ARRAYED FORCE PHENOTYPING OF SINGLE-CELLS FOR HIGH-THROUGHPUT QUANITIFICATION OF PHAGOCYTIC FORCES BY HUMAN MACROPHAGES

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ABSTRACT

We describe a high-throughput platform to assay the ability of cells to apply contractile forces that is composed of arrays of uniformly shaped adhesive molecular patterns that deform in response to cell-induced forces. Unlike other methodologies used for such measurements, our platform confines cells to specific shapes designed to focus their traction forces to precise points creating deformations in ultra-soft, planar substrates that can be characterized with a single measurement, allowing for direct comparisons between cells. Here, we employ the platform to measure the forces involved in phagocytosis by human macrophages for the first time. Importantly, we are able to the vary chemical composition of these patterns to mimic various opsonins macrophages encounter in the body.

KEYWORDS: Contractility, Macrophages, Micro-patterning, Phagocytosis

INTRODUCTION

The ability of cells to exert forces is key in the roles of many physiological systems, such as in the digestive system, and in cardiac tissue. This ability is also important at the cellular level where it helps maintain homeostasis, particularly with specialized contractile cells such macrophages which generate forces during phagocytosis to clear cellular debris and engulf pathogens. Due to an organism's dire dependence on cell-generated forces, a variety of disorders can arise directly as a result of faulty force generation. Therefore, there is a need to screen for drugs which regulate force generation, but also to counter-screen potential candidates against non-specific cell targets. Here, for the first time, we use our Force-Phenotyping platform to assay the phagocytic force of human macrophages in response to challenges by various physiologically relevant ligands in high-throughput.

To date, there has been no adequate method of measuring forces applied by macrophages in a controlled way that yields statistically significant results. Jeong *et al.* measured the forces with which macrophages pulled on micropipettes probes[1], and Evans *et al.* determined the contractile forces neutrophils applied when phagocytosing a yeast cell by opposing this force with a suction pressure through an aspirator [2]. Although innovative, each method was severely limited in throughput as micropipettes had to be manually manipulated to assay one cell at a time.

Previously, we presented our Force-Phenotpying platform for assaying forces of single-cells in high-throughput [3]. Briefly, the platform is composed of arrays of uniformly shaped adhesive molecular patterns covalently coupled to a thin-film of soft PDMS, that deform in response to cell-induced forces (Fig. 1A). Custom software then records this deformation in automated way (Fig. 1B). We previously validated our platform by identifying contractile differences within populations of stem cells and cancer cell, and by accurately determining the IC_{50} value for a known myosin inhibitor. Here, we employed the platform to study the phagocytic response of macrophages to various surface ligands.

EXPERIMENTAL

The platform consists of an ultra-soft layer of PDMS patterned with fluorescent adhesive molecules such as extracellular matrix (ECM) or other biomolecules that is supported by a rigid glass substrate. The molecule comprising the patterns is either, itself, fluorescent or is co-patterned with a fluorescent molecule, and is covalently embedded into the soft substrate using a sacrificial technique we developed [4]. Seeded human macrophages derived from the monocytes of healthy blood donors (hMDMs) adhere to the adhesive patterns and attempt to phagocytose the surface, (known as frustrated phagocytosis) and thus de-

form the patterns. Fluorescent microscopy is used to image the resulting patterns and custom-written image analysis software measures the dimensions of the deformed patterns (Fig 1). Following image analysis, finite element method modeling is used to transduce the pattern deformations into force values.

