

MASSIVELY PARALLEL MICROFLUIDIC CELL-PAIRING PLATFORM FOR THE STATISTICAL STUDY OF IMMUNOLOGICAL CELL-CELL INTERACTIONS

Melanie M. Hoehl¹, Stephanie K. Dougan², Hidde L. Ploegh², Joel Voldman³

¹Harvard-MIT Division of Health Sciences and Technology, MIT, USA; ²MIT Whitehead Institute, USA; ³Department of Electrical Engineering and Computer Science, MIT, USA

ABSTRACT

Variability in cell-cell interactions is ubiquitous and particularly relevant for the immune system, where the reliability of cell-cell interactions is critical for the prevention of disease. This variability is poorly understood mainly due to the limitations of current methods. We have therefore designed a highly parallel microfluidic cell-pairing device and optimized its pairing-efficiency using fluids modeling. The optimized device can hydrodynamically pair hundreds of primary mouse immune-cells at an efficiency of ~50%. We measured T cell activation dynamics of ~130 primary mouse T cells paired with B cells. Our findings represent the first time that variation has been observed in T cell activation dynamics.

KEYWORDS: Microfluidics, Cell-Pairing, Immune Cells, Ratiometric Imaging, Fura-2, Calcium

INTRODUCTION

Many immune responses are mediated by cell-cell interactions. In particular, cytotoxic T cells form conjugates with pathogenic and cancer cells in order to fight disease. Moreover, T cell maturation and activation is governed by direct cell interactions with antigen-presenting cells (APCs). Errors in these processes can lead to the progression of severe diseases, such as multiple sclerosis (MS) and type 1 diabetes. The study of these intricate cell-cell interactions at the molecular scale is therefore crucial for understanding the dynamics and specificity of the immune response. One important feature of cell-cell interactions is variability across populations. Cell-to-cell variability in presumably homogeneous populations has been neglected in immunology due to the limitations of conventional assays, which typically measure bulk responses at the population level [1-2]. Current methods to image immune cell-cell interactions randomly mix cells on a substrate and then use microscopy to scan and find cell pairs (Fig. 1A) [3-4]; entire papers are often written based upon analysis of ~5-10 cell pairs [5], making it difficult to understand activation dynamics and variability across the population. We have overcome these limitations by developing a microfluidic platform that can control cell-pairing across >100 immune cell pairs while allowing visualization of the resulting responses (Fig. 1B).

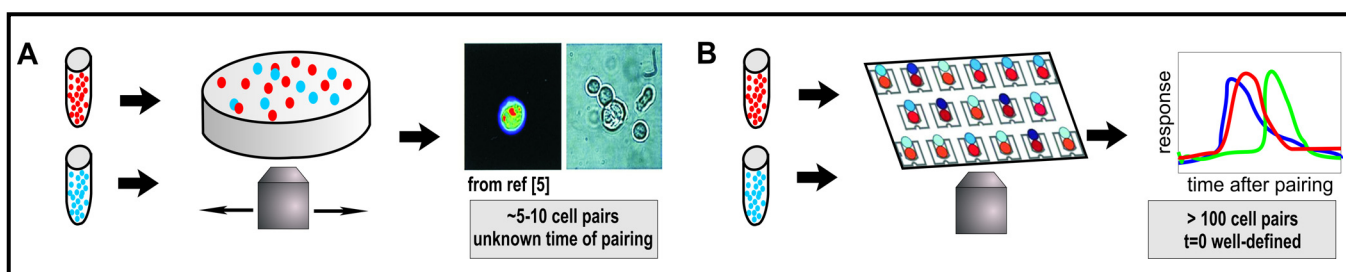


Figure 1: Methods to study immune cell-cell interactions **A)** Current methods to image cell-cell interactions in immunology randomly mix cells on a slide, giving very few cell pairs and making it impossible to study dynamics and variability. **B)** Our microfluidic immune cell-pairing device overcomes these limitations by controlling cell-pairing across >100 immune cell pairs simultaneously while allowing visualization of the resulting responses.

RESULTS

Device Optimization

We previously developed a microfluidic device that used hydrodynamic trapping to create many cell pairs for studying cell reprogramming [6]. The device pairs cells in a three-step loading procedure (Fig. 3). Immune cells are substantially smaller (~3×) than stem cells, and because of the nonlinear scaling of fluid flow with geometry and lithographic limitations, we had to significantly alter the device design for use with primary immune cells. Maximizing pairing efficiency requires maximizing the fractional flow through the capture cup (Q_c/Q_t , Fig. 2A). We performed hydrodynamic fluids

modeling (COMSOL) to determine how to alter the geometry to maximize Q_c/Q_t (Fig. 2B). We found that altering the cup-to-cup spacing transverse to the flow (W_c) had the greatest impact on flow through the cups (Fig. 2B). We fabricated redesigned traps according to our modeling results (Fig. 2C) and have achieved ~50% pairing efficiency with primary immune cells (Fig. 3). This is the highest pairing efficiency reported for such cells.

