PARALLEL SCREENING OF SINGLE ZOOSPORE GERMINATION AND GERM TUBE PROTRUSIVE FORCES

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ABSTRACT

This paper reports the parallel screening of zoospore germination and protrusive forces of germ tubes on a lab-on-a-chip platform, integrating valve-based single zoospore compartmentalization with micropillar force sensing. Individually-controllable, normally-closed microvalves were optimized to facilitate zoospore compartmentalization in measurement channels containing traps and sensing pillars. Performance was evaluated using microspheres and zoospores of the oomycete *Achlya bisexualis.* By parallelizing the screening, the platform will support fundamental studies and aid in the discovery of new compounds to control fungal and oomycete pathogens.

KEYWORDS: Microfluidic, Membrane valve, Zoospore, Fungi and oomycetes.

INTRODUCTION

Although only a small minority of fungi and oomycetes are pathogenic, those that are have a rich diversity and increasingly serious impact on human health, food security and ecosystem resilience.[1] The germination of zoospores or conidia is a crucial stage in the pathogenic process, leading to new infections and penetration into a host.[2] Therefore, it is important to understand the mechanisms that underlie the germination of fungi and oomycete spores, and the invasive growth of resulting germ tubes. To enable the parallel screening of germination of individual zoospores and force sensing on resulting germ tubes, we have developed a triple-layer PDMS platform combining capture and culture of single zoospores with in-channel micropillar force sensing.[3,4] In the current work, we integrate normally-closed membrane microvalves with microfluidic trapping and sensing structures to overcome sealing limitations of previously-used sieve valves.[4] The resulting design allows for high-throughput compartmentalization of individual spores for parallel screening in response to exposure to various biocontrol strategies.

EXPERIMENTAL

The platform consisted of a gas layer and a fluidic layer, separated by a thin PDMS membrane. The gas layer contained six air chambers with separate gas inlets, forming pneumatic valves via valve seats on the fluidic layer and the membrane (see Fig. 1(a)). The fluidic layer included six parallel measurement channels, each with a valve seat close to the entrance, a zoospore trap-site and a freely-bending micropillar as force sensor. Both, gas and fluidic layer of the platform were fabricated by PDMS casting from photoresist masters.[4,5] The gas layer was first bonded to PDMS membrane, and then together onto the fluidic layer with continuous vacuum to avoid the membrane bonding with the valve-seats. Microparticles or zoospores were introduced from the zoospore inlet and

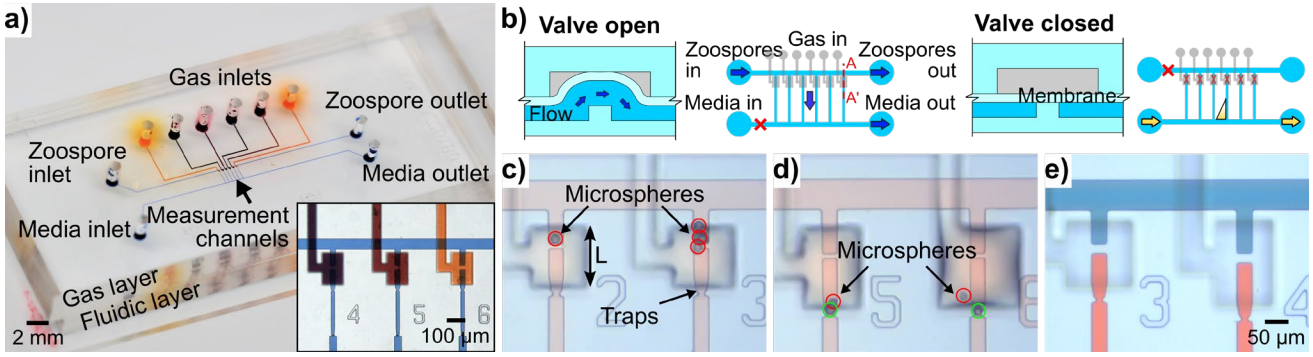

Figure 1: Monolithic PDMS platform. (a) Photograph of assembled platform with separate gas inlets for each valve. Light micrograph of measurement channels and individually-addressed valves, filled with different colour epoxy dyes. (b) Schematic diagram of platform operation *while introducing (valve open) and culturing zoospores (valve closed). Valve driving test with various air chamber sizes (c & d), and sealing test (e) using food colouring.*

hydrodynamically captured while microvalves were open (see Fig. 1(b)). Then, media was supplied from the media inlet during the entire culture after completely closing all microvalves. The growth of germlings and movement of pillar tops were recorded using an inverted microscope, and analyzed using ImageJ and Matlab.[4]

