High-throughput single-cell contractility measurements using FLECS Technology and imaging with the ImageXpress Micro System

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OVERVIEW

The ability of mammalian cells to generate mechanical forces – to push, pull, or squeeze – is an intrinsic capability that is used by cells both individually, and together as tissue to perform important physiological functions. In order to better understand the mechanisms behind cellular force generation, to identify new drug targets or candidates, and to validate existing candidates thought to affect force generation, quantitative screening approaches to functionally evaluate cellular force must be employed. Importantly, since the functional cellular output drives many disease processes, measuring force generation itself, and not a non-specific molecular surrogate such as calcium flux, is critical for maximizing the success of drug discovery and development.

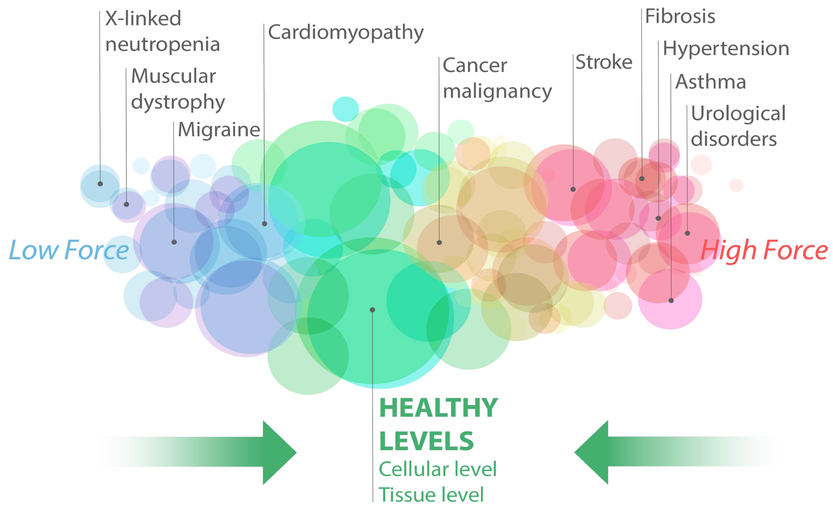


Figure 1: Role of mechanical cell force generation in various diseases. High-throughput assays are needed to identify new compounds that can correct dysregulated force generation.

This study reports our development of an automated functional single-cell functional contractility assay called ***FLECS*** (Forcyte Biotechnologies) for assessing both the tonic contractility of cells and the ability of test compound to modulate the forces applied by the cells. To help validate this technology, primary human airway smooth muscle cells obtained from 6 asthmatic and 6 healthy donors were compared in terms of contractile tone, responsiveness to agonist bradykinin, and finally bronchodilator, formoterol. Assessment of single-cell contractility for all 12 cell lines was performed using a single FLECS 384-wellplate and imaged on the ImageXpress Micro (IXM) system (Molecular Devices) which is capable of capturing an entire 384 well at 4x magnification and thereby enables measurements of 100,000s of cells per experiment.

EXPERIMENTAL

FLECS 384-wellplates (Forcyte Biotechnologies) containing uniform arrays of fluorescently labeled extracellular matrix (ECM) micropatterns covalently embedded into soft base films are seeded with cells to be evaluated (Fig. 2). The cells are allowed to adhere to these patterns and various treatments can be applied. Cell contraction generates mechanical forces onto the underlying elastic film and produces well-calibrated displacements at the pattern's peripheries. The patterns are imaged using a 4X objective on the IXM and the displacements are calculated using an in-house-developed computer algorithm (Fig. 2). Wide-field imaging and rapid laser-based autofocus enabled by the IXM allows a full 384-well plate to be read in <10 mins.

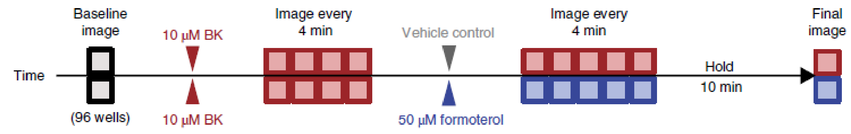


Figure 3: Graphical depiction of the experimental procedure following cell seeding. A baseline image is acquired before cells are given bradykinin to induce contraction followed by treatment with formoterol or control. The evolution of their contractile responses is monitored every 4 mins using the IXM.

