

Review

Microdevice-based mechanical compression on living cells

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SUMMARY

Compressive stress enables the investigation of a range of cellular processes in which forces play an important role, such as cell growth, differentiation, migration, and invasion. Such solid stress can be introduced externally to study cell response and to mechanically induce changes in cell morphology and behavior by static or dynamic compression. Microfluidics is a useful tool for this, allowing one to mimic *in vivo* microenvironments in on-chip culture systems where force application can be controlled spatially and temporally. Here, we review the mechanical compression applications on cells with a broad focus on studies using microtechnologies and microdevices to apply cell compression, in comparison to off-chip bulk systems. Due to their unique features, microfluidic systems developed to apply compressive forces on single cells, in 2D and 3D culture models, and compression in cancer microenvironments are emphasized. Research efforts in this field can help the development of mechanocuticals in the future.

INTRODUCTION

Cells in tissue experience different types of forces that they either can accommodate or respond to by deforming accordingly. Experimental platforms can be established to mimic these conditions and to study cell response to applied forces in a controlled environment. There are various types of physical forces that can be applied on living cells, with fluid shear, pressure, and stretch being the most common ones.^{1,2} Different types of forces occur depending on the means and substrates used during force application. For example, while fluid flow mainly causes shear stress on cells, it can also generate combined forces depending on the substrate the cells are adhered to, such as rigid surfaces, flexible membranes, post arrays or 3D gels.^{3,4} Compressive stress with or without confinement^{2,5} or with gradient strain⁶ constitutes a further example of occurring forces. Application of strain/stretch (tensile stress) on living cells by mechanical stretching on the other hand varies depending on the stretchable substrates used, and whether these contain microelectrodes or microposts.^{7,8}

Studying biomechanics through the application of external forces on living tissues in *in vitro* systems provides a suitable model for *in vivo* mechanotransduction pathways between intrinsic mechanical forces and the respective biological responses. A prior review by Polacheck et al. provides an in-depth description of how mechanotransduction takes place in the body.¹ Briefly, mechanical inputs, such as shear stress, interstitial flow, substrate strain, confinement, and compression, are all transduced into biological outputs, such as altered gene expression, protein secretion, cell migration, and differentiation. During this transduction, intrinsic mechanical properties of cells, such as cytoskeleton stiffness or cell morphology, may change. These changes provide feedback to the system and may alter the effect of the mechanical input. For example, shear stress causes the polarization and elongation of epithelial cells. This biological output changes the fluid flow profile and shear stress on the epithelial monolayer as a feedback.¹ When comparing the aforementioned force types, less is known about the impact of compressive forces on cells and tissue microenvironments. Whereas in physiological conditions a combination of forces may act upon tissue, the examples reviewed in this work predominately focus on compressive force applications on cells in *in vitro* models to reduce complexity. Related examples of compression being applied to cells are summarized and discussed in detail in the following sections and [Tables 1 and 2](#).

While a wealth of literature reviewing mechanobiology and the general types of forces applied to cells and tissues exists,^{1,3,4,30–35} mechanical compression and the systems used to apply this type of force remain less well covered. Here, we aim to close this gap by providing a comprehensive review particularly focused on

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Table 1. Summary of compressive forces applied on living cells in bulk platforms including force application system, force magnitude, and observed cell responses

