ELASTOMERIC MICROPILLAR ARRAYS FOR THE STUDY OF PROTRUSIVE FORCES IN HYPHAL INVASION

V. Nock[1*], A. Tayagui[2] and A. Garrill[2]

[1] Biomolecular Interaction Centre, Department of Electrical and Computer Engineering, University of Canterbury, NEW ZEALAND and

[2] School of Biological Sciences, University of Canterbury, NEW ZEALAND

ABSTRACT

Fungi and oomycetes are microorganisms whose pathogenic growth causes significant economic losses and disease. The growth of the hypha of these organisms involves turgor pressure, cell wall yielding and a dynamic cytoskeleton. To study the role of microtubules, actin and turgor in the generation of protrusive force, we have developed a Lab-on-a-Chip platform with integrated force-sensors based on elastomeric micro-pillars. The oomycete *Achlya bisexualis* was cultured on-chip and a maximum total force of 16 μN was recorded for this organism. The platform provides a useful tool to study the molecular mechanisms for generating protrusive force, such as cytoskeletal dynamics.

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

INTRODUCTION

Fungi and oomycetes extend by the process of tip growth [1]. This increases the surface area over which nutrients can be absorbed and enables exploration of the environment. While most fungi and oomycetes are saprophytic, a number grow as pathogenic species on both plants and animals. In this situation they can have significant effects on human affairs through fungal infections and crop losses. One of the key processes in pathogenicity is the ability of hyphae to grow invasively [2]. This is likely to involve an interplay of enzymatic breakdown of host tissue and a protrusive force generated at the tip of the growing hypha. The protrusive force will be influenced by the turgor pressure of the hypha [3] and the yielding capacity of the tip. Development of a platform capable of measuring this protrusive force and determining the factors that underlie it is the main aim of this work.

Measurements of protrusive force have been made using a variety of techniques, including most recently with narrow openings made in polydimethylsiloxane (PDMS) microchannels. These were presented as obstacles to pollen tubes growing on a chip. By coupling with finite element modelling, this method used the measurement of the dilating force exerted normal to the gap wall to deduce the penetration pressure [4]. Previous work by us, which involved the use of PDMS micro-pillar arrays for the characterization of force patterns in nematodes and the influence of the microenvironment on their locomotion parameters [5,6], inspired us to adapt these for use with fungi and oomycetes. The resulting new sensor platform is unique in its simplicity and capability of being able to measure both magnitude and direction of protrusive forces.

EXPERIMENTAL

To demonstrate protrusive force measurement with elastomeric micro-pillars, we have developed a PDMS-based platform for use with the oomycete *Achlya bisexualis*. Figure 1(a) shows a schematic of the measurement principle. PDMS devices were fabricated using replica-molding of a two-layer SU-8 master, as described in detail previously [5]. The design included chamber outlines, measurement pillars (15 μm diameter; 30 μm height) and a 5 μm spacer-layer to enable free movement of the pillar tops. Figure 1(b) shows a photograph of the fabricated chip, illustrating the pillar array and adjacent pillar-free seeding area. Hyphae of the *Achlya* were cultured on peptone-yeast-glucose (PYG) plates (containing [in % w/v] Peptone [0.125], Yeast extract [0.125], Glucose [0.3], and Agar [2]) covered with sterile cellophane paper for about 24 h at 26 ºC [3]. A plug of the culture, approximately 3 mm, was transferred to the PDMS chips, which previously had been filled with PYG broth media (containing [in % w/v]

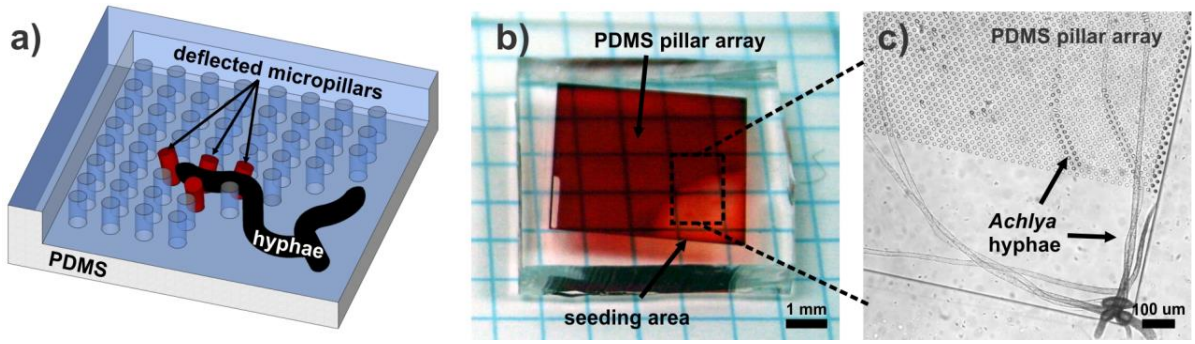

Figure 1: Experimental setup: (a) Schematic of the measurement setup showing fungal hyphae growing into an array of micro-pillars. Pillar deflection is recorded using an imaging setup and converted into force magnitude and direction. (b) Photograph of the fabricated PDMS chip containing an array of sensing pillars and a seeding area (red-colored water used for visualization). (c) Optical micrograph of Achlya hyphae extending from a mycelial plug into the micro-pillar array.