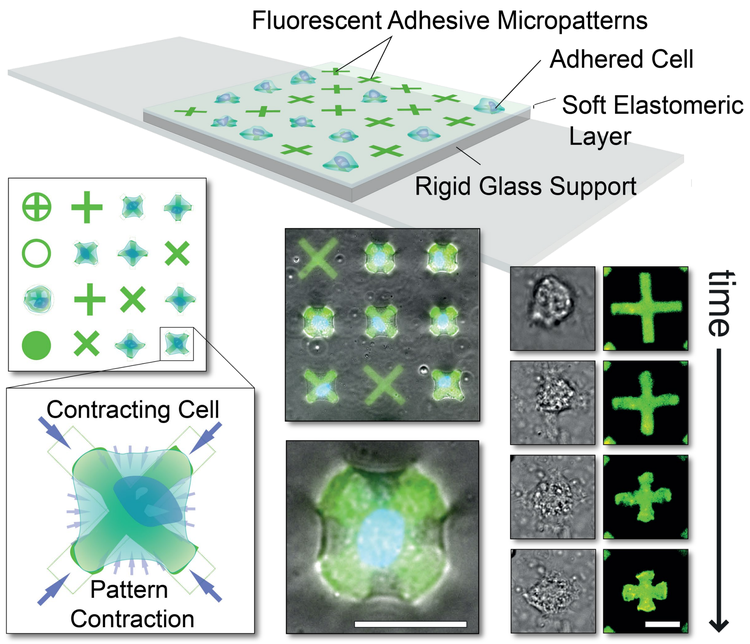
The present study evaluated 12 lines of airway smooth muscle cells derived from 12 unique donors, of which 6 were asthmatic and 6 were non-asthmatic, and each pair is age-, gender-, and race- matched. Basal tone, contractile responsiveness to bradykinin, sensitivity to the bronchodilator formoterol and the kinetics of these responses were measured following the procedure shown below.

MATERIALS

- F LECS assay kit
  - 384-well FLECS-plate (Forcyte Biotechnologies)
  - Hoechst 33342 nuclear stains (ThermoFisher)
- Primary Human Airway Smooth Muscle Cells
  - 6 Healthy donors
  - 6 Asthmatic donors
- Chemicals
  - Bradykinin Acetate (Millipore Sigma)
  - Formoterol (Millipore Sigma)
- Molecular Devices HCS imaging system
  - ImageXpress® Micro 4 High-Content Imaging System

FLECS (Fluorescent Elastomeric Contractible Surfaces) FUNCTIONAL CONTRACTILIY ASSAY PRINCIPLE

Platform Overview:



Automated Data Processing:

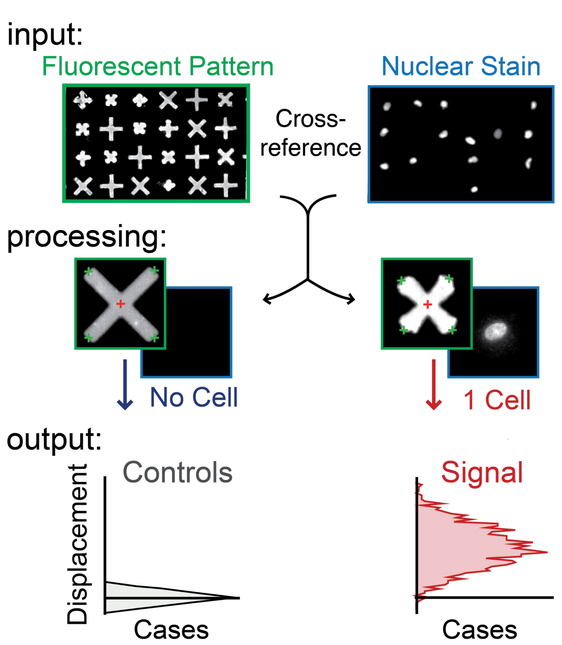
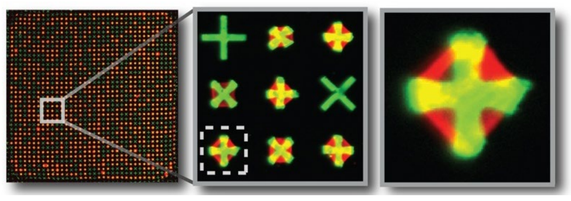


Figure 2. Left: Schematic of the FLECS assay architecture. Right: Image analysis algorithm locate cells and to perform quantitative contractility measurements.

384 Well-plate Format:



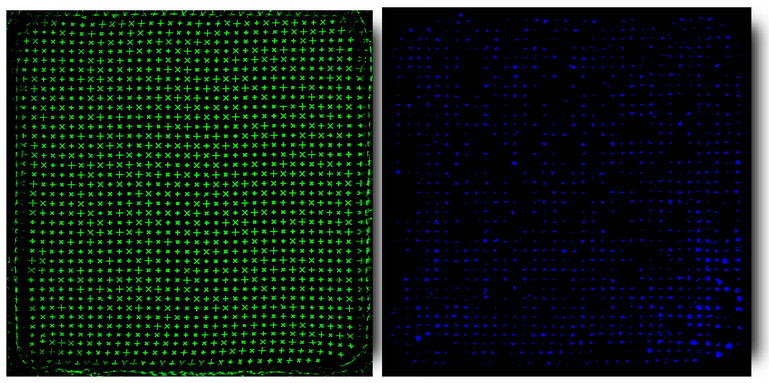


Figure 4. Images of single full well of a 384-well FLECS-plate acquired with the IXM. Fluorescent micro-patterns (green) are adhered to and squeezed by single-cells (actin stained in red), resulting in regular and quantifiable dimensional changes, relative to empty micropatterns, corresponding to the magnitude of the generated force.

