# MICROFLUIDIC ASSAY TO COMPARE SECRETION VS CONTACT BASED CELL-CELL INTERACTIONS USING DYNAMIC ISOLATION CONTROL

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## ABSTRACT

We report cell-cell interaction studies utilizing a microfluidic chip capable of capturing cell pairs of different types into culture microwells and isolating the captured cells for a given amount of time using pneumatic valves. We have quantified the capture efficiency and confirmed cell viability and interaction in the microwells. We successfully distinguished interaction modes in the fabricated devices: secretion-based and contact-based. C2C12 cells, when co-cultured with PC3, showed increased proliferation due to PC3 secretions. Also, it was indentified that C2C12 proliferation is increased through contact with endothelial cells by comparing proliferation in both secretion only and contact only systems, respectively.

**KEYWORDS:** Cell Interaction, Microwells, Secretion vs. Contact, Pneumatic Control

## INTRODUCTION

Studying cell-to-cell interactions has become increasingly important in order to elucidate cellular processes such as stem cell proliferation and differentiation [1]. Unfortunately, most conventional methods are limited to the collective measurement of thousands of cells; therefore, important interactions can remain hidden by this averaging process and make high-throughput screening problematic. [2]. Recent studies have addressed this problem by allowing observation of single cell pairs in close proximity [3][4]; however, these studies could not distinguish whether the interaction was induced by secretion or contact from other cells. In this work, we expand on this previous effort by presenting a device for cell-cell interaction analysis that allows researchers to determine the exact contribution of interaction modality (secretion vs. contact). Any combination of mixed cell pairs in either contact or close proximity can be captured and isolated in a microwell. Using dynamic pressure control, secreted signals can be concentrated in the small well volume or washed away by continuous media perfusion.

## METHODS

Figure 1A shows the microfluidic chip used in this study that has been characterized in our previous report [5]. The device consists of two separate polydimethylsiloxane (PDMS) layers bonded to each other and then to a glass slide. The bottom layer serves as the culture microwell and is kept to a total thickness of only 50 microns so that it is easily manipulated by the upper channel (Figure 1B). The upper layer serves as the valve control via pressure changes. Each microwell consists of a front valve, a rear valve, and a culture surface surrounded by a wall (Figure 1C).

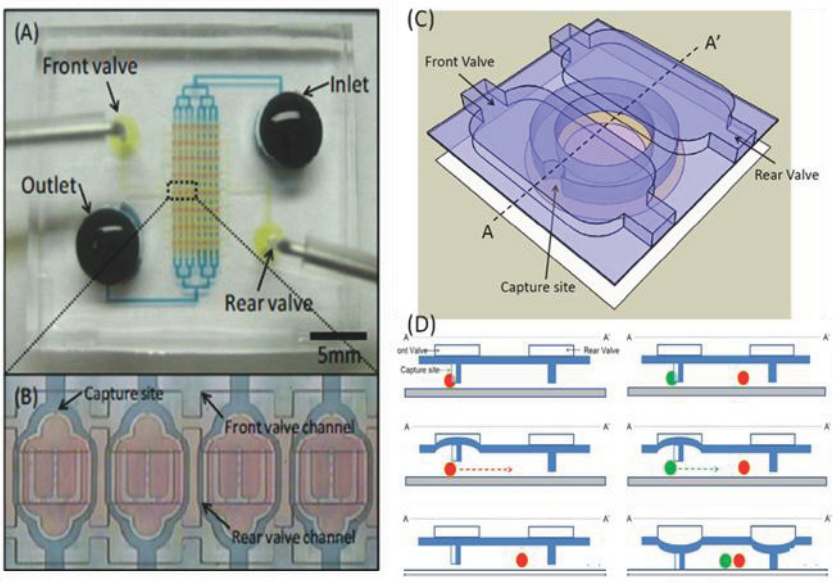


Figure 1: 1A) Device overview with connections, 1B) 2 Layer device with valve channel on top and capture site below, 1C) 3D device schematic, 1D) Principle of device operation

To load the device (Figure 1D), cells are introduced from the inlet driven by gravity force, while no pressure is exerted on the upper channel, leaving a small gap that allows fluid to pass through. This allows single cells to be trapped in the indented capture site. Applying negative pressure to the valve channel pulls the capture wall up for cells to flow into the well. Repeating this process with multiple cell types enables the customization of co-culture pairs. Conversely, applying positive pressure causes the wall to close, completely isolating the cell pairs.

Using the pneumatic valve operation during culture allows one to control the contributions from each interaction modality (secretion or contact). By changing the fabrication layout of the

culture chamber to be either partitioned (Figure 2A) or "open" (Figure 2B) for cell-cell contact, we can control the way in which the cells are interacting. During secretion studies, positive pressure is applied to valve channel to seal the microwell below. This results in the buildup of secreted factors within the micro environment and fresh media can be introduced at any time by releasing the pressure. On the other hand, contact interaction studies require constant media perfusion to wash away any secreted factors. Comprehensive interaction studies can be performed using the "open" device layout and dynamically altering between isolation and perfusion states.