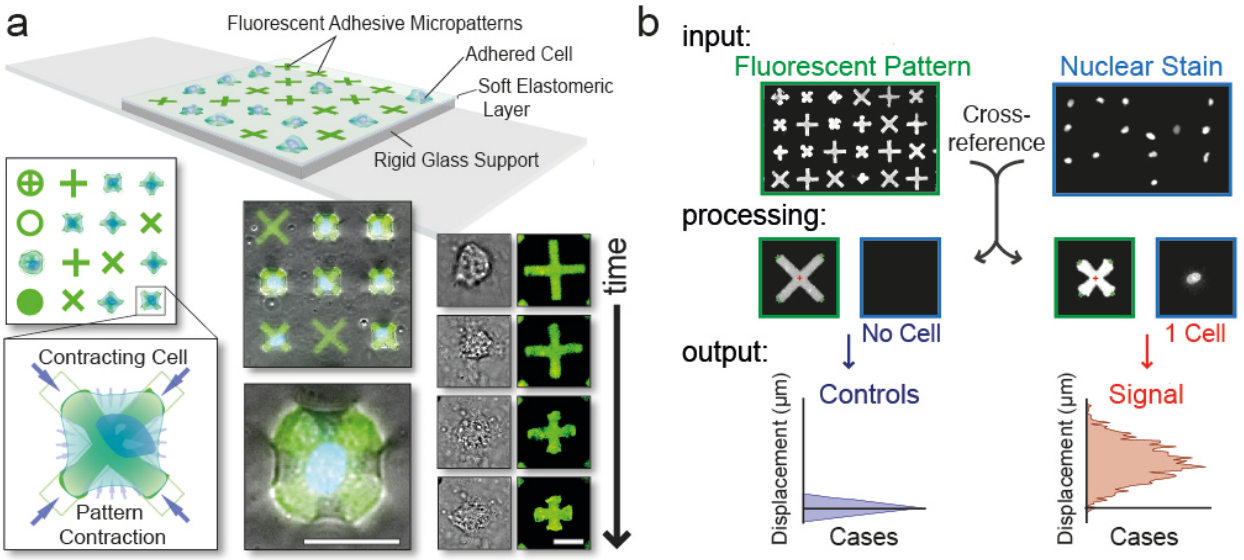

Figure 1: (a) Platform schematic. Cells adhere to adhesive fluorescent molecular patterns on the ultra-soft substrate and apply traction forces causing deformations in the patterns. (b) Data processing. The pattern array and cell nuclei are imaged. Custom software counts cells on each pattern and measures pattern dimensions. C) Plots of pattern deflections with 0 (control) or 1 cell present are generated.

Here, we assessed the phagocytic responses of hMDMs to opsonin density and opsonin type. To test for the effect of opsonin density, we patterned unique human-mouse chimeric IgG antibodies (hIgG) in either a hollow ring formation, a semi-filled ring formation or a filled circrle formation, all with equal diameters. To test the phagocytic response to various opsonins, hIgG, BSA, vitronectin (VN) and fibrinogen (FN) were patterned in 50 micron cross patterns. In each case, HMDMs were seeded and given 6 hrs to initiate and sustain frustrated phagocytosis of these patterns before being imaged.

RESULTS AND DISCUSSION

We used our platform to assay 200 to 800+ macrophages per experiment and did not find the relative density of the ligand to have any significant effect on the forces applied by phagocytosing macrophages (Fig. 2C). We did however find that there is a large differential response to the different ligands, likely representing differential urgency in response to the various scenarios *in vivo,* with the force response being highest against IgG (Fig. 2D). Together with substrate stiffness measurements and COMSOL modeling we calculated the median energy expenditure per cell to be as high as 6.4 pJ on IgG surfaces, and as low as 0.18 pJ on fibrinogen surfaces (Fig. 2E).

