

YEAST ON A CHIP

Single-cell Analyses of MAPK Signaling Pathways in Saccharomyces Cerevisiae using Cell Chips

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Abstract: The mitogen-activated protein kinase (MAPK) signaling pathways are essential for cell growth, cell differentiation and survival in eukaryotes. The MAPK signaling pathways transmit signals from the cell surface to nucleus. The mating and high osmolarity responses in the budding yeast, *Saccharomyces cerevisiae*, depend on the MAPK signaling pathways. Here we analyzed the mating and high osmolarity responses in the budding yeast, *S. cerevisiae* at single-cell level using cell chips. The cell chip analyses of the mating and high osmolarity responses were performed using fluorescent proteins fused to genes whose transcription is specifically upregulated by each signaling. Using the technique, we have determined the real-time gene expression patterns of the mating and high osmolarity responses at single-cell level. In this study, we observed that the mating and high osmolarity MAPK signaling showed a non-uniform, fluctuating flux in the population of yeast cells analyzed.

1 INTRODUCTION

Cellular behavior has been typically investigated by utilizing bulk-scale methods that measure average values for a population of cells. For example, commonly used methods for high-throughput, cell-based assays are adapted to 96- and 384-well plate (recently 1536-well plates) formats (Hertzberg & Pope, 2000). Despite the success of these assays, such population-wide studies mask the behavior of individual cells and are often insufficient for characterizing biological processes in which cellular heterogeneity plays a key role (i.e., ensemble averaging problem).

Single-cell measurements are necessary for investigating the stochasticity of gene expression because cell-to-cell variation cannot be quantified using population measurements. Flow cytometry and automated microscopy are some of the most widely used techniques for single-cell measurements. Owing to the stochastic nature of gene expression, the optimal experimental setup for analyzing gene

expression dynamics will be capable of both monitoring the behavior of a large population of cells and of tracking individual cells. Flow cytometry can be used to obtain gene expression data for thousands of cells, but only provides a snapshot of gene expression at single time points. Traditional microscopy experiments can track gene expression dynamics in individual cells, but can only monitor a relatively small population of cells. Microfluidics or “lab-on-a-chip” technologies can be used to track gene expression changes in individual cells, enable large populations of cells to be monitored, and allow the precise control of the cellular microenvironment.

These microfluidic “lab-on-a-chip” technologies offer the ability to work with smaller reagent volumes, shorter reaction times, and the possibility of high-throughput analysis (Figeys & Pinto, 2000; Reyes, Iossifidis, Auroux, & Manz, 2002). Utilizing these technologies, one possible approach to analyze individual cells is based on cell-trapping including hydrodynamic confinement (Wheeler et al., 2003), negative dielectrophoresis (Voldman, Gray, Toner,

& Schmidt, 2002), optical tweezers (Ashkin, 1997), and microwells etched at the tip of a fiber-optic bundle (Biran & Walt, 2002). These methods, however, would have some limitations for easy, cheap, high-throughput microscopic studies of single cells.

Recently, we reported highly improved version of the soft lithographic approach using surface tension driven cell seeding and subsequent cell docking induced by receding meniscus (Park, Hur, Kwon, Park, & Suh, 2006). Using this method, single to multiple yeast cells can be accurately deposited onto microwells depending on the size of the microwell with a cheap, easy and high throughput manner. Here, we incorporated the receding meniscus induced docking method into high-throughput automated fluorescent microscopy for analyzing stochastic nature of the MAPK signaling pathways in the budding yeast, *S. cerevisiae*. Using the technique, we have determined the real-time gene expression patterns of the mating and high osmolarity responses at single-cell level. In this study, we observed that the mating and high osmolarity MAPK signaling showed a non-uniform, fluctuating flux in the population of yeast cells analyzed.

2 RESULTS AND DISCUSSION

2.1 Receding Meniscus Induced Docking

Inside a microfluidic channel, receding meniscus can be a powerful tool for arraying yeast cells at single-cell level in a high-throughput manner. To utilize the receding meniscus induced docking method, we fabricated PUA microwells onto glass substrate using capillary molding (Suh, Kim, & Lee, 2001), and the patterned glass substrate was bonded to a PDMS microfluidic mold (Khademhosseini et al., 2004) (Fig. 1).