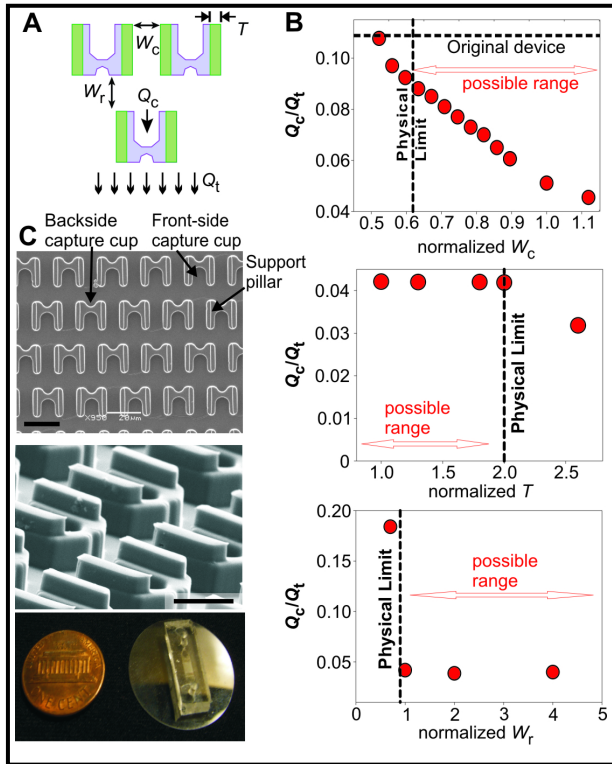


Figure 2: Modeling *A*) Schematic of the hydrodynamic cell trap, showing the parameters that we varied (W_r , W_c , and T) to try to maximize the fractional flow through the capture cup (Q_c/Q_t). *B*) Plots of the fractional flow through the capture cup as we vary geometry. Each parameter (W_r , W_c , and T) is normalized to a nominal value, and can only vary up to a physical limit (e.g., W_r cannot be smaller than the size of a cell). The model predicts that decreasing horizontal cup spacing (W_c) can increase flow through the capture cup. *C*) Images of the immune cell-pairing devices based upon the modeling design. Scale bars = 20 μ m.

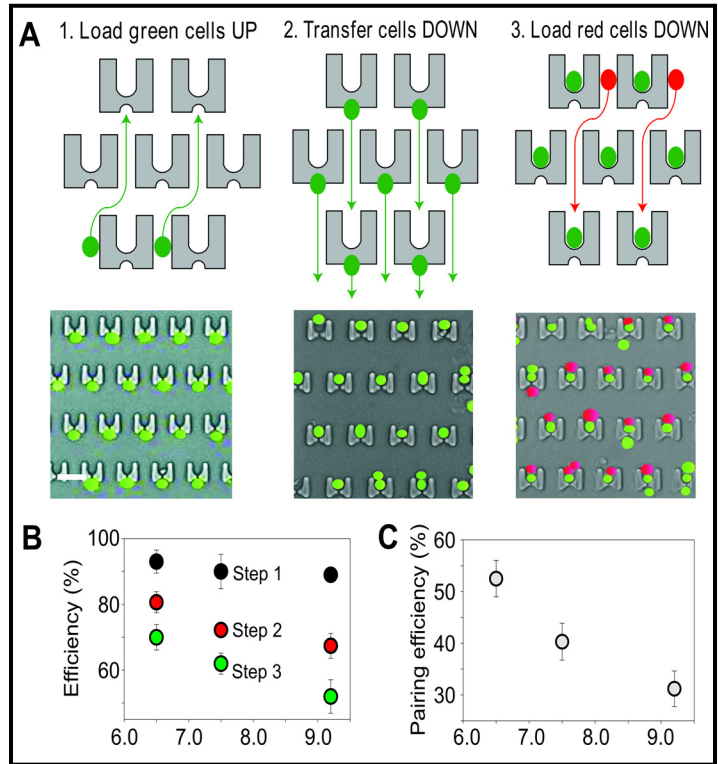


Figure 3: Pairing Efficiency *A*) Schematic and images of 3-step loading. (1) Cells are first loaded 'up' toward the smaller back-side capture cup. (2) The direction of the flow is reversed, and the cells are transferred 'down' into the larger front-side capture cup two rows below. (3) The second cell type is loaded in from the top, and cells are captured next to the first cell type. Images show pairing of dye-labeled immune cells. Scale bar = 20 μ m. *B*) Measured efficiencies of steps 1-3 as a function of cup spacing W_c . Efficiency is defined as the fraction of cups that successfully complete each step (e.g., transfer cells in step 2). In accordance with modeling, the pairing efficiency increases with decreasing W_c . *C*) Measured overall pairing efficiency increases with decreasing W_c .