Force application system	Magnitude	Cell response	Reference
Uniform compression method via glass cylinder placed over confluent cell layers in 6-well plates	~0.05-0.3 kPa (0.5-3.0 g/cm ²)	Induced osteoclastogenesis by receptor activator of nuclear factor κ B ligand upregulation via prostaglandin E ₂ synthesis	Kanzaki et al., ⁹
	~0.05-0.3 kPa (0.5-3.0 g/cm ²)	Induced bone formation via increasing bone sialoprotein and prostaglandin E ₂ production, and via increase in bone morphogenetic protein and decrease in their antagonist production	Mitsui et al., ^{10,11}
	~0.05-0.3 kPa (0.5-3.0 g/cm ²)	Bone matrix turnover via increasing matrix metalloproteinases and their inhibitors	Mitsui et al., ¹²
Cup of weight acting as a piston to apply constant forces on tumor spheroids embedded in agarose gel cultured on a transwell membrane	0-8 kPa (0-60 mmHg)	Suppressed cell proliferation and mechanically induced apoptosis under high solid stress	Cheng et al., ¹³
As above, constant forces on agarose disk in contact with cell monolayer cultured on a transwell membrane	0-0.77 kPa (0-5.8 mmHg)	Coordinated migration of cancer cells by stimulated formation of leader cells and enhanced cell-substrate adhesion	Tse et al., ¹⁴
	0.13-0.8 kPa (1.0-6.0 mmHg)	Stimulated fibroblast activation, upregulated Growth Differentiation Factor-15 (GDF15) expression, and promoted pancreatic cancer cell migration	Kalli et al., ^{15,16}
Custom apparatus consisting of piezo actuator and control system to apply cyclic compression stress	0.1-1.0 kPa (smaller loading) at 1-30 Hz, 5.1-18.7 kPa (larger loading) at 30 Hz	Mechanical stress-induced cell death (MSICD) in breast cancer cells, with mixed mode of 5-35% apoptosis and 5-95% necrosis dominant (smaller loading), and of 0.1-4.4% apoptosis and 5-98% necrosis dominant (larger loading), respectively	Takao et al., ¹⁷
Commercial BioPress compression plates (Flexcell Corp.)	Moderate compressive force (CF) 5%, 20 kPa (2 cN/mm ²); high CF 10%, 40 kPa (4 cN/mm ²)	Moderate CF enabled active tissue remodeling and tooth movement; high CF in orthodontics caused tissue damage	Nettelhoff et al., ¹⁸
	3.18-3.53 kPa (~24-26.5 mmHg)	Altered expression of epithelial-mesenchymal transition genes and dispersal of compressed ovarian cancer multicellular aggregates on collagen gels	Klymenko et al., ¹⁹
	20 kPa	Increased cell proliferation and expression of regulators of epithelial-mesenchymal transition in the Rho/ROCK-dependent manner, indicating the role of compressive stress in cancer progression	Boyle et al., ²⁰

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Table 1. Continued

Force application system	Magnitude	Cell response	Reference
	1-3 kPa	Increased ovarian cancer cell adhesion to peritoneum, enhanced interaction between peritoneal mesothelial cells and cancer cells via induction of tunneling nanotubes (TNT), altered peritoneal microenvironment with more linear anisotropic alignment of collagen fibers, and promoted metastatic ovarian cancer progression	Asem et al., ²¹

mechanical compression, including an extensive and critical summary of existing studies and methods, as well as a future perspective for this emerging field. Thus, this review introduces and expands on compressive forces, the concept of applying mechanical compression, and engineering-based tools and methods to apply compression on cells. Studies using off-chip systems in bulk settings are compared to on-chip systems with microtechnologies and microdevices to apply cell compression. Due to their unique features, microfluidic systems developed to apply compressive forces in 2D and 3D culture models and compression in cancer microenvironments are emphasized in this review. As discussed in the following, existing cell compression studies demonstrate the necessity to develop and use novel microfluidics-based flexible microdevices, which are also referred to as on-chip compression systems.

MECHANICAL COMPRESSION ON CELLS AND TISSUE MICROENVIRONMENTS

Various cell and tissue types experience compressive forces through interactions with other cells inside the body. During development, tissue compression and resulting stresses regulate tissue size and cell fate specification, and, in turn, their functions.³⁵ For example, mechanical compressive stress has been found to control tissue size by producing homogeneous proliferation rates across tissues during *Drosophila* imaginal disc growth.³⁶ Similarly, it has been shown in *Drosophila* embryos that mechanical compression of the stomadeal primordium induces β -catenin release from cell junctions in a Src-dependent manner and this, in turn, upregulates primordial twist expression.³⁷

Another mechanism that can lead to mechanical compression is condensation.^{35,38} For instance, the condensation of mesenchymal stem cells during tooth formation in vertebrates, together with the resulting mechanical compression, regulate cell fate specification and differentiation.³⁹ Dental epithelium within the embryonic tooth organ produces fibroblast growth factor (FGF) to attract mesenchymal cells to migrate to under the dental epithelium. At the same time, dental epithelium also secretes a short-range repulsive signal, Sema3f, which locally repulses mesenchymal cells. This attraction and repulsion lead the mesenchymal cells to condense and compact under the dental epithelium. The mechanical compression formed in this way causes cell rounding, loss of stress fibers, and a decrease in RhoA activity. Reduced RhoA activity, in turn, upregulates the expression of odontogenetic genes such as Pax9, and thus forms the odontogenetic cell fate induction in those cells.³⁹

