A MICROFLUIDIC PLATFORM FOR APPLYING LOCALIZED AND DYNAMICALLY-CONTROLLED COMPRESSION ON CANCER CELLS

Sevgi Onal, Maan M. Alkaisi, and Volker Nock*

MacDiarmid Institute for Advanced Materials and Nanotechnology, Electrical and Computer Engineering, University of Canterbury, Christchurch, NEW ZEALAND

ABSTRACT

In this work we report a microfluidic cell-culture platform with an integrated, actively-modulated actuator for the application of compressive forces on cancer cells. We show fabrication of the platform, mechanical characterization of the actuator and observed mechanotactic behavior of a monolayer of SKOV-3 ovarian cancer cells under static conditions. We further show compression and lysing of the cells, demonstrating suitability for mechanical stimulation with various pressures to study compressive forces in cancer microenvironments.

KEYWORDS: Microfluidics, Micro-piston, Localized and controlled compression, Cancer cells

INTRODUCTION

Biomechanical forces regulate tumor microenvironment by solid stress, matrix mechanics, interstitial pressure and flow [1]. While recent studies have shown that tumorigenic and metastatic events can be induced by mechanical forces transduced by the surrounding microenvironment, the nature and level of forces have yet to be quantified [2]. Force application systems capable of inducing compressive stress on living cells exist in literature [3,4], but none have yet been used to apply compression on cancer models in a localized, flexible and controlled manner. Our system achieves the latter by use of a polydimethylsiloxane (PDMS) micro-piston actuated via a membrane. As such, it constitutes a new biomechanical tool to investigate responses of cancerous and healthy cells to applied stress with regards to changes in cell morphology, viability and mechanobiological protein profile.

EXPERIMENTAL

To demonstrate localized and dynamically-controlled compression of cancer cells we fabricated a microfluidic platform with flexible actuators composed of PDMS. Dry-film lamination on silicon wafers and photolithography were used to fabricate masters [5]. Micro-pistons, monolithically-integrated onto a PDMS membrane, were obtained by spin-coating PDMS onto the resist masters. Similarly, channels and the control layer were fabricated by exclusion- and replica-molding of PDMS on 300 and 200 µm thick resist masters, respectively. Devices were assembled by plasma-bonding the membrane with piston to the control layer and bottom channels (Fig. 1(a)). The micro-piston was actuated by pressure controller with pressure sensors (Elveflow, see Fig. 1(b)).

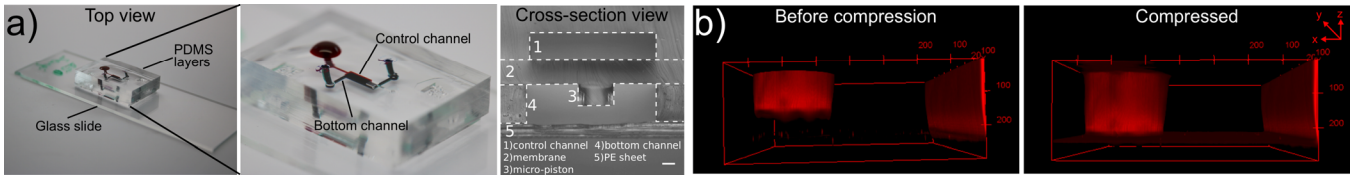

Figure 1: (a) Top view and cross-section view of the micro-piston device (Scale bar 100 µm). (b) Confocal images illustrating the concept of applying compression with an integrated PDMS micro-piston in a microfluidic channel. PDMS was stained with DiD (lipophilic carbocyanine DiIC18(5) solid) for visualization.

RESULTS AND DISCUSSION

Vertical displacement measurements via optical profilometry (Profilm3D) were used to quantify the deflection of 100, 215 and 345 µm thick membranes (Fig. 2(a)). Characterization of the 215 µm thick membrane deflection was followed by piston actuation to demonstrate position recovery regardless of pressure loading profile (see Fig. 2(b&c)). Cancer cells (SKOV-3) showed a directional movement and growth towards the region under micro-pistons, proposing that cells are mechanotactic to the suspended, static micro-pistons (see Fig. 3(a)). Cell viability was tested with 4 µM Ethidium Homodimer (EthD-1) and found to be 98.1% in channels and 99.4%