Peptone [0.125]-Yeast extract [0.125] and Glucose [0.3]. Hyphae were left to grow from the seeding area into the pillar arrays for approximately 12 h. The deflection of the pillar tops in contact with the hyphae was recorded using a microscope-based imaging setup (Nikon Eclipse 80i). A custom image-processing algorithm implemented in MATLAB was used in conjunction with pre-calibrated mechanical pillar properties to convert the measured deflection into force values [5].

RESULTS AND DISCUSSION

Pillar-based force-sensing chips are made from hydrophobic PDMS material, while hyphal organisms of interest tend to grow through aqueous environments. Through seeding *Achlya* on the PDMS chips and observing its growth, we have established that it is possible for this organisms to grow on this material. Prior to seeding, PDMS had to be plasma-treated to increase hydrophilicity. Preliminary experiments have shown that both mycelial plugs and zoospores of *Achlya* can be transferred onto the chips using stenciled solid agar medium or cellophane paper. Seeded hyphae have been observed to grow from and through both aqueous and agar-containing media on the chips. Since hyphae of *Achlya* can grow to a width of more than 30 µm, the spacing between the pillars and the pillar dimensions are crucial to successfully measure protrusive forces. Figure 2(a) shows the measurement of forces of a microtubule originating from a mycelial plug of *Achlya* on chip. This demonstrates that, by simply shrinking the pillar dimensions and spacing of an existing pillar array chip designed for use with nematodes [5], we can obtain pillar deflection and resolve forces exerted by growing hyphae. What can also be observed in Fig. 2(a) is that the growth direction of the hyphae is guided by the pillar arrangement. In the future we will use this behavior to selectively guide hyphae to increasingly narrower pillar spacings , which can be used to determine force limits.

One major advantage of using micro-pillar arrays is that, by arranging pillars appropriately and recording the deflection of each pillar, multi-point forces along a hyphae can be recorded. An example of a hyphal force pattern measured using the device is shown in Fig. 2(b). Both force magnitudes and directional components can be extracted from each measurement pillar. Directions plotted in Fig. 2(b) indicate that the hypha shown in Fig 2(a) was undergoing contraction during this particular measurement, a behavior difficult to determine using other force sensing designs. Total force magnitudes up to a maximum of 16 µN were recorded. While these values compare well to measurements for invasive *Camellia japonica* pollen tubes obtained using a micro-gap approach [4] similar to our setup, even higher forces were recorded for an *Achlya* hypha with its tip growing directly against a miniature silicon strain gauge [7]. It is expected that a future device design will allow us to in a similar fashion guide a hyphae tip onto a single pillar, which will enable us to directly compare measurements to this method.

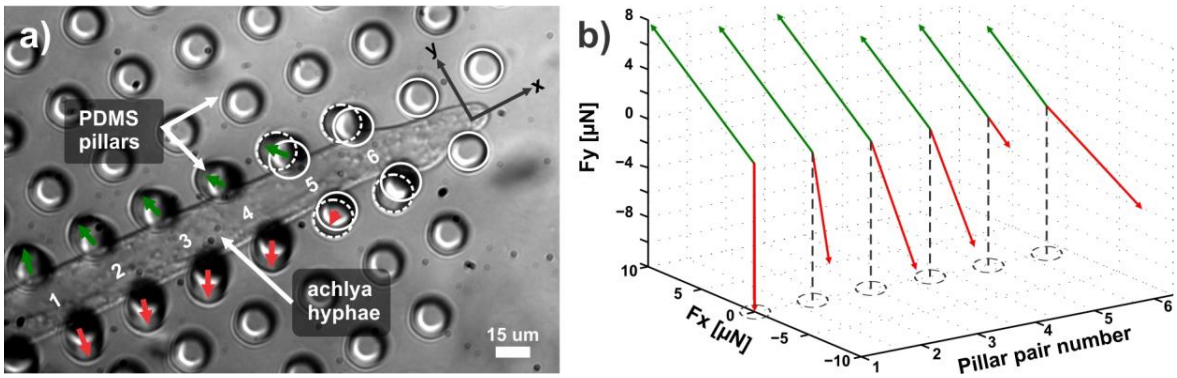

Figure 2: Force sensing on living hyphae. (a) Micrograph of an Achlya hypha growing through an elastomeric micro-pillar array. Full and dashed circles indicate tracking of initial and final positions of pillar tops, respectively. Red and green arrows show the derived force magnitudes and directions from pillar pairs 1 to 6. (b) Plot of force magnitudes and directions along the length of the hyphae as function of pillar pair number. Green arrows correspond to top, red to bottom pillar row.

CONCLUSION

We have demonstrated the use of elastomeric micro-pillar arrays for the study of protrusive forces in hyphal invasion. Devices containing arrays for force measurement were fabricated using a double-layer SU-8 mold and PDMS soft-lithography. Following plasma treatment, growth media was introduced into the devices and *Achlya bisexualis* was seeded as mycelial plug and zoospores. Growth was monitored on-chip and hyphae were observed to grow into micro-pillar arrays. Force measurements were performed by recording pillar deflection and magnitude and force directions were compared to existing methods. A maximum total force of 16 µN was measured. The current platform provides a useful tool to study the molecular mechanisms enabling protrusive force and may help to address the many diseases and infections that occur due to invasive fungal and oomycete growth.

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CONTACT

* volker.nock@canterbury.ac.nz