Above-mentioned examples of mechanical stress during development showcase the impact of compression in shaping the biomechanics of tissue and organ formation. Such biomechanical processes occur with mechanical compression as an input, and a feedback loop via resulting changes in cell and tissue properties, leading eventually to cell response as an output. Importantly, such mechanical impact can exist both in healthy and diseased tissues. For the latter in particular, and to supplement animal models and *in vivo* studies, advanced engineering-based *in vitro* tools are being developed to further study the impact of compression on cells and tissue microenvironments.

As such, the concept of applying mechanical compression on living cells, such as cancer cells, stromal cells (e.g. fibroblasts), neurons, and chondrocytes has continued to gain interest in recent years. For instance, various studies have shown the application of cell compression using a conventionalized method where a certain *in vitro* compression device is used to apply a pre-defined compression on cancer cells and stromal cells via an agarose cushion and cup of weight in the shape of a piston.^{12–16,40,41}

Table 2. Compressive forces applied on living cells in microfluidic platforms

Applied force	Force application system	Magnitude	Cell response	Reference
Compressive stress	Microfluidic biomechanical device including membrane for closing fluid channel and loading membrane for applying compressive stress	10-35 kPa external pressure	Deformation and lysis of mammary gland epithelial (MCF7) cells and decreased viability in response to increasing applied stress	Kim et al., ²
	Valve-based microfluidic compression platform: pressure application by regulated compressed gas	0-250 kPa contact (internal) pressure	Axon deformation and growth prior to, during, and immediately after focal mechanical injury	Hosmane et al., ²²
	Cell compression microdevice with a diaphragm	0-2 kPa external pressure	Calculated Young's modulus of osteoblasts calculated ranging from 3.8 to 4.2 kPa based on measurements of cell strain	Yokokura et al., ²³
	PDMS balloons-based pneumatic microfluidic cell compression device	14 kPa gauge (external) pressure	Chondrocyte mechanobiology - effect of compressive stress on cell viability and transcriptional networks	Lee et al., ²⁴
Gradient static-strain	Gradient strain hydrogels in microfluidic chip - applying compressive gradient forces on cell-laden hydrogels by stretching of circular hydrogel (in the radial direction) under strain or stress from PDMS membrane by releasing liquid pressure	Compressed strain 35-80% for Circle1, 5-40% for Circle12 on chip	Cell alignment in 3D	Hsieh et al., ⁶
Gradients of multi-modal compression	Monolithic microfluidic platform exerting gradients of compression on cell-laden hydrogels by vertical membrane actuated by three adjacent pressurized chambers creating multi-modal stimulation patterns	5-12% strain, 0-100 kPa (0-1000 mbar) external pressure	Resultant gradients of chondrocyte deformation in accordance with gradients of compression ranging from healthy to hyper-physiological conditions	Paggi et al., ²⁵
Compressive strain	Mesofluidic device combining the high-throughput automation and precision of microfluidics with the biological relevance of live meso-scale embryos	0-22% strain, vacuum to 6.9-34.5 kPa (1-5 psi) external pressure	Embryo survival and development (no induction of anoxic arrest) and dose- and duration-dependent mechanical induction of twist during early <i>Drosophila</i> development	Shorr et al., ²⁶
Cyclic compression	Microfluidic platform of microcontact printed fibronectin patterns for single-cell spreading and elastomeric membrane block geometry to apply compression on single cells	68.9-103.4 kPa (10-15 psi) valve (external) pressure at 0.5 Hz	Non-permanent plastic deformation of MCF10A cells	Ho et al., ²⁷
Sequential cyclic compression	Flexible microdevice composed of top microchannel for controlling external force and PDMS membrane underneath, with monolithically integrated actuators suspended in the bottom microchannel to apply force on cells	0-140 kPa contact (internal) pressure in dynamic modes	Retained viability after dynamic cell compressions at physiological pressures and endpoint mechanical cell lysis at high pressures; actin and nuclei deformation profiles in control vs. compressed cells exposed to sequential cyclic compression	Onal et al., ^{28,29}