Arrays of fluorescently labeled extracellular matrix (ECM) micropatterns of defined shapes and sizes are covalently embedded into soft substrates with controlled elasticity. Cells are allowed to adhere to these patterns and various treatments can be applied. Nuclear stain Hoechst 33342 is used to identify single cells bound to micropatterns. Cell contraction generates mechanical forces onto the underlying elastic film and produces well-calibrated displacements at the pattern's peripheries. A single field-of-view captured an entire well and the standard TIF image format allowed direct import into Forcyte's proprietary analysis software for sophisticated analysis of the displacement results.

RESULTS: DYNAMIC CONTRACTILITY OF PATIENT-DERIVED AIRWAY SMOOTH MUSCLE SINGLE-CELLS

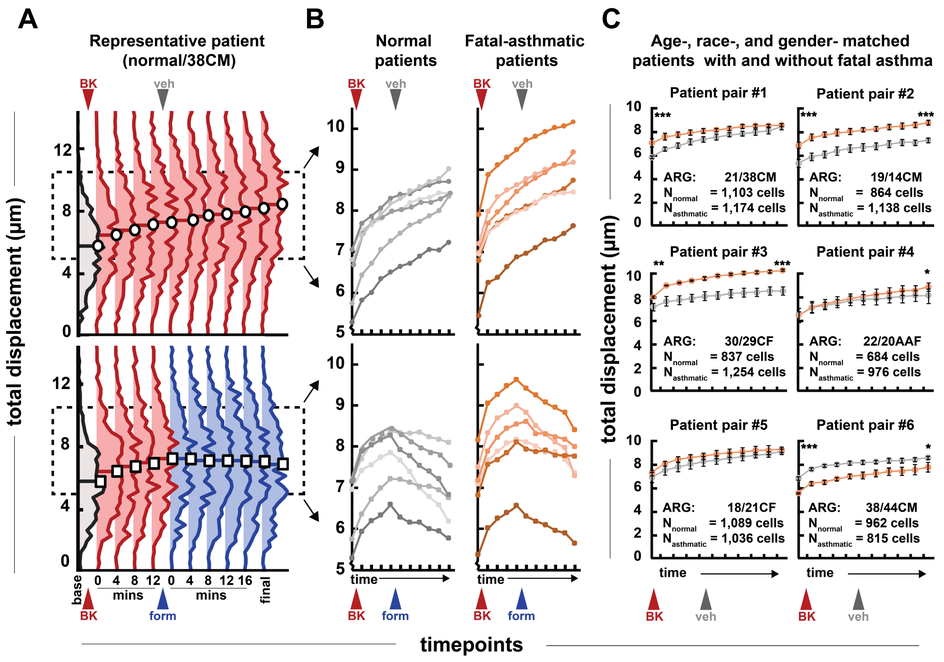


Figure 5. (A) Population-level histograms of single-cell contractility measurements evolving with time after treatment with pro-contractile agonist bradykinin, and later, bronchodilator formoterol or vehicle control (one pat). (B) Median values derived from population-wide histograms for all 12 donors Pair-wise comparison of age-, gender-, and race-matched donor cells.

Contractility for each of the ~31,000 cells (from the 12 cell lines) was tracked over time and cells exhibiting increased contraction at 16 min relative to baseline were selected for further analysis (~72% of cells on average). We tracked the evolving contractile distributions for all selected cells (**Fig. 5**) and found the distributions showed robust upward shifts following treatment with bradykinin that were un-attenuated following addition of vehicle, but were halted or reversed following treatment with formoterol . The median contractility value of each cell population was tracked over the course of pharmacological treatment (**Fig. 5b**) and it was observed that for 5 out of 6 pairs of age-, race-, and gender- matched patients with or without fatal asthma, the asthmatic patients' cells exhibited either greater tone, greater BK-induced contraction, or both 4 of these cases, the differences were statistically significant (**Fig 5c**).

CONCLUSIONS

Using the FLECS well-plate assays for single-cell contractility, functional force generation can be rapidly evaluated for primary cells or cell lines. Imaging with the ImageXpress Micro system enables rapid acquisition of whole 384-wells at a time with sufficient spatial resolution to observe minute differences in cellular contractility. Together, the FLECS well-plate products and the ImageXpress Micro system provide a means to functionally screen large drug libraries for effects on cellular contractility known to be involved in disease processes. This study is part of a larger study previously published [1].

[1] Pushkarsky, I. et al. Elastomeric sensor surfaces for high-throughput single-cell force cytometry. Nature Biomedical Engineering 2, 124–137 (2018).

We would like to recognize Molecular Devices for their collaboration on this project.