ON-CHIP MEASUREMENT OF PROTRUSIVE FORCE EXERTED BY SINGLE HYPHAL TIPS OF PATHOGENIC MICROORGANISMS

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ABSTRACT

Invasive growth is a process used by fungi and oomycetes to find sources of nutrients. To study the protrusive forces generated as part of this process, we have developed a Lab-on-a-Chip platform capable of measuring forces exerted by individual hyphal tips during hyphal growth. Force measurement was demonstrated by culturing the oomycete *Achlya bisexualis* on-chip. Protrusive forces of 7.5 µN and bending forces of 19 µN were recorded for this organism. The platform provides a useful tool to better understand the mechanisms enabling fungi and oomycetes to grow invasively.

KEYWORDS: Fungi and Oomycetes, Hyphal Tip, Protrusive force, Elastomeric pillar arrays.

INTRODUCTION

An unprecedented number of diseases caused by fungi and oomycetes have in recent times been found responsible for some of the most severe die-offs and extinctions of plants and animals ever witnessed [1]. Invasive growth (i.e. growth through host tissue) of these organisms is a process that requires a protrusive force [2]. This force arises from a complex interplay of hydrostatic pressure in the hyphae and of yielding at the cell apex, which is likely to involve dynamic changes in the cytoskeleton [3]. In this paper we report the first elastomeric-micropillar measurement of protrusive forces generated by individual hyphal tips of *Achlya bisexualis* on a lab-on-a-chip platform. Previously, we detailed the growth of fungi and oomycetes on polydimethylsiloxane (PDMS) devices and determined the suitability of elastomeric micropillars for the measurement of forces exerted by hyphae [4]. Modifications to the platform mean we can now measure protrusive forces at the very tips of individual hyphae.

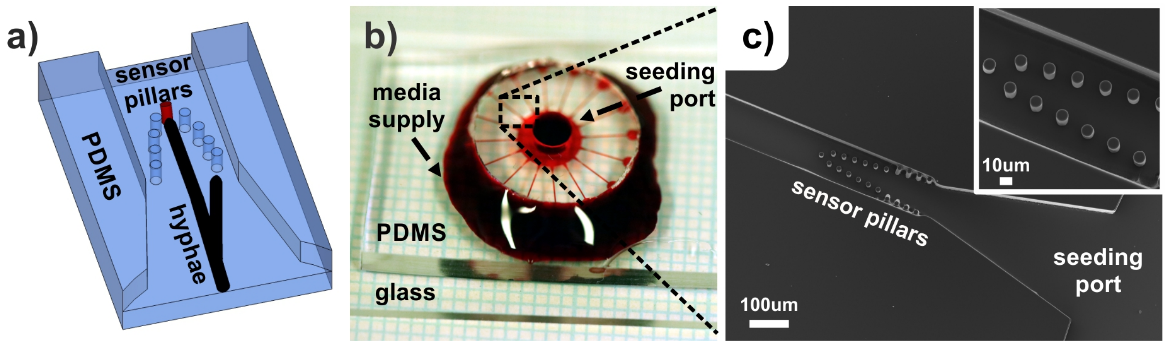

Figure 1: Force measurement devices. a) Oomycete hyphae grow into a polydimethylsiloxane (PDMS) microchannel containing flexible micropillars. b) Photograph showing the PDMS device containing the pillar sensors in 16 channels leading off from a central seeding area. An external ring of liquid around the PDMS lid provides media to the channels. c) Scanning electron micrograph of the pillar setup inside a channel. Inset shows the 10 µm diameter and 30 µm high pillars. Channel walls suspend a PDMS lid 5 µm above the pillars.

EXPERIMENTAL

Tips of hyphae grown on previous large-area arrays evaded pillars and only "squeezing" forces in subapical regions of the hyphae could be measured. To demonstrate single hypha measurements, existing PDMS devices were modified to include offset pillar arrays placed at constrictions within microchannels. Hyphae grow into the channels (Fig. 1) and the tips were guided against the measurement pillars. Devices were fabricated by replica-moulding of a two-layer SU-8 mould and consecutive hydroxypropylmethylcellulose-assisted PDMS double-casting. A PDMS lid with a cored seeding port for inoculation was bonded onto the channels. From this seeding area an array of 16 channels containing pillars (diameter 10 µm, height 30 µm), radiated out with the measurement area about 400 μm from the channel inlets. Pillar deflection by the tip of the hypha was recorded using an external imaging setup, and converted into force magnitude and direction via calibrated mechanical parameters and custom image processing in MATLAB [5].

RESULTS AND DISCUSSION

Upon seeding into the platform, hyphae of *Achlya bisexualis* grow into the surrounding channels towards the measurement area. A video showing hyphal extension into a channel and pillar actuation is available at https://youtu.be/thKkG5-Vx1A. Figure 2 shows an example measurement of the force exerted by a single tip on a PDMS micropillar. The force was observed to increase to a maximum of 7.5 μN during tip contact and its direction could be tracked through different stages of hypha-pillar interaction. The hypha was observed to grow into the pillar and changed its direction of growth as a result of the contact. The bending force after tip deflection reached a maximum of 19 μN, which compares to the squeezing forces measured in our previous work [4].

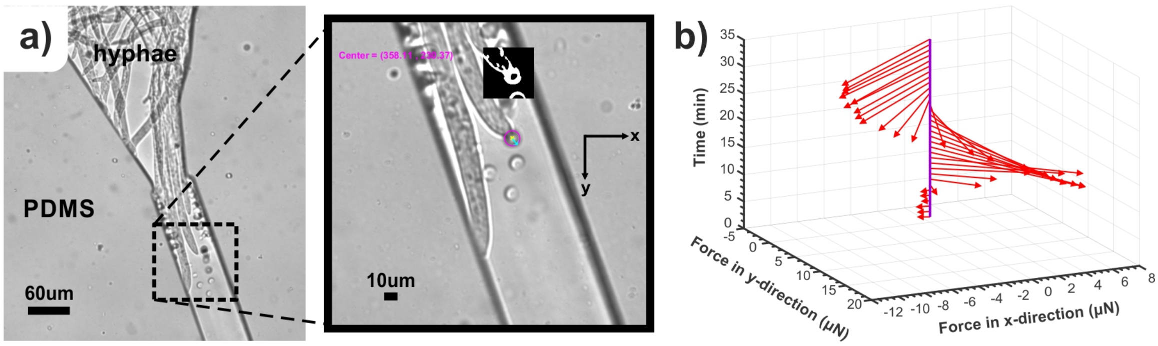

Figure 2: Force sensing on single hypha. a) Micrograph of multiple Achlya hyphae growing into a PDMS pillar array inside a channel constriction. Close-up shows automated tracking of a pillar top during actuation by a single hyphal tip. b) Plot of force exerted on the pillar by the hypha. The force initially increases to 7.5 μN during tip contact and reaches a maximum of 19 μN during bending. Force magnitude and the change in direction upon tip deflection can be observed.

CONCLUSION

This paper demonstrates the first measurement of protrusive force exerted by individual hyphal tips during growth using elastomeric micropillars. The devices presented here will enable the coupling of force measurements with the imaging of any underlying cytoskeletal changes. Thus, we now have the experimental tools to better understand the mechanisms enabling fungi and oomycetes to grow invasively. In the future this understanding may enable us to combat loss of biodiversity caused by these organisms.

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