Researchers have so far performed compression applications to study bone formation (osteogenesis), bone matrix turnover, and bone resorption,^{9–12} neuron injury,²² chondrocyte compression,^{24,25} stromal cell differentiation, such as fibroblast-to-myofibroblast differentiation and fibroblast function (i.e. fibroblasts activation),¹⁶ solid stress and associated tumor and stroma interactions in tumor microenvironment,^{34,42–45} apoptosis and necrosis of cells,^{13,17} mechanical deformation and lysis of cells,^{2,27,28} cell motility, invasion and migration,^{14,19,21,40,41,46,47} and cell alignment under compression.⁶ The studies discussed here collectively show that compression plays no small part in the mechanics of cells and tissues. At the same time, when reviewing existing methods used in the cell compression field to date, it becomes clear that there remains an opportunity to develop more advanced tools to further understand the impact of mechanical compression.

Compressive stress in tissues can be studied by applying external forces on living cells and tissues in *in vitro* systems similar to the development of *in vivo* mechanotransduction pathways from the intrinsic mechanical forces (input) to the biological response (output).¹ Compression, for instance, alters the cell deformation and causes mechanical lysis of the cells. Mechanical compressive stress can also induce the necrosis mode of cancer cell death, which results in autolysis. Danger-associated molecular patterns released during necrosis play an important role in recruiting immune cells to the site of cancer.^{17,48} Thus, the investigation of cell lysis under mechanical compression in a controlled manner in *in vitro* microenvironments could be an intriguing approach to better understand interactions of cancer and immune cells, as well as immunogenic cell death in cancer therapeutics.^{48,49}

Apart from cell lysis, compression-induced cell deformation can also result in intracellular Ca^{2+} signaling in chondrocytes, which could mediate a series of mechanotransduction events.⁵⁰ In another example, compressive force induction *in vitro* facilitates stromal cell differentiation and their activation to cancer-associated form (e.g. fibroblast-to-myofibroblast differentiation) depending on the magnitude of the applied stress.^{16,34} While static mechanical compression on living cells is readily attainable in bulk systems, controlled and dynamic compression can be achieved particularly well in microfluidic settings, mimicking *in vivo* conditions. Microfluidic systems can further be integrated with physical structures to introduce static and dynamic physical inputs and gradients on living cells, all while enabling real-time imaging of the process. As such, microfluidics-based compression platforms provide useful tools to study the biomechanics of living cells.

In addition to microfluidics-based platforms, atomic force microscopy (AFM)-based methodology has also been used to apply controlled compressive forces to single cells or 3D spheroids. For instance, Stewart et al. analyzed cell shape to understand the forces that drive the cell shape deformation or rounding during mitosis.⁵¹ They simultaneously employed AFM and transmission light microscopy to measure cell height and monitor cell width, respectively. To quantify the force of cell rounding, a tipless cantilever was positioned over a prophase HeLa cells and held there while the cell underwent mitosis. Additionally, they compared the effect of long-term constraint under the AFM cantilever with measurements on a shorter timescale using a simple rheological compression assay.⁵¹ In another study, Andolfi et al. modified the AFM cantilevers to allow for larger planar contact areas with the biological samples, thus enabling more accurate characterization of mechanical properties.⁵² 3D multicellular tumor spheroids at different growing stages and human oocytes were tested with the developed AFM macro-probes. The authors estimated oocyte and tumor spheroid deformations during stress-relaxation measurements by evaluating the vertical deformation under vertical compression.⁵²

As exemplified, AFM-based compression methods are advantageous in that they can be used for both applying and measuring forces in a versatile way.^{51–53} However, AFM-based tools naturally have limitations, for example in regards to setup portability and cost, as well as the need to physically access samples with the probe, which makes the technique unsuitable for use with enclosed microenvironments used to mimic *in vivo* conditions. AFM techniques also usually only operate locally on a single cell or part of it, although recent developments promise to expand the technique to larger biological systems such as human oocytes or 3D cell spheroids.^{52,53} In contrast, microfluidics-based platforms have shown to be able to easily apply global compression to cells cultured in various platforms and at various scale, including single cells, monolayers, hydrogel-based 3D culture models, as well as spheroids. It is expected that the development and integration of force sensors on-chip and/or computational modeling to measure forces generated inside chips^{22,24,28,29} will further close any functional gaps between AFM and microdevices. As discussed in the

following, the latter already incorporate advanced capabilities of controlled compressive force application in both static and dynamic manner, portability, and ease of use with different microscopes.