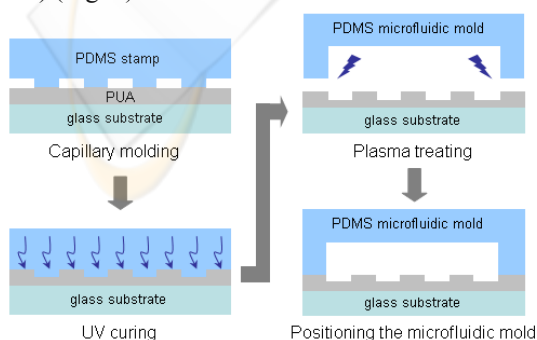


Figure 1: Fabrication of a patterned microfluidic channel.

Some representative SEM images of the fabricated PUA microwells are shown in Fig. 2. The pattern dimension of circular wells was $8\ \mu\text{m}$ in diameter, allowing for a feature density of $3906\ \text{wells}/\text{mm}^2$ which is similar to Affymetrix GeneChipTM. A higher-magnification ($\times 3500$) right column SEM images shows the well-defined PUA structures with good edge definition. The depth of each PUA microstructure was measured to be $8\ \mu\text{m}$ corresponding to the original height of the silicon master (not shown).

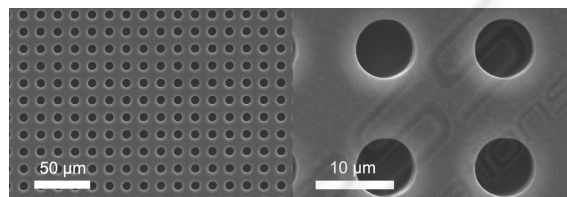


Figure 2: SEM images of PUA microwells.

As previously described (Park et al., 2006), yeast cells were docked into the microwells at single-cell level. As shown in Fig. 3, the docking efficiency is more than 90 % that allows high-throughput and high-content single-cell analysis.

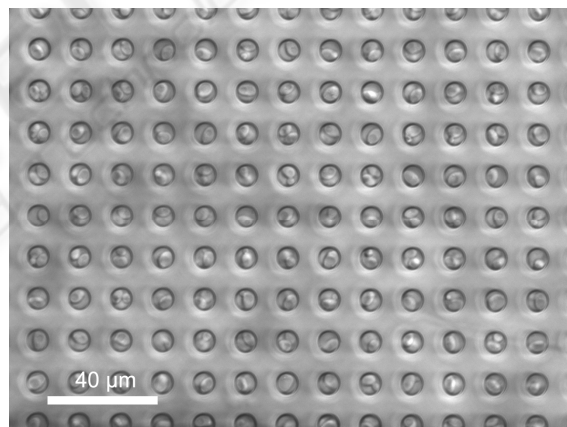


Figure 3: Single-cell docking of yeast cells in large-area.

2.2 Monitoring Gene Expression

Using this cell chip platform, we monitored the mating (α -factor) and high osmolarity (KCl) responses in the budding yeast, *S. cerevisiae* at single-cell level over time. The cell chip analyses of the mating and high osmolarity responses were performed using fluorescent proteins fused to genes whose transcription is specifically upregulated by each signaling. To do this, we constructed three kinds of yeast strain such as SH129 (*MATa*, *leu2*, *trp1*, *met15*, *P_{Fus1}-EGFP*, *P_{Gpd1}-Tdimer2*), SH133 (*MATa*, *leu2*, *trp1*, *met15*, *P_{Fus1}-EGFP-Cln2(PEST)*,

P_{Gpd1} - $Tdimer2$) and SH135 ($MATa$, $leu2$, $trp1$, $met15$, $Kar4$ -EGFP, P_{Gpd1} - $Tdimer2$) using homologous recombination.

For microscopic monitoring, we used DeltaVision™ system (Applied Precision, LLC.) which provide real-time live cell imaging. In order to image multiple fields of cells automatically and repeatedly over time, we controlled the microscope and camera with commercially available software, softWorx™ suite (Applied Precision, LLC.). We monitored 20 image fields which contain 42 ~ 49 well so that the total number of monitored cells were about one thousand. Some representative merged fluorescent images of each yeast strain are shown in Fig. 4 (scale bars are not shown).

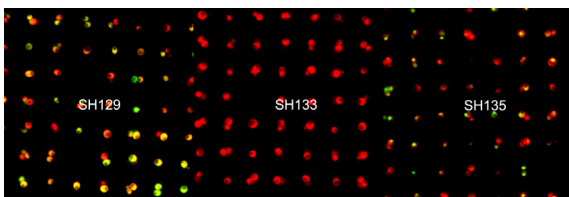


Figure 4: Merged fluorescent images of yeast strains.