Statistical T Cell Activation

By coupling the device with ratiometric fluorescent microscopy, we studied variation in APC-induced T cell activation, critical to adaptive immunity. We paired OT-1 T cells with B cell blasts loaded with a peptide derived from ovalbumin (SIINFEKL). The OT-1 cells come from transgenic mice whose T cells only respond to this peptide and are, by standard measures, immunologically homogeneous. Our three-step loading procedure (Fig. 3A) allowed the cells to be paired synchronously and enabled comparison of activation profiles. We continuously imaged cell pairs and analyzed their calcium response dynamics using the ratiometric calcium dye Fura-2 (calcium rise is an early indicator of T cell activation). We recorded, for the first time ever, T cell activation dynamics from ~130 cell pairs (Fig. 4). We quantified the extent of T cell activation using clustering of the response dynamics, determining that the nominally homogeneous cells actually fall into four clusters (Fig. 4B-C). The first two clusters represent cells with delayed responses that do not decay much, while the last two clusters are cell pairs with wide and narrow peaks. Our results represent the first time that T cell activation has been

studied for hundreds of cells simultaneously. Our single-cell, statistical measurements also show, for the first time ever, heterogeneity amongst this CD8 T cell pool.

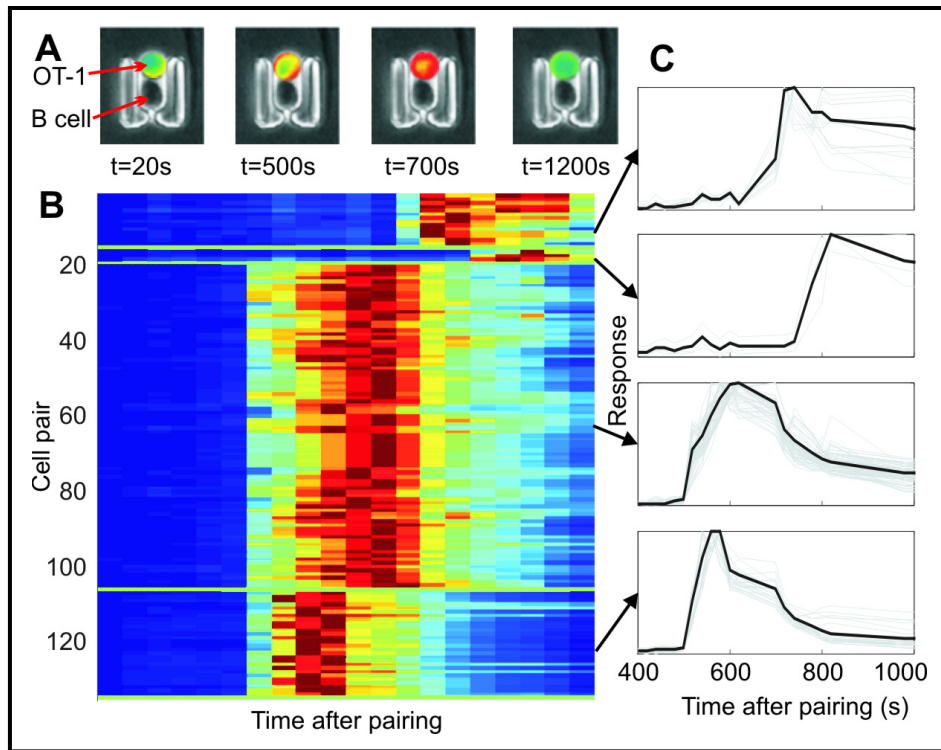


Figure 4: T cell activation
A) Combined fluorescence and phase images showing calcium activation of an OT-1 T cell in response to pairing with a B cell. **B)** Heat map showing k-means clustering of the activation profiles of ~130 synchronously paired cells. The T cell activation responses fall into four clusters. **C)** Centroid (black) and all (gray) response curves for the four clusters in (B). The first two clusters represent cells with delayed responses that do not decay much, while the last two clusters are cell pairs with wide and narrow peaks.

CONCLUSION

We have developed a microfluidic immune cell-pairing device that can hydrodynamically trap hundreds of primary mouse immune cells. By performing hydrodynamic modeling and optimization we achieved a pairing efficiency of ~50%. We used this device to pair primary mouse B and T cells. Our findings represent the first time that variation has been observed in T cell activation dynamics. Although all OT-1 T cells carry the identical receptor, the T cells obtained from OT-1 transgenic mice are not necessarily functionally homogeneous. Our measurements are consistent with heterogeneity amongst this CD8 T cell pool. This new platform presents a significant improvement in our ability to study variability in cell-cell interactions in immunology.

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