In the next sections, we review the compressive forces cells experience inside the body and methods used to investigate the impact of the compressive forces on cells. A particular focus is placed on the use of compression platforms in cancer research due to the role of compression in cancer microenvironments. Mechanical compression applications on cells are summarized with a broad focus on those using micro-technologies and microdevices to apply cell compression. For the applications of force types other than compression, the reader is referred to existing reviews by.^{1,4,31–33}

Note that in [Tables 1](#) and [2](#), unit conversions to kPa were applied to the respective study results for ease of comparison. Original magnitude and units are given in parentheses for each reference if other than kPa.

OFF-CHIP COMPRESSION SYSTEMS

The off-chip compression systems to apply mechanical compression on living cells have been built in bulk settings and operated in static conditions in general. These are historically among the first cell compression systems to be reported and now can be used as conventional methods.^{9,41,54} Studies using such systems are summarized in [Table 1](#).

Existing bulk compression systems

The principles of, and a selection of cell compression results obtained with the bulk systems in literature, including the uniform compression method, *in vitro* compression device, and BioPress compression culture plates (Flexcell) are introduced here briefly. These are representative examples of static weight systems.

The uniform compression method, shown in [Figure 1A](#), was applied via a glass cylinder/plate placed over confluent cell layers in 6-well plates or 100-cm² culture dishes.^{9–12} Compressive force was generated and controlled by the number of lead granules added into the cylinders. Cultures were compressed statically and continuously. In an example study, after periodontal ligament (PDL) cells were compressed at 0.5–2 g/cm² for 6 h or 24 h using this compression method, glass cylinder, weight, and culture medium were removed.⁹ Compressed PDL cells were then cultured with peripheral blood mononuclear cells (PBMCs). An increased number of the tartrate-resistant acid phosphatase-positive (TRAP+) multinucleated cells was observed, which indicated up-regulated osteoclastogenesis from PBMCs by mechanical stress ([Figure 1B](#)). It was further found that osteoclastogenesis was induced by receptor activator of nuclear factor κ B ligand up-regulation via significantly increased prostaglandin E₂ synthesis in the compressed PDL cells compared to control.⁹

Another example of a static bulk compression system is the *in vitro* compression device initially proposed by Cheng et al.⁴¹ and since used by others.^{13–16,40,55} It consists of a 6-well transwell insert, agarose cushion, and a cup or piston filled with adjustable weight, as shown in [Figure 1C](#). Cancer cells or tumor spheroids were first cultured on the transwell membrane and then compressed statically via the cup. [Figure 1D](#) shows that the migration of mammary carcinoma cells was induced under compression applied using this device. As the indication of cell migration, wound closure rate was measured for five different mammary epithelial cells, MCF10A, MCF7, 67NR, 4T1, and MDA-MB-231, with increasing invasion potential, respectively. Comparison was made between the corresponding stress-free (control) and the cells subjected to a compressive stress of 5.8 mmHg for 16 h in each group. Results from this study by Tse et al. also showed coordinated migration of cancer cells by stimulated formation of leader cells on patterned protein islands and enhanced cell-substrate adhesion within compression applications using this platform.¹⁴

BioPress compression culture plates (Flexcell International Corporation, Hillsborough), shown in [Figure 1E](#), have foam sample holders of six-well plates with silicone elastomer well bottoms where cells or multicellular aggregates and hydrogel constructs or tissue explants could be placed.^{18–21} The wells were then loaded with culture medium. Adjustable platens, which were physically contacting the samples from above and adjusted to ensure even loading conditions, were inserted into the culture plates. Static compression was applied to the samples with a Flexcell Compression System. In [Figure 1F](#), results from the study conducted by Klymenko et al. using this platform are presented as the quantitation of lateral dispersal of OvCa433 ovarian cancer multicellular aggregates (MCAs) on collagen gels at 72 and 96 h of