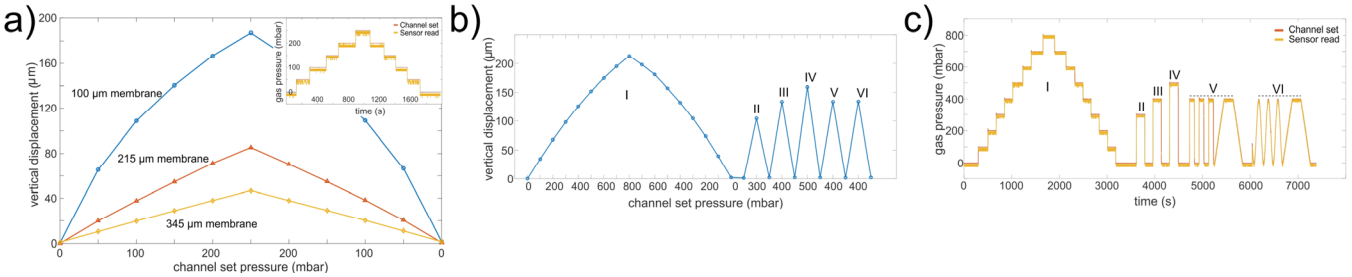

Figure 2: (a) Vertical deflection of membranes of different thicknesses as function of applied pressure. Inset: Corresponding pressure profile. (b) Micro-piston actuation with higher pressures and different loading profiles (I-VI) for the 215 µm membrane. (c) Pressure loading profiles I-VI used for (b).

under the static micro-pistons (9 devices). Finally, the system was tested for dynamically-applied compression on cell monolayers by selectively lysing the cells under pistons and characterizing cell viability with EthD-1 epi-fluorescence (see Fig. 3(b & c)). A video of this process can be found at https://youtu.be/WKRZbPKR-Lw.

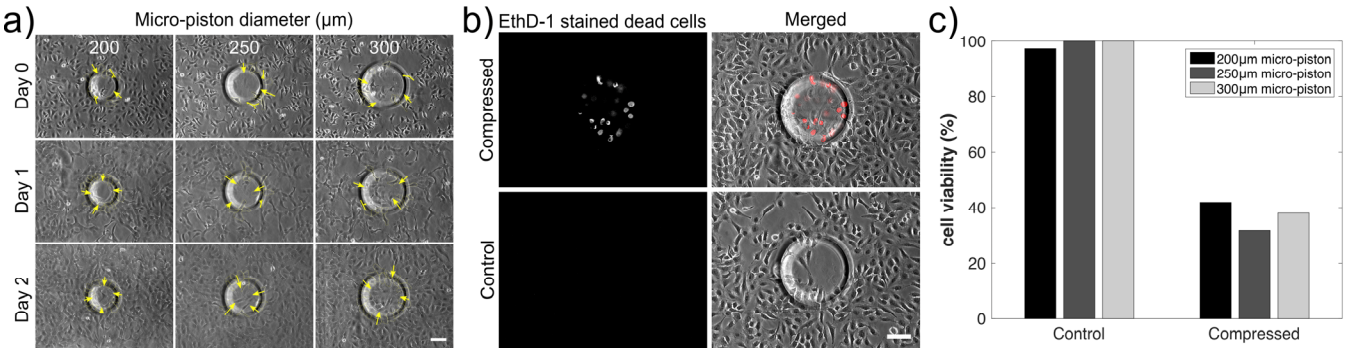

Figure 3: (a) Representative images for SKOV-3 cancer cells occupying the area under micro-pistons. Cells aligned across the periphery of and grew under the pistons (Scale 100 µm). Arrows illustrate the main direction of cell elongation, indicating mechanotactic response to the suspended pistons. (b) After mechanical lysing using the pistons, dead cells (red) were imaged with EthD-1 epi-fluorescence and merged with phase contrast images (Scale 100 µm). (c) Cell viability decreased by on average 62% under pistons (1 h compression, 640 mbar) compared to non-compressed (control) devices.

CONCLUSION

Overall, our system provides a novel mechanical tool to elucidate the role of compressive forces in cancer microenvironments, as well as for studies of cell-cell, cell-microenvironment and cell-drug interactions.

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CONTACT

* V. Nock; phone: +64-3369-4303; volker.nock@canterbury.ac.nz