2.3 Analyses of Stochastic Gene Expression

The acquired time-course merged fluorescent images were pre-processed for more accurate extraction of single-cell expression level. The pre-processing includes background subtraction, HiGauss filtering, Sharpen filtering and Flatten filtering. After image pre-processing, we extracted quantitative gene expression information with ImagePro™ software (Media Cybernetics, Inc.) (Fig. 5).

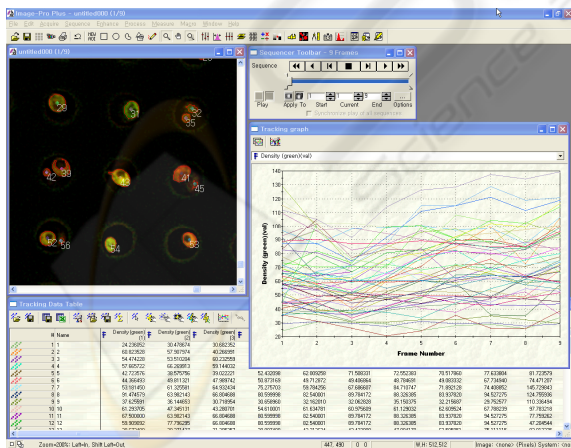


Figure 5: Extraction of quantitative information.

Genetically identical cells exhibit remarkable diversity even when they have identical histories of

environmental exposure (Elowitz, Levine, Siggia, & Swain, 2002; Raser & O'Shea, 2004).

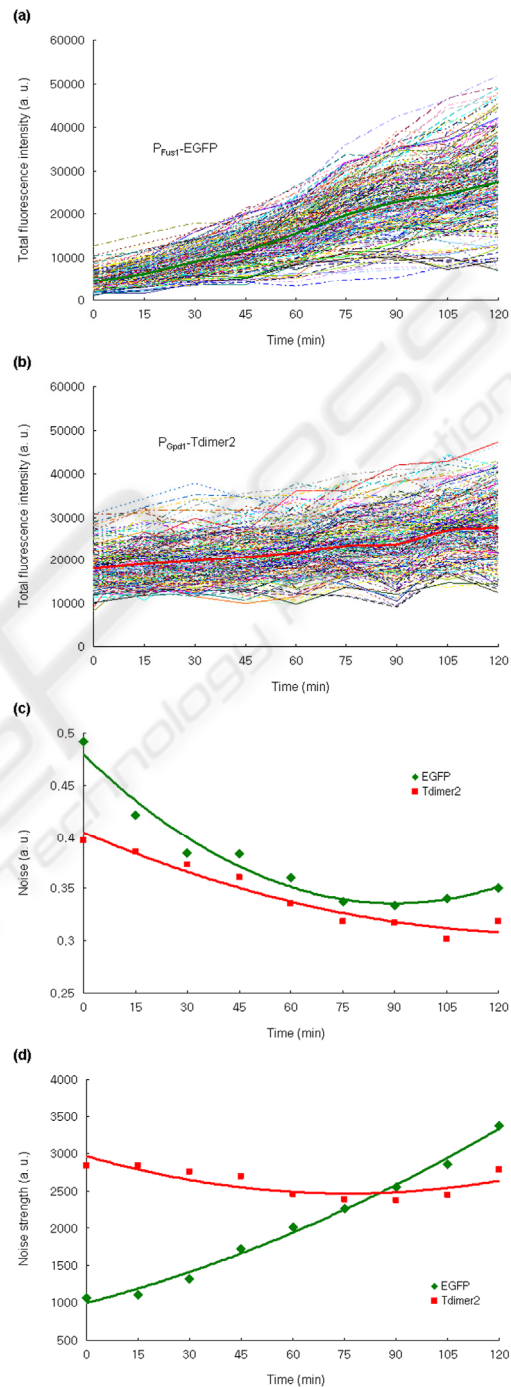


Figure 6: Analysis of stochastic gene expression in SH129.

As expected, we observed that the mating and high osmolarity MAPK signaling showed a non-uniform, fluctuating flux in the population of yeast cells analyzed. For example, when we used strain

with EGFP and Tdimer2 reporters driven by the α -factor-responsive P_{Fus1} promoter or by the α -factor-independent P_{Gpd1} promoter (i.e., SH129), the total fluorescence intensity, noise and noise strength upon stimulation of 10 μ M α -factor are characterized as shown in Figure 6.

Figure 7 shows the stochastic gene expression in SH133 strain which contain C-terminal residues Cln2 (yeast G1 cyclin) PEST motifs. The Cln2 (PEST) destabilized EGFP so that it allows dynamic monitoring of transcription over time. Figure 7a shows dose-dependent gene expression of P_{Fus1} -EGFP upon stimulation of α -factor. Interestingly, the mating MAPK signaling has different kinetic gene expressions as increasing cellular area (Fig. 7b).