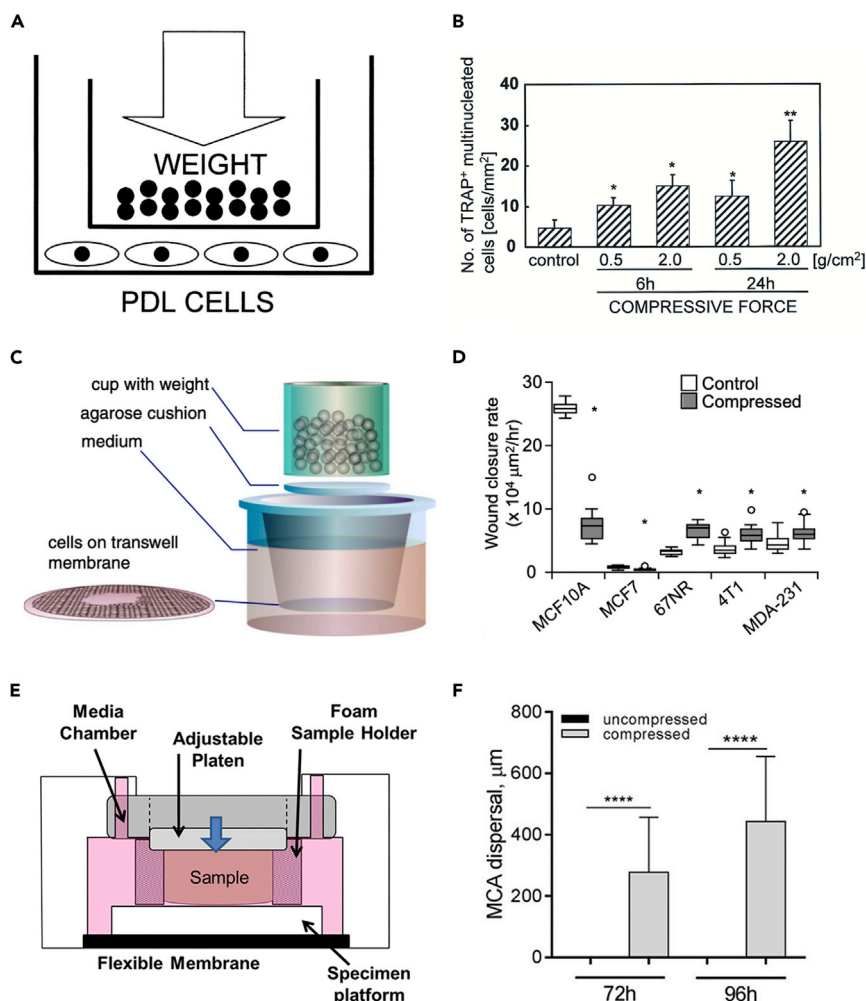


Figure 1. Cell compression in bulk platforms

(A) Uniform compression method via glass cylinder used to apply compression to periodontal ligament (PDL) cells. Force was controlled by the number of lead granules placed in the cylinder.

(B) After compression for an indicated force and time, glass cylinder, weight, and culture medium were removed. Compressed PDL cells were then cultured with peripheral blood mononuclear cells (PBMCs). An increase in the tartrate-resistant acid phosphatase-positive (TRAP⁺) multinucleated cells indicated up-regulated osteoclastogenesis from PBMCs by mechanical stress.

(C) Schematic of *in vitro* compression device to apply constant compressive forces on a mammary carcinoma cell monolayer via a cup with adjustable weight and monitor cell migration on a transwell membrane. Cell migration under compression was assessed by monitoring the closure of a wound of around 1 mm in diameter scraped at the center.

(D) Wound closure rate for five different mammary epithelial cells of increasing invasion potential, with comparison between the corresponding stress-free (control) and the cells subjected to a compressive stress of 5.8 mmHg for 16 h in each group, indicating that compression-induced mammary carcinoma cell migration.

(E) BioPress compression well plate and Flexcell Compression Plus System for applying compressive stress to hydrogel/multicellular aggregates (MCAs).

(F) Quantitation of OvCa433 MCA dispersal at 72 and 96 h of culture after compression. Reproduced, with permission, from⁹ for (A) and (B); from¹⁴ for (C) and (D); from¹⁹ for (E) and (F).

culture after compression, compared to control (uncompressed) MCAs which did not disperse. For this application, the hydrogel/MCA samples were subject to compression at 3.18-3.53 kPa (~24-26.5 mmHg) for 48 h and then removed from the hydrogel and placed into collagen-coated wells where they were imaged for 0-96 h. Results showed significantly enhanced lateral dispersal and migratory behavior of OvCa433 MCAs due to compression, indicating the role of compressive stress in cancer metastasis.¹⁹

Advantages and disadvantages of using bulk systems in compressive force application

As exemplified, bulk static compression systems have produced interesting biological results. They are easy-to-use and can treat a large amount of samples with uniform forces. They also have influenced the development of more complex and controlled systems via the results they have yielded. Despite the emergence of dynamically controlled bulk compression application systems, such as custom apparatus consisting of piezo actuator and control system to apply cyclic compression stress,¹⁷ they have some limitations in general.