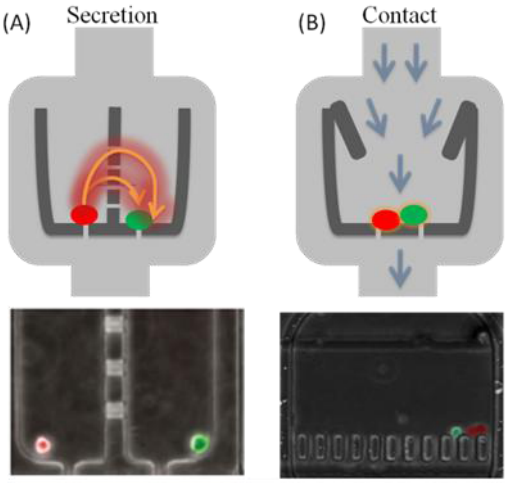


Figure 2: 2A) Principle of secretion interaction studies, 2B) Principle of secretion contact studies

## RESULTS

Following the capture procedure described above, C2C12 myoblasts and PC3 prostate cancer cells were suspended and loaded into the device for pairwise culture. The cells are hydrodynamically captured prior to the pneumatic actuation and a pairwise capture efficiency of ~65% has been achieved [5]. Figure 3A shows the high capture efficiency in the microfluidic array with green fluorescence labeled C2C12 and red fluorescence labeled PC3.

In order to validate the ability of the platform to modulate contact modalities, two separate studies were performed. First, PC3 and C2C12 cells were used to demonstrate the ability of our assay to study secretion interactions. Previous work has demonstrated the ability of PC3 to increase cell proliferation through a secreted factor [6]; as such, this device can be an appropriate model. To validate the system for contact interaction studies, C2C12 cells and bEnd (brain endothelial) cells were co-cultured. It has been shown that co-culture of these cells will increase proliferation in both cell types [7], and it is believed that cell-cell contact is the most important mediating factor. By subjecting them to either secretion only or contact only conditions and monitoring their proliferation, we can determine the contributing interaction type.

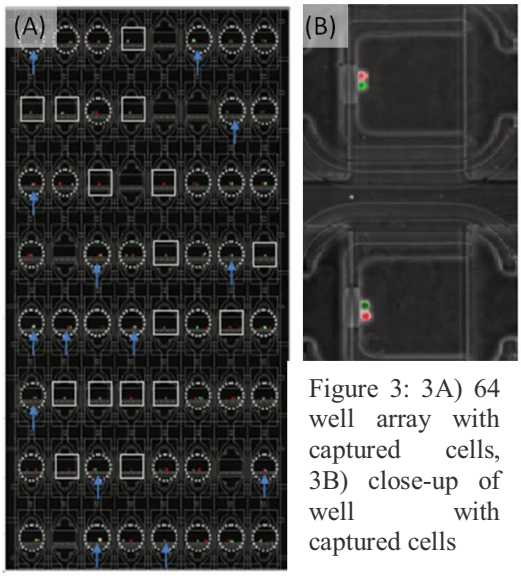


Figure 3: 3A) 64 well array with captured cells, 3B) close-up of well with captured cells

In the first study, PC3 cells were loaded from gravity driven wells and captured in the chambers. This process was repeated for C2C12 cells in order to capture a pair of each cell types in a microwell. Control devices were prepared to load only two C2C12 cells instead. The device was connected to a custom-made pneumatic controller, which closed the valves for 3 hours twice a day, and then was placed in an incubator for 3 days of cell culture. Media was continuously perfused from the inlet to the outlet but only introduced into the microwells when valves were open. Additional fresh media was added to the inlet every 20 hours. As expected, increased proliferation of C2C12 was observed only when the secreted factors from PC3 cells were allowed to accumulate, with no increase when the same cells (two C2C12 cells) were loaded, as shown in Figure 4.

By varying the isolation period and monitoring the resulting proliferation rate, the optimal isolation time has been determined. Trying times between 1 and 9 hours for 1 or 2 times per day, it was found that the twice daily, 3 hour isolation time was the optimal balance between secretion build up for stimulation and the fresh media needed for growth. This period of isolation and perfusion was used as a standard protocol for the rest of experiments for secretion-based inter-action studies.