RESULTS AND DISCUSSION

As shown in Fig. 1(c) & (d), 20 µm microspheres did not pass valve-seats of open normally-closed valves with 140 µm long (L) air chambers. Based on this, air chamber sizes were optimized to 180 µm in length for zoospore trapping. No leakage of microvalves was observed when completely closed (see Fig. 1(e)). Zoospores of *A. bisexualis* were captured in traps after passing through valve seats, corresponding valves were then partially closed, avoiding subsequent zoospores flowing in (see Fig. 2(a)). During culture on the platform (Fig. 2(b)),

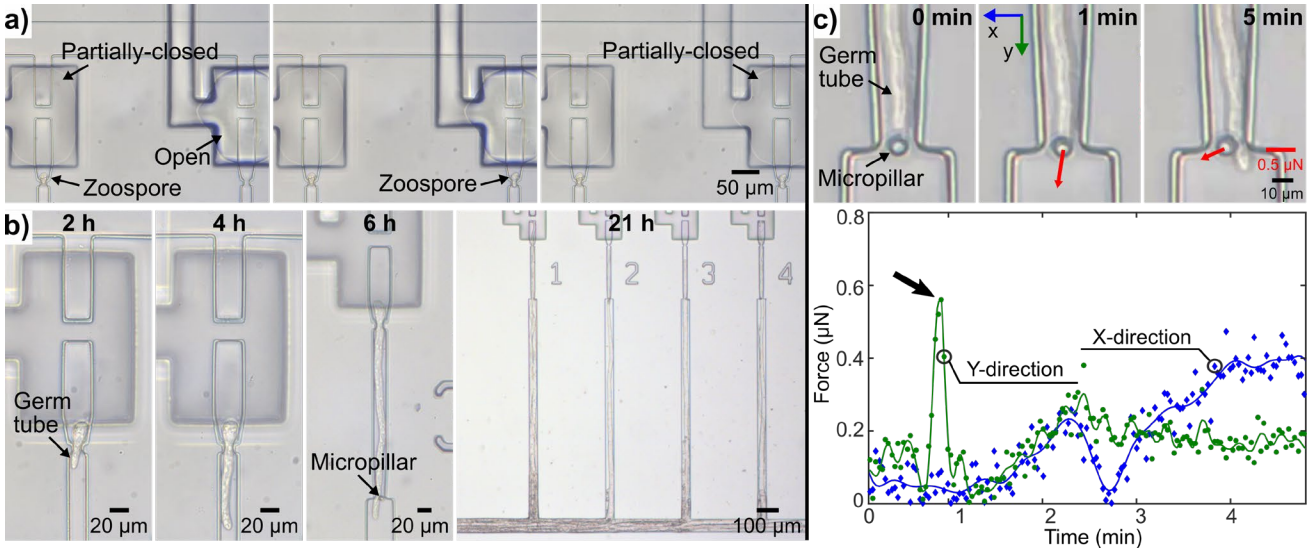

Figure 2: Capture and germination of A. bisexualis zoospores on the platform. (a) Light micrographs showing trapping of single pre-cultured zoospores using separately-controlled microvalves. (b) A sample zoospore germinating and resulting germ tube on-chip. (c) Time sequence showing a single germ tube impacting on a micropillar and resulting force magnitudes in X- and Y-direction plotted as a function of time. Arrow indicates germ tube – pillar impact.

zoospores germinated and resulting germ tubes grew along channels, impacting on force sensing pillars. Normally-closed valves successfully stopped backwards growth of hyphae. Protrusive force (Y-direction in example Fig. 2(c)) of germ tubes was recorded up to 0.56 µN while directly impacting on sensor pillars.

CONCLUSION

We have developed and characterized an improved microfluidic platform, combining normally-closed microvalves and force sensing pillars, to achieve single zoospore compartmentalization for biocontrol screening applications.

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REFERENCES

[1] J. B. Konopka *et al.*, *ASM*, 2019.
[2] S. Kamoun *et al.*, *Mol. Plant Pathol.*, vol. 16, no. 4, pp. 413–434, 2015.
[3] A. Tayagui *et al.*, *Lab Chip*, vol. 17, no. 21, pp. 3643-3653, 2017.
[4] Y. Sun *et al.*, *J. Microelectromech. S.*, vol. 27, no. 5, pp. 827-835, 2018.
[5] Y. Sun et al., *Transducers & Eurosensors XXXIII*, pp. 409–412, 2019.

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