Bulk systems have larger areas in which the cells are exposed to compression. Thus, with large sampling, these systems allow for the collection of significant amounts of media and extracts from the high cell number involved in the compression process, for instance, for proteomic analysis to profile mechanical stress-induced signaling cascades driving the motility of cancer cells.⁵⁵ On the other hand, given that the compressed regions are large, bulk platforms are less likely to allow the test (compressed) and control (non-compressed) cells to be assayed on the same sample. For instance, Tse et al. compressed a cell monolayer with an intermediate layer of agarose cushion, on top of which a cup with weight was used as the compression source, while their control group was a separate cell monolayer with an agarose cushion only.¹⁴

Typically bulk compression systems are easy to produce and operate when a constant force is to be generated. The latter in particular can be advantageous for long-term compression as the compression unit relies solely on a constant material source, such as a cup with weight. Conversely, while a recent system by Takao et al. appeared to be controlled by an external, bulk piezo actuator and control system, bulk systems are inherently difficult to operate in a controlled and dynamic manner due to their size.¹⁷ As a result of the latter, a number of more flexible, integrated and, in general, more miniaturized platforms have been proposed, which are discussed in more detail in the following section.

ON-CHIP COMPRESSION SYSTEMS

The examples described so far all employ similar static bulk setups for compression on cancer and stromal cells. While these systems reliably apply a constant force, they typically lack the automation required to modulate the applied pressure, which helps to better mimic *in vivo* processes. On-chip compression systems have differentiated from off-chip ones mainly by size and deflection capability. In on-chip systems, the size of the compression unit could be easily downscaled from millimeter scale to micrometer scales using microfluidics, mimicking cell and tissue microenvironments in a physiologically relevant manner, as compared to bulk systems. Microdevices have a deflectable part of the chip, generally in form of a flexible membrane layer, while bulk systems have the compression unit created out of the weight of a rigid material such as a glass cylinder with lead granules. Of the earlier on-chip force application systems capable of inducing compressive stress on living cells described in the literature,^{2,6,22,24} only a limited number have been used to apply on-chip compression in a dynamic setup.

Microfluidics and flexible microdevices for studying cell compression on-chip

As alluded to, microfluidics is a useful tool to study the biomechanics of living tissues as it allows static and dynamic physical inputs and gradients, such as varying amounts of mechanical forces, in both 2D and 3D to be applied, all while enabling real-time imaging. Lab-on-a-chip (LOC) systems incorporating microfluidic technology can be used to mimic *in vivo* microenvironments for both, 2D and 3D *in vitro* culture, and force application in these can be controlled spatially and temporally.^{6,22,27,28}

Flexible microdevices have been named so, as part of the device, for example, the force loading membrane, tends to be deflectable to apply compression under either static or dynamic conditions. In fully static compression, when one cannot alter the setting of the device once the compression application is initiated, no temporal control of the device or compression (e.g. to decrease, increase or remove the pressure within certain time intervals) is possible. The only temporal control that exists is in regard to how short or long the static compression is applied. This attribute is most common in bulk compression systems. However, it can also be observed in microdevices, such as the one introduced by Hsieh et al.⁶ Once the liquid pressure was released in this device, the hydrogel became compressed and device operation appeared not to be reversible in that the membrane could not be brought back to its previous buckled state.⁶ In contrast, with dynamic compression, especially on platforms that have actuation capability, the duration that cells are

exposed to compression can typically be controlled by on-off valves or adjustable applied pressure amounts and rates. Thus, the platform can dynamically compress the cells with various amounts of force, for various durations and/or following pre-designed force application profiles, including for example fully automated cycling, alternating, and recovery.