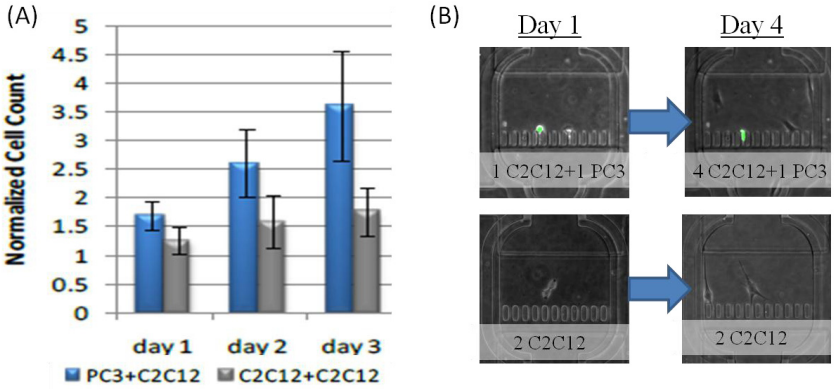


Figure 4: 4A) C2C12 growth under influence of secreted factors, 4B) Images of C2C12 growth over 3 days

In the subsequent contact-mode cell interaction studies, C2C12 and bEnd cells were loaded pairwise as the positive experiment. For the control, C2C12 cells were loaded with 10T1/2 (fibroblasts) which have been previously shown not to increase C2C12 proliferation [7]. Half of the devices were connected to the pneumatic actuator to be subjected to 2 separate 3 hour isolations per day, and the other half were not. In this manner, secretion-only and contact-

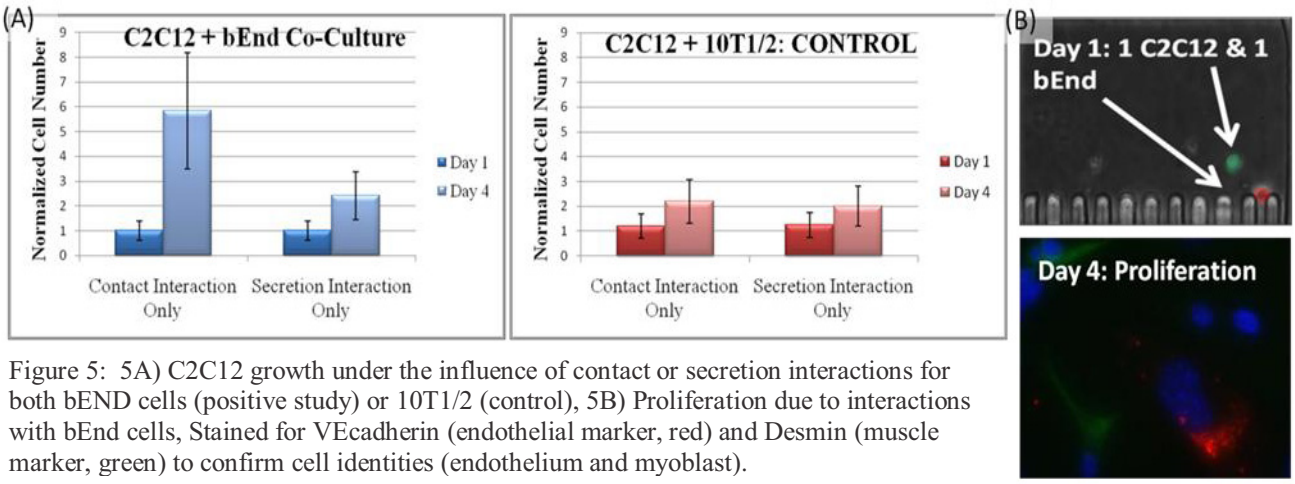


Figure 5: 5A) C2C12 growth under the influence of contact or secretion interactions for both bEND cells (positive study) or 10T1/2 (control), 5B) Proliferation due to interactions with bEnd cells, Stained for VEcadherin (endothelial marker, red) and Desmin (muscle marker, green) to confirm cell identities (endothelium and myoblast).

only interactions would be distributed through the two groups of devices. The results are shown in Figure 5 and suggest that the interaction is mainly contact based and secreted factors play only a marginal role. This can be concluded from the fact that a significantly higher proliferation was observed in the continuously perfused wells, where cells remained in close contact. In the case that the cells were retained in separation but the secreted factors were allowed to accumulate, proliferation remained almost same and was not statistically different from that observed in the controls (both contact and secretion interactions with 10T1/2 cells).

## CONCLUSION

In this work, we have successfully demonstrated the application of a novel microfluidic array chip for pairwise cell interaction studies. The fabricated device can provide high-throughput cell culture platform for multiple cell types at a pairwise cell resolution. The enabling control of interaction modality was validated by studying C2C12-PC3 secretion interactions and C2C12-bEnd contact interactions. An increased proliferation rate of C2C12 cells was observed when exposed to the secreted factors from PC3 cells. When C2C12 cells were in contact with bEnd in continuous perfusion, proliferation rate was significantly enhanced. As a research tool, the proposed device is useful to distinguish cellular interactions in a complicated niche where both contact interactions and secreted factors can contribute in determining cell fate. It is expected that after identifying a niche of multiple cell types, many other cell combinations and types can be tested as desired, utilizing the stepwise cell loading and the dynamic control of interaction modalities throughout the study.

## ACKNOWLEDGEMENTS

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