \gamma \left(\frac{1}{r_1} + \frac{1}{r_2} \right) \quad (1)$$

In a rectangular channel with four sides, the radii of curvature are calculated for two perpendicular planes, which intersect the lowest portion of the meniscus (Fig S1. B). By simple trigonometry, the contact angle of the liquid, θ , and the width of the cross section, h_c and w_c , can be used to calculate the radius of curvature:

$$\cos \theta = \frac{h_c}{2r_1} \quad \cos \theta = \frac{w_c}{2r_2} \quad (2,3)$$

Equations (2) and (3) yield:

$$r_1 = \frac{h_c}{2 \cos \theta} \quad r_2 = \frac{w_c}{2 \cos \theta} \quad (4,5)$$

The pressure can thus be calculated using equation (1), by:

$$\Delta P_{channel} = 2 \gamma \cos \theta \left(\frac{1}{h_c} + \frac{1}{w_c} \right) \quad (5)$$

The pressure differential of the post is calculated in a similar fashion, but differs since only three walls are present in the channel (Fig. S1. C). Similarly to the channel, two cross sectional planes are created. The radius of curvature, r_1 , of the first plane, plane A, is calculated in a similar way to what was performed previously, with the contact angle, θ , and the width of the channel, d_p :

$$r_1 = \frac{d_p}{2 \cos \theta} \quad (6)$$

However, because there is only one wall present in plane B, the liquid will display a negative radius of curvature in the plane perpendicular to the plane of r_1 , thus:

$$r_2 < 0 \quad (7)$$

This negative radius of curvature will assist in keeping liquid in the channel, and takes at most a value of 0 if the interface were straight. Thus, we can write the pressure drop for the post, ΔP_{post} :

$$\Delta P_{post} < \frac{2 \gamma \cos \theta}{d_p} \quad (8)$$

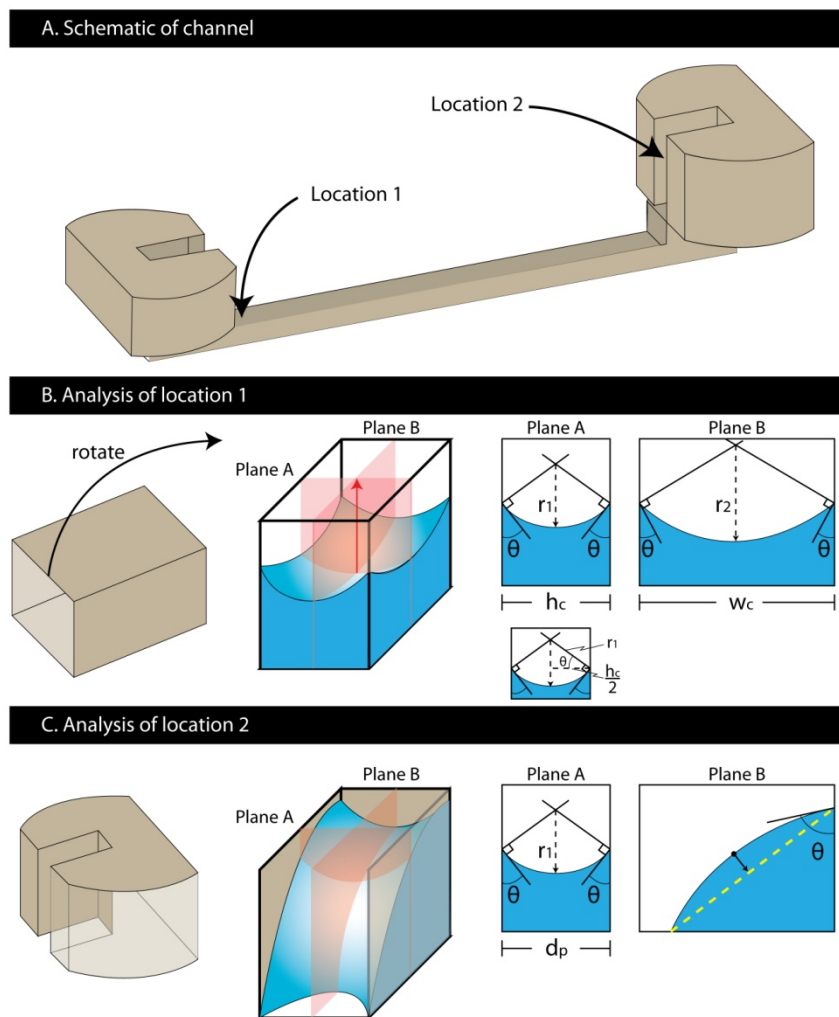


Fig. S1. **A.** Schematic of a KOALA microchannel containing a horseshoe-shaped post on each side. **B.** Analysis of the capillary pressure generated in the channel section of the KOALA microchannel. **C.** Analysis of the capillary pressure generated in the horseshoe post section of the KOALA channel.

Alternative KOALA bases and lids:

To expand the KOALA platform as well as enable and accelerate alternative assays we have developed a variety of alternative lids and bases. The first base, as presented in the paper, is a comb type of channel that enables filling a variety of wells in one simple step. (Fig S2 A) The base is formed in polystyrene and houses a variety of wells which are interconnected by a channel. When liquid is flowed into the channel, the liquid successively fills each of the wells. Once the liquid reaches a pad at the end of the channel, the pad wicks the excess fluid out of the channel, thereby disconnecting each of the wells from one another, and leaving a series of wells with a precise volume of liquid. This base is then used with a standard KOALA lid in the same, in the same fashion as described in the main article.

