Sequential cyclic compression profiles applied on cancer cells in a flexible microdevice

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While some cells experience permanent plastic deformation after a repetitive mechanical tensile loading and unloading, the impact of such repetitive compression on plastic deformation of cells remains unknown [1,2]. As such, the ability to apply cyclic compression is crucial for any experimental setup aimed at the study of mechanical compression taking place in tissue [3,4].

In this paper we present the use of a microfluidic platform to apply cyclic cell compression, mimicking biologically-relevant compression profiles occurring in tissue microenvironments *in vitro*. We demonstrate applicability of the platform by using it for the chronic exposure of ovarian cancer cells to compressive stress. Fabrication and characterization of the multilayer microfluidic platform, the observation of directional growth of cells and mechanical cell lysis as an end point assay under static state and highly pressurized state within this platform were demonstrated previously [4,5]. In the current work we extended the use of the platform to the application of cyclic compressions at and beyond physiological pressure values in a sequential fashion. Based on the experimental data obtained from optical profilometer measurements of micro-piston actuations (Fig. 1(a-b)), COMSOL simulations were performed to predict the piston contact pressure (Fig. 1(c)) and pistons used to perform cyclic cell compression. Details of the sequential cyclic compression process at mild pressures, up to cell lysis at severe pressures, are shown in video 1, together with cell viability. Controlled micro-scale mechanical cell compression up to lysis, as demonstrated, will provide more accurate replication of cell-physiological mechanisms for cell biology over bulk mechanical, chemical or electrical lysis.

We further show the application of sequential cyclic compression to record the dynamic changes of GFP-tagged live cell actin compressed at various applied pressures (see video 2). For these experiments, SKOV-3 ovarian cancer cells were transduced on-chip with CellLight Actin-GFP, BacMam 2.0 baculovirus (Invitrogen) to study the dynamic biomechanical processes under compression (Fig. 2(a-c)). The actin cytoskeleton profile was quantified for the cancer cell response at the compressed and at rest stages during the compression process (Fig. 2(d)). The compressed cell actin-GFP signal decreased under the piston with increasing applied pressure. Applicability of the cyclic compression on the platform is further demonstrated by recording the actin and nuclei deformation as an end point assay in cancer cells fixed at zero time or after 24h-recovery following 1 hour-long cyclic compressions. To this end, we show progression of the compressed cells when live culture was maintained for 24h-recovery after compression. No significant change was observed between the control and compressed cells at 15.6 kPa for areal and axial cell nuclei deformation, while compressed cells had larger nuclei than 24h-recovered compressed cells (Fig. 3). Circularity of the cell nuclei, on the other hand, was reduced in the compressed cells compared to the control (non-compressed) cells at zero time after compression, while not significant in 24h-recovered compressed group compared to its control (Fig. 3(c)). Circularity of nuclei was also less for the compressed cells compared to 24h-recovered compressed cells. Differences between the compressed cells fixed at zero time or after 24h-recovery suggest that cells show deformations at the time of the compression, a proposed mechanism the cells use to prevent mechanical damage [1], while deformations were not permanent morphologically and can be recovered at mild pressures such as 15.6 kPa.

As demonstrated, our platform can control for strength and duration of cyclic compression, while enabling the observation of morphological and cytoskeletal changes in cells, thus providing a powerful new tool for the study of mechano-biological processes in cancer and cell biology.

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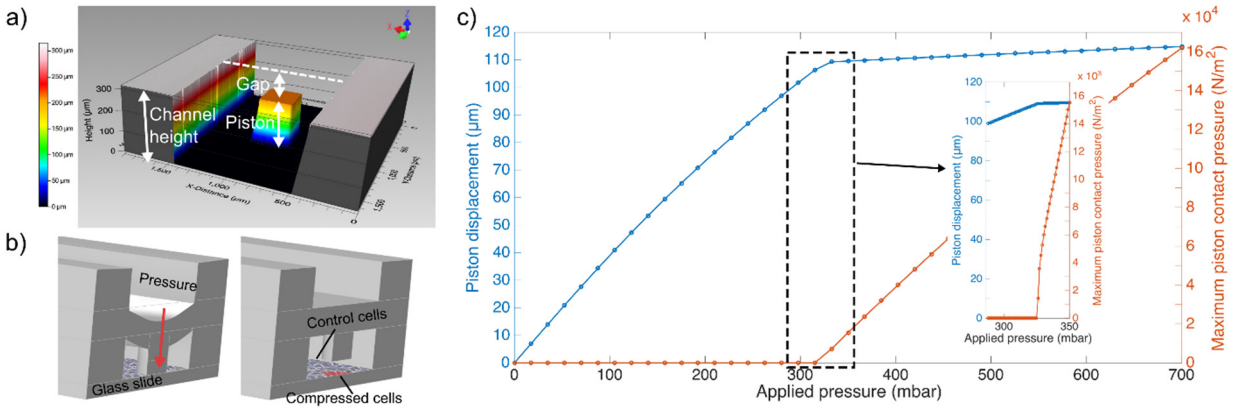

Figure 1. (a) 3D optical profilometer image of the micro-piston device showing the compartment including total height of the channel where the micro-piston is suspended, height of the micro-piston and the gap between the piston and surface of the channel. (b) Compression on cells is illustrated by the membrane deflection and micro-piston brought onto the cells by the pressure applied through the control channel and retracted back after compression. (c) Plot of simulated vertical separation of the micro-piston top and the bottom glass substrate, and maximum contact pressure under the micro-piston as a function of applied gas pressure (boundary load).