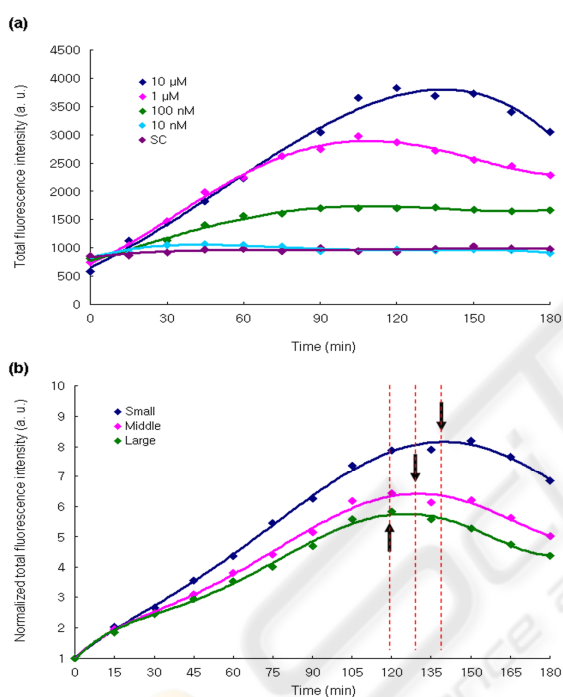


Figure 7: Analysis of stochastic gene expression in SH133.

Similarly, the SH135 strain whose character is protein localization exhibits the stochastic gene expression (not shown).

3 CONCLUSIONS

We have presented an optimal experimental setup for analyzing gene expression dynamics which would be capable of both monitoring the behavior of a large population of cells and of tracking individual cells. It was composed of yeast strain construction, single-cell docking, automated image acquisition,

extraction of quantitative information, analyzing and modelling of the stochastic gene expression. Using this cell chip platform, we could successfully have an insight into the stochastic nature of gene expression, so we hope that many other investigators also will have such insight more easily aided this high-throughput and high-content single-cell analysis method.

REFERENCES

- Ashkin, A. (1997). Optical trapping and manipulation of neutral particles using lasers. *Proceedings of the National Academy of Sciences of the United States of America*, 94(10), 4853-4860.
- Biran, I., & Walt, D. R. (2002). Optical Imaging fiber-based single live cell arrays: A high-density cell assay platform. *Analytical Chemistry*, 74(13), 3046-3054.
- Elowitz, M. B., Levine, A. J., Siggia, E. D., & Swain, P. S. (2002). Stochastic gene expression in a single cell. *Science*, 297(5584), 1183-1186.
- Figeys, D., & Pinto, D. (2000). Lab-on-a-chip: A revolution in biological and medical sciences. *Analytical Chemistry*, 72(9), 330a-335a.
- Hertzberg, R. P., & Pope, A. J. (2000). High-throughput screening: new technology for the 21st century. *Current Opinion in Chemical Biology*, 4(4), 445-451.
- Khademhosseini, A., Suh, K. Y., Jon, S., Eng, G., Yeh, J., Chen, G. J., et al. (2004). A soft lithographic approach to fabricate patterned microfluidic channels. *Analytical Chemistry*, 76(13), 3675-3681.
- Park, M. C., Hur, J. Y., Kwon, K. W., Park, S. H., & Suh, K. Y. (2006). Pumpless, selective docking of yeast cells inside a microfluidic channel induced by receding meniscus. *Lab on a Chip*, 6(8), 988-994.
- Raser, J. M., & O'Shea, E. K. (2004). Control of stochasticity in eukaryotic gene expression. *Science*, 304(5678), 1811-1814.
- Reyes, D. R., Iossifidis, D., Auroux, P. A., & Manz, A. (2002). Micro total analysis systems. I. Introduction, theory, and technology. *Analytical Chemistry*, 74(12), 2623-2636.
- Suh, K. Y., Kim, Y. S., & Lee, H. H. (2001). Capillary force lithography. *Advanced Materials*, 13(18), 1386-1389.
- Voldman, J., Gray, M. L., Toner, M., & Schmidt, M. A. (2002). A microfabrication-based dynamic array cytometer. *Analytical Chemistry*, 74(16), 3984-3990.
- Wheeler, A. R., Thronset, W. R., Whelan, R. J., Leach, A. M., Zare, R. N., Liao, Y. H., et al. (2003). Microfluidic device for single-cell analysis. *Analytical Chemistry*, 75(14), 3581-3586.