The second base is designed to enable a series of dilutions in two simple pipetting steps. (Fig S2 B) The first channel functions in the same manner as the previously described, where the liquid goes in the channel, fills a series of wells, then is wicked into the pad. However, each well on the first side varies in size, to control to level of dilution. After the first well is filled, and the channel is empty, liquid is flowed into the second channel. As the fluid flows through the main channel, it fills each adjacent well, and pins at a constriction, adjacent to that of the first liquid. Next, fluid flows through a small channel, which fluidically connects the second liquid with the first liquid. As the fluid continues through the small channel, it creates a short between the fluid of the first well, and the floor of the larger circular well. This allows both liquid 1 and liquid 2 to flow into the larger well, and mix into solution. Ultimately, the volume is controlled by the size of the larger well, and the levels of dilution are controlled by the size ratio of the smaller wells.

Finally, we developed a lid to perform neutrophil chemotaxis, to demonstrate the amenability of the KOALA platform to alternative channel designs and alternative assays. (Fig S2 C) This lid uses the same channel and post format as the lid described in the main article. However, in this case, each device utilizes two inlet ports and one outlet port. The first step is to apply the lid to a base containing media in alternating wells. This will preload the channel with media, from one inlet port, to assure the entire channel is filled and no bubble are trapped in the migration channel. The second step is to apply the lid to a base containing a chemoattractant in alternate channels. In this case, the chemoattractant was mixed into a hydrogel to reduce convective mixing and unwanted flow. The third step is to apply the lid to a base containing neutrophils which are in alternating wells, opposite that of the chemoattractant. A gradient of chemoattractant then formed in the cross migration channel, and migration was observed over a 30min time period. The KOALA platform enables simultaneous loading of the microchannels, which is beneficial to ensure that the gradient is consistent between each channel.

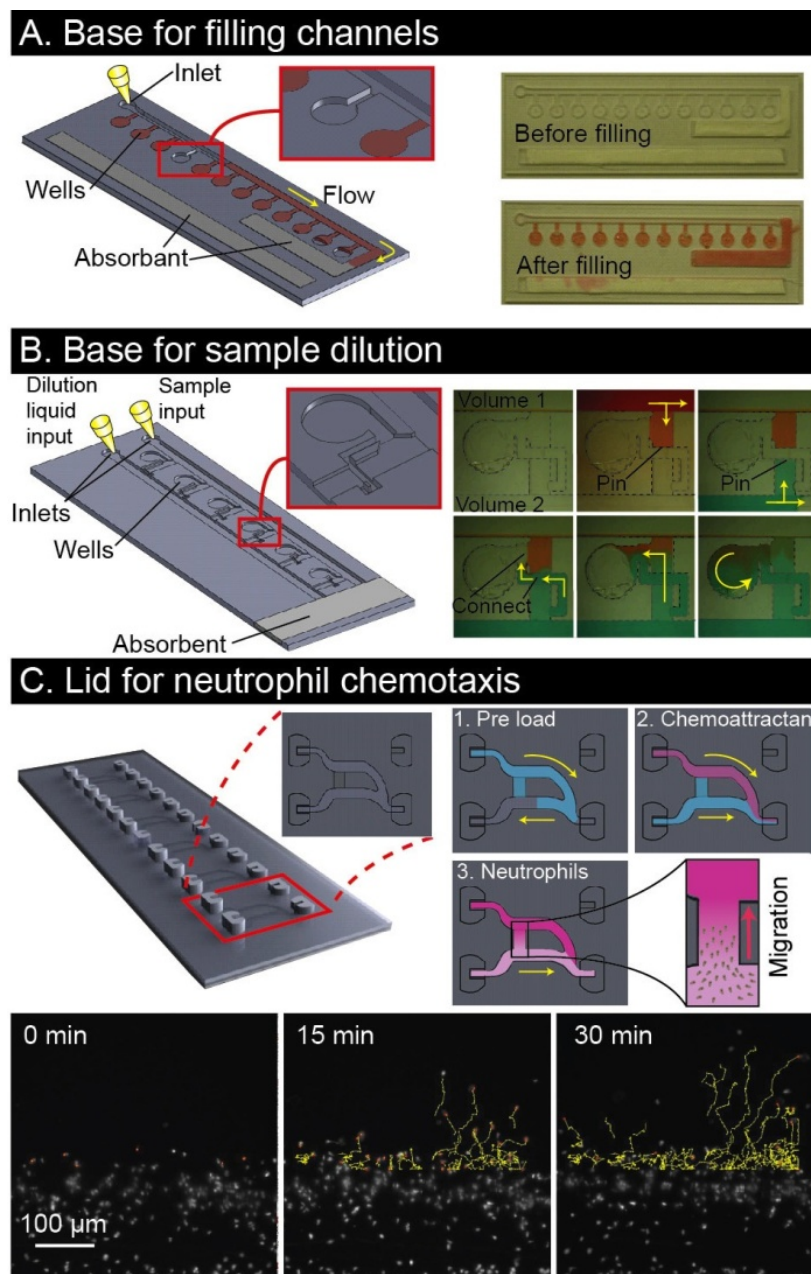


Fig. S2. **A.** Schematic of the cell cryopreservation embodiment where cell are contained in a well enclosed by a tape and a microporous membrane. **B.** The device is rocked back and forth to facilitate dialysis for replacing the freezing media with fresh media, after which the tape is removed and the lid is placed on the base for seeding cells **C.** Quantification of the dialysis of Alexa488 dye in PBS. After 6 minutes ~97% of the dye was removed from the wells. **D.** Viability of various cell lines through the KOALA freezing and thawing process. Microscopy images of the live/dead stain for the HEK cell line is shown.