Fabrication of flexible microdevices

Microfluidic devices can be designed based on the intended compression application, for instance, whether it is in 2D or 3D; on single cells or multicellular structures; or at culture standards of microorganisms or mammalian cells. They are fabricated using various microfabrication techniques that can meet design dimensions, as well as material and structural constraints defined by the application type. Among those, photolithography, 3D printing (e.g. stereolithography), or micro-milling are mostly used for designs at micro- and millimeter scales. While printable flexible materials are slowly starting to become available, the above techniques are generally used to first fabricate a master out of hard and/or hardened materials, such as Si and photoresist in case of photolithography, resin for printing, or metals and hard plastics for micro-milling. Flexible microdevices for compression applications, as their name says, however need flexible and thus deflectable materials. Thus, until 3D printing of flexible materials matures, multi-step processes for replica molding of flexible materials on pre-fabricated mold masters remain the method of choice.

Even with the emergence of techniques that do not require photomasks, such as 3D printing and micro-milling, photolithography of a photoresist layer on a silicon wafer still provides the highest feature resolutions over a wide range of dimensions and surface finishes. For cell compression devices with larger overall dimensions, masters with designs at sub-millimeter ranges are difficult to fabricate in multilayer photoresist form which would allow for single-step multilayer casting. An example for this is the device shown in Onal et al., for which PDMS layers were ultimately fabricated using separate photoresist masters and then assembled into a multilayer flexible device.²⁸ The advantage of this method is that a modification can be easily introduced to any of the device layers as the dimensions of the compartments are not linked to each other via a single multilayer photoresist master. Thus, when a compartment of the device needs to be changed, it is sufficient to change the dimension of one layer while the others can be kept the same.²⁸ Comparable small dimensions and aspect ratios in flexible polymer molds can also be achieved with the type of multilayer photoresist masters used in the study of Hosmane et al.²²

In general, viscoelastic polymer materials, such as PDMS, are ideal for the fabrication of flexible microdevices. Mechanical properties of the material can be optimized during the fabrication process through, for example, the mixing ratio of the PDMS base and curing agent, curing temperature, and duration.^{28,56} PDMS transparency and the ease of bonding it to glass or thermoplastic (e.g. polystyrene) surfaces, make assembled devices compatible with imaging via either upright or inverted microscopes.³³ Good biocompatibility also makes it an ideal material for use in contact with biological cells.⁵⁷ Specifically in compressive force applications, where the deformation of the material is integral to cell compression device functionality, PDMS is a superior choice given its high compliance, or low stiffness, in contrast to other materials. In general, PDMS has an elastic modulus of $\sim 1\text{--}3$ MPa, which is four orders of magnitude lower than glass (~ 50 GPa) and three orders of magnitude lower than typical thermoplastics such as polystyrene (~ 3 GPa).⁵⁸ Less advantageous properties of PDMS include evaporation (i.e. gas permeability, which also can be highly advantageous when used in a CO₂ cell culture incubator), absorption of hydrophobic compounds, and leaching of uncrosslinked oligomers. Furthermore, hydrophobic recovery caused by surface diffusion of low molecular weight chains can also influence study results, as it can render PDMS surface treatment or functionalization unstable over time.⁵⁹ All these need to be taken into consideration for cell-based applications, as they may cause issues depending on the type and duration of the application of interest. For instance, natively hydrophobic PDMS surfaces are typically plasma-treated and converted to hydrophilic surfaces which are favorable for bonding between two layers, surface functionalization (e.g. coating with protein solutions), microchannel filling, and cell culture. To minimize experimental variability due to naturally occurring hydrophobic recovery, surface-treated devices can for example be prepared freshly and used as soon as possible after treatment. Alternatively, hybrid devices can be formed to incorporate non-PDMS culture surfaces. For a more detailed discussion on the use and limitations of PDMS, the reader is referred to the review by Berthier et al.⁵⁸

Using soft lithography, the flexibility of the microdevices can be easily achieved for dynamic compressions. Devices are prepared layer-by-layer in a top-down approach for designs with a membrane positioned

horizontally in a vertical multilayer device and deflected vertically to apply compression to a sample in a lower layer. Although the layers can be aligned precisely, the timing of the fabrication relies on several fabrication steps to finalize a multilayer device. In contrast, a device design with a membrane positioned vertically to an adjacent channel and deflected horizontally to apply compression to a sample in that channel does not need the alignment of the flexible components as they are fabricated within the same layer in a time-saving single-step process.²⁵

Cell compression in flexible microdevices

Effