MICROFLUIDIC INVESTIGATION OF CELLULAR MECHANICAL DYSFUNCTION IN CAMPOMELIC DYSPLASIA

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ABSTRACT
Abnormal cellular mechanics are often indicative of pathological states. In the analysis of disease it is useful to have methods to quickly and reproducibly test cellular mechanical properties. Microfluidic tools have been used to probe cellular elasticity [1-3]; however, none have tried to differentiate between cytoskeletal and nuclear mechanics of adherent cells. Here we report the first microfluidic platform for the elucidation of cytoskeletal and nuclear deformability of primary human fibroblasts. Moreover, we perform the first microfluidic investigation of Campomelic Dysplasia (CD), a severe disorder associated with abnormal skeletal development. We also provide novel evidence implicating cellular mechanical dysfunction in CD. Finally, we report a semi-automated method for data analysis, characterize cellular deformation through multiple constriction sizes, and investigate the effects of cytoskeletal disruptive compounds on deformation.

KEYWORDS: Channel microfluidics, cell mechanics, Campomelic Dysplasia, fibroblast, biophysical cytometer

INTRODUCTION
Disease states are often associated with cellular mechanic defects. Multiple experimental systems have been developed for the evaluation of cellular mechanical properties including bulk membrane filtration, micropipette aspiration, and atomic force microscopy. Ultimately these methods are challenged by a combination of a lack of single cell data, challenges in performing the highly specialized technique, or low throughput. Microfluidic devices have recently been used to evaluate changes in mechanical properties, typically by observing cellular deformation through constrictions, with the majority of these systems testing suspension cells of the hematopoietic system.

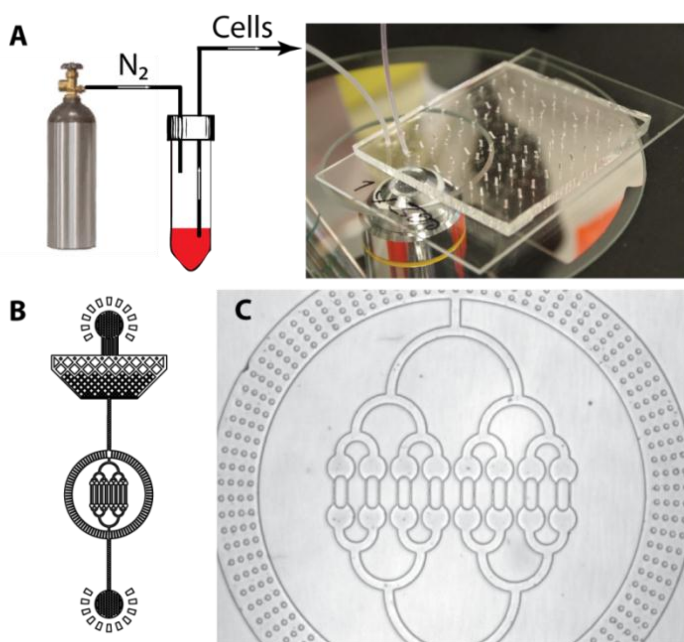

Figure 1: Device operation. (A) Schematic and photograph of set-up. Cells are flowed through the device by pressure driven flow using a 50 mL screw cap tube as a pressure vessel. Each 2" x 3" substrate contains 40 individual devices. (B) The device inlet is connected to a filter and the deformation area. A bypass balances flows through the device during operation. (C) Brightfield microscopic image of the deformation area.

In this work we examined the implementation of a microfluidic biophysical cytometer for the mechanical analysis of wild-type and diseased primary skin fibroblasts derived from healthy patients and those afflicted with the rare congenital disorder Campomelic Dysplasia (CD). The disease is characterized by abnormal development of the skeletal and reproductive systems. Genetically, it is associated with a mutation in the mechanically sensitive transcription factor Sex-Determining Region Y Box 9 (SOX9). With this in mind we hypothesized that cells derived from these patients would present a mechanically dysfunctional phenotype.

We report here the first microfluidic platform for the elucidation of cytoskeletal and nuclear deformability of primary human fibroblasts. Moreover, we perform the first mechanical investigation of CD and implicate mechanical dysfunction as a potential contributor to the disease phenotype. Finally, we report a semi-automated method for data analysis, characterize cellular deformation through multiple constriction sizes, and investigate the effects of cytoskeletal disruptive compounds on deformation.

EXPERIMENTAL
Cell culture, treatment, and preparation
Primary human fibroblasts from healthy (0969) and Campomelic Dysplasia affected (CD04329) donors were cultured according to methods described by the Progeria Research Foundation. Prior to each trial, cells were serum starved at 0.5% fetal bovine serum (FBS) for 18 hours. For certain conditions cells were treated with cytoskeletal disruptive drugs

for one hour. Specifically, cells were exposed to either cytochalasin D (Cyt D, 20 µM in deionized water), colchicine (COL, 4 µM in DMSO), or blebbistatin (BLEBB, 20 µM in DMSO). The cells were diluted to 500,000 cells/mL in fresh media supplemented with 0.02% Pluronics F68 to 500,000 cells/ ml for use in the biophysical cytometer.

Biophysical cytometer fabrication, design and operation

A PDMS device comprising 16 parallel channels with 100 µm long constrictions 3, 5, 7, 9 µm wide was designed and fabricated according to standard protocols (Fig. 1). Cell suspensions were aliquoted to 50 mL tubes with modified caps for use as pressure vessels. Cells were flowed through the device at 5 and 15 psi. Experiments were recorded by microscopy with a high speed camera (200 fps). The amount of time (τ) required for single cells to move through the length of the constriction was analyzed using a semi-automated Python script developed in-house. More than 100 single-cell data points were collected for each condition evaluated.

RESULTS AND DISCUSSION

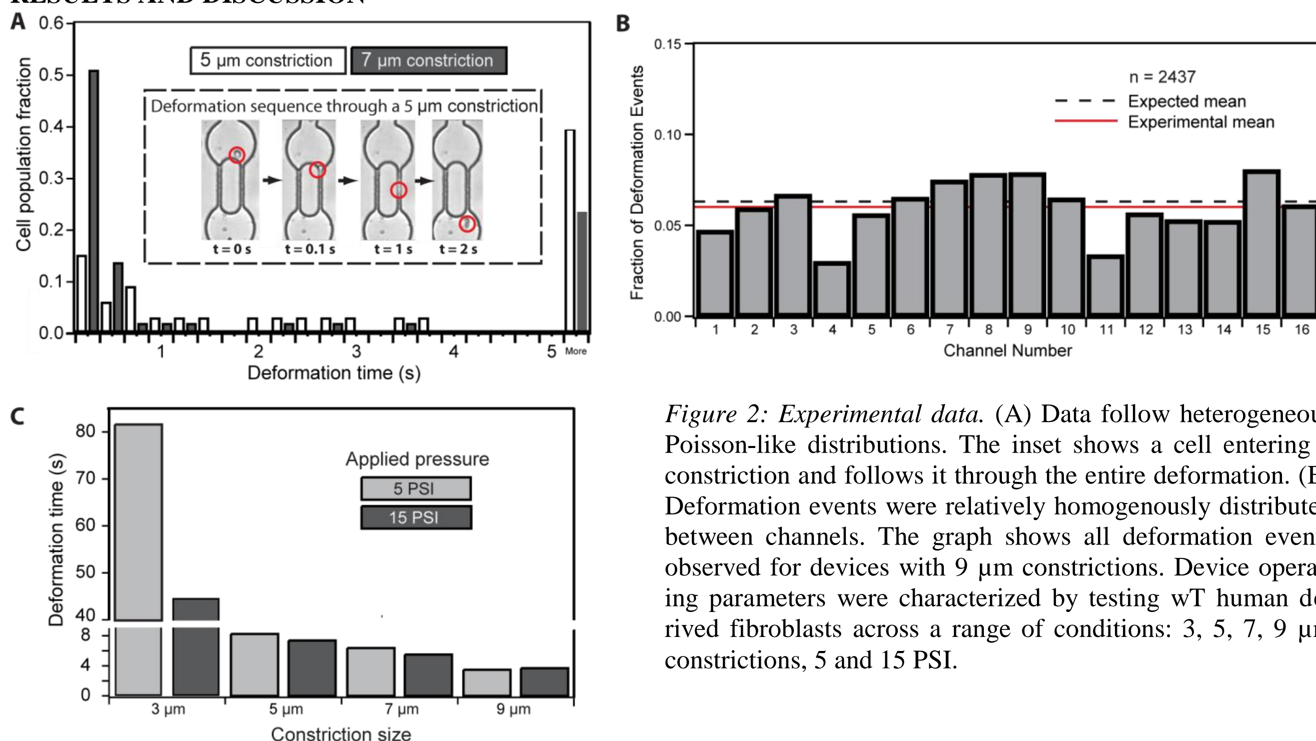

Figure 2: Experimental data. (A) Data follow heterogeneous Poisson-like distributions. The inset shows a cell entering a constriction and follows it through the entire deformation. (B) Deformation events were relatively homogenously distributed between channels. The graph shows all deformation events observed for devices with 9 µm constrictions. Device operating parameters were characterized by testing wT human derived fibroblasts across a range of conditions: 3, 5, 7, 9 µm constrictions, 5 and 15 PSI.

Initial screening experiments with healthy or wild-type (wT) human fibroblasts demonstrated significant heterogeneity in transit times (τ) that could be approximated by Poisson distributions (Fig. 2A). The distribution of deformation events (n = 2437) for all experiments on devices with 9 µm constrictions demonstrated channel independence with an expected fraction of events of 0.0625 per channel and an experimentally observed mean fraction of 0.0595 per channel (Fig. 2B). Varying constriction size and applied pressure shifts distributions as would be expected; increasing pressure and constriction size decreases τ from 82 s (3 µm, 5 psi) to 3.3 s (9 µm, 15 psi) (Fig. 2B). Based on this data we selected constrictions of 5 and 7 µm for further experimentation as 9 µm did not demonstrate differences between the applied pressures of 5 and 15 psi, while τ for 3 µm constrictions were not amenable for high-throughput operation.

We then tested the effects of cytoskeletal disruptive agents on τ. Specifically, cells were exposed to either cytochalasin D (Cyt D), an inhibitor of actin filament polymerization, colchicine (COL), an inhibitor of microtubule polymerization, or blebbistatin (BLEBB), a myosin II inhibitor. For all treatments on wT fibroblasts, τ decreased for 7 µm constrictions (Fig. 3A), in agreement with previous results [2], For 5 µm constrictions this phenomenon was not conserved and deformation did not decrease with the addition of cytoskeletal inhibitors (Fig. 3B). We hypothesize that this effect is due to nuclear mechanics, as the nucleus is the most rigid component of the cell (~5 µm in diameter). Building on these findings, we used the device to probe the mechanical properties of fibroblasts derived from CD patients. Fig. 3C demonstrates that these cells respond similarly to cytoskeletal disruptive agents (increasing in deformability) as those derived from wT patients. However, when flowed through 5 µm restrictions, CD fibroblasts are sensitive to actin inhibitors (Cyt D, BLEBB) but not microtubule inhibitors (COL). This is the first evidence that mechanical anomalies may contribute to CD.

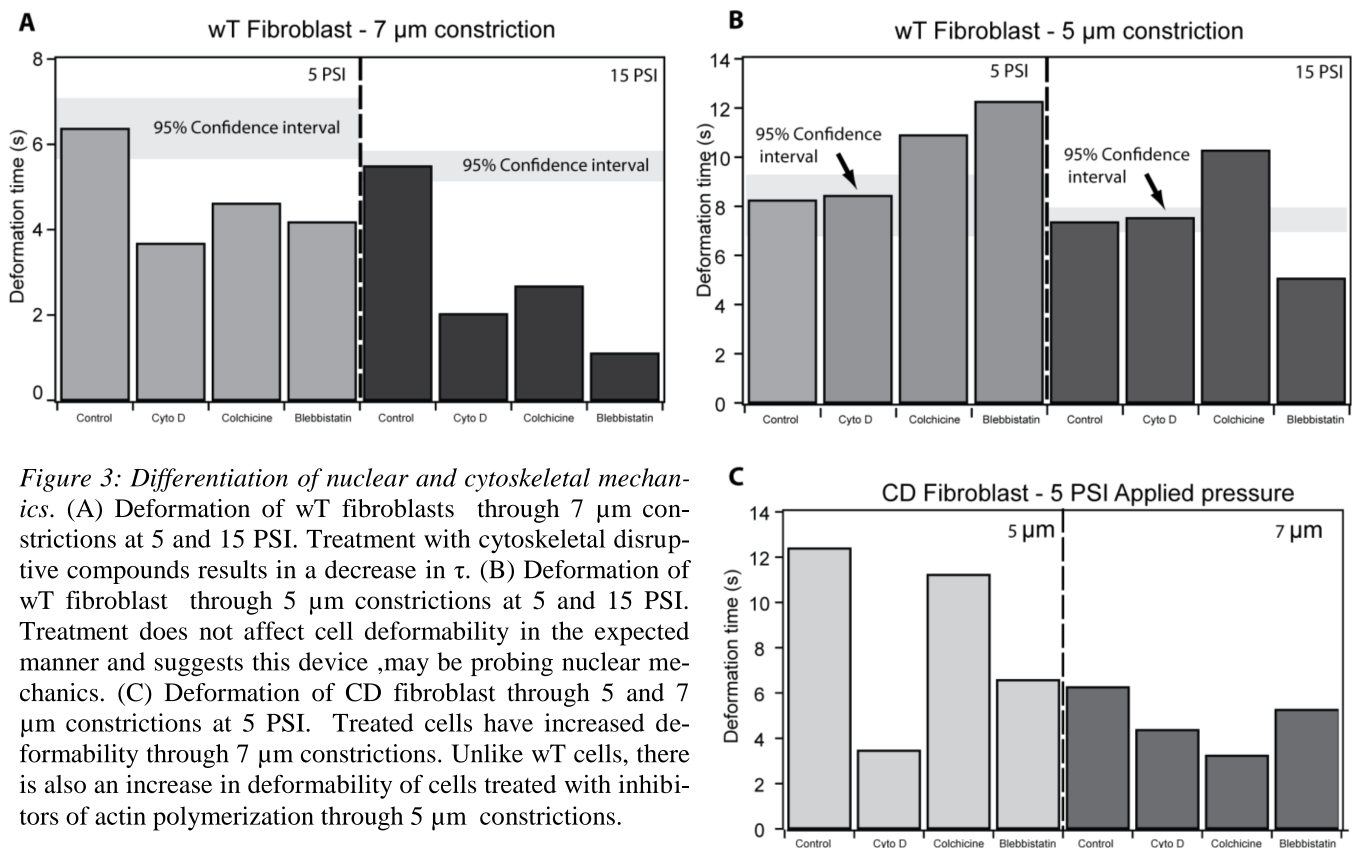

Figure 3: Differentiation of nuclear and cytoskeletal mechanics. (A) Deformation of wT fibroblasts through 7 μm constrictions at 5 and 15 PSI. Treatment with cytoskeletal disruptive compounds results in a decrease in τ. (B) Deformation of wT fibroblast through 5 μm constrictions at 5 and 15 PSI. Treatment does not affect cell deformability in the expected manner and suggests this device ,may be probing nuclear mechanics. (C) Deformation of CD fibroblast through 5 and 7 μm constrictions at 5 PSI. Treated cells have increased deformability through 7 μm constrictions. Unlike wT cells, there is also an increase in deformability of cells treated with inhibitors of actin polymerization through 5 μm constrictions.

CONCLUSION

Microfluidics is proving to be a robust and useful tool for the mechanical characterization of cells. This work demonstrates a biophysical cytometer with the ability to elucidate differences between cytoskeletal and nuclear deformability. We also report the first implication of cellular mechanical defects in Campomelic Dysplasia.

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