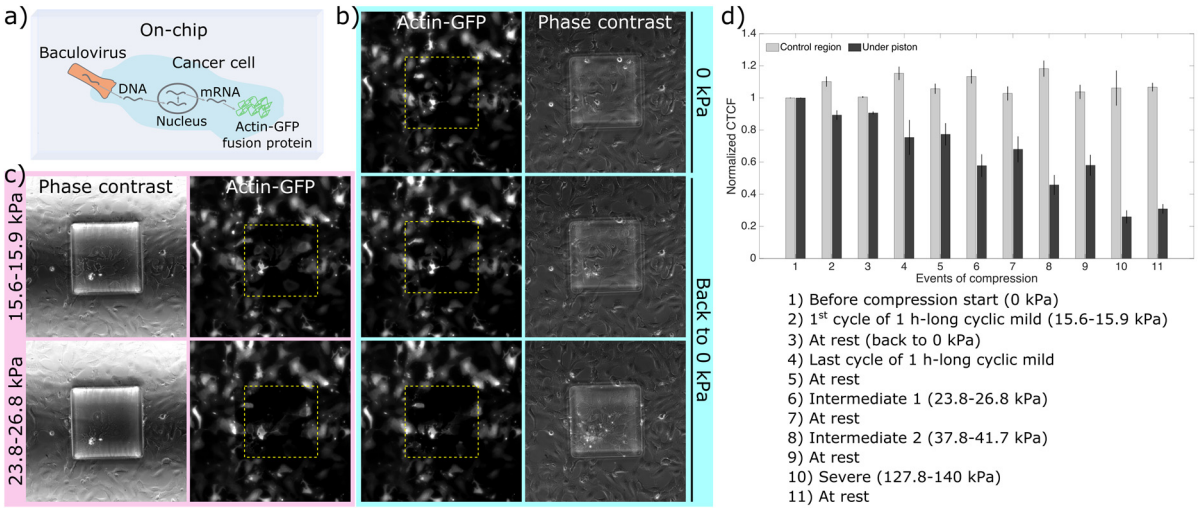

Figure 2. Representative fluorescent microscopy images for live imaging of GFP-tagged actin cytoskeleton of cells. (a) Schematic of the on-chip BacMam transduction into cancer cell for the expression of GFP-actin fusion protein. (b) Phase contrast (right) and fluorescent (left) images of the GFP-tagged actin expressing SKOV-3 ovarian cancer cells at static state (0 kPa) or retracted back to static state (0 kPa) after compression at the given pressure range. (c) Phase contrast (left) and fluorescent (right) images showing the change in GFP-tagged actin of the cells at the compressed state by the applied pressure. The pressure was applied sequentially in the ascending order of the given ranges. (d) Corrected total cell fluorescence (CTFC) calculated from images of GFP-tagged actin cytoskeleton of cells during sequential cyclic compression at the indicated pressures.

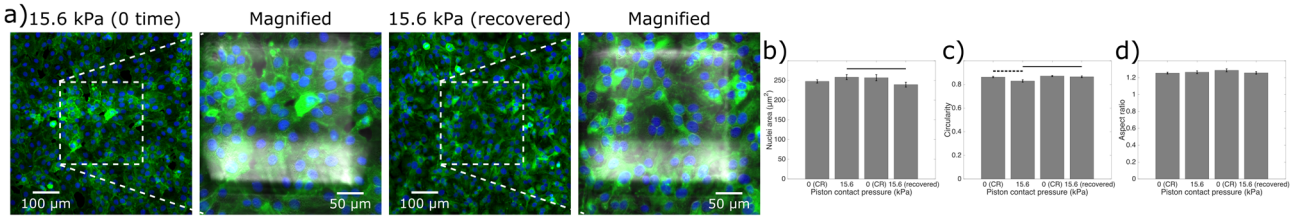

Figure 3. End point assay staining and analysis for actin and nuclei of cancer cells fixed at zero time or after 24h-recovery, following 1 hour-long cyclic compression. (a) Control and compressed cell groups stained for actin (green) and nuclei (blue). Dashed areas are under piston, while the surrounding is control region (CR). The region under piston imaged at high magnification, merged with phase contrast images. Nuclei deformation in control cells at control regions (CR) versus in compressed cells under piston at zero time or after 24h-recovery measured for area (b), circularity (c) and aspect ratio (d) of the nuclei.