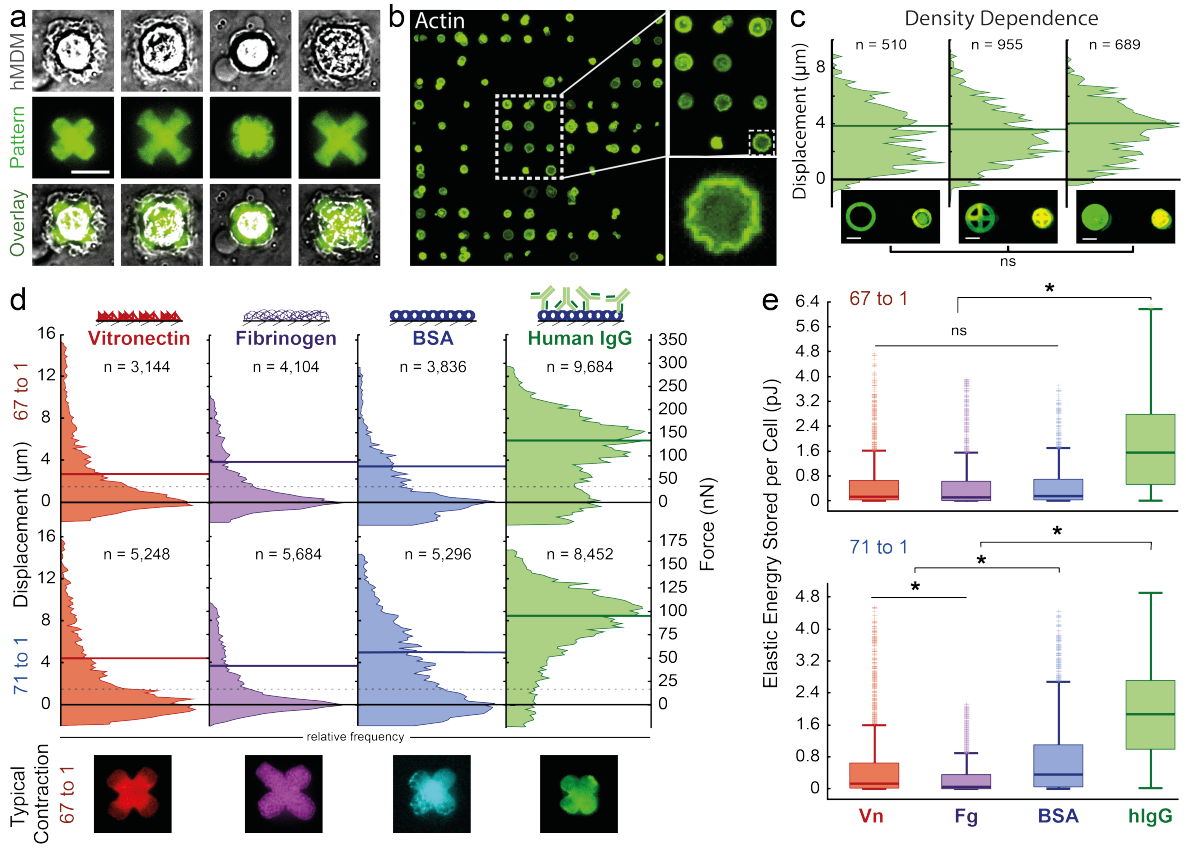

Figure 2: (a) hMDMs on hIgG cross patterns showing a range of phagocytic responses. (b) Actin-stained hMDMs spread over circular patterns in an array. (c) Phagocytic contraction of i) ring, ii) cross and iii) filled hIgG circular patterns.. (d) Opsonin-dependence in phagocytic contraction. Vitronectin, fibrinogen, BSA, and hIgG were patterned in 50 μm cross shapes on a stiffer, 67:1 base:crosslinker (top) and softer 71:1 (bottom) substrate. Left Y-axis represents displacement in μm; Right Y-axis represents applied forces in nN. (e) Elastic strain energies corresponding to the displacements in (d).

CONCLUSION

We demonstrated the unique ability of our platform to directly measure the forces involved in phagocytosis by human macrophages at the single cell level for the first time, achieving high N while performing a direct comparison between the responses to different opsonins in a manner never before done. We expect this method of assaying phagocytic forces to further our understanding of immune response in different physiological scenarios. Importantly, given the necessary biological role of phagocytes, the method will be crucial to the counter-screening step of the drug discovery process for force-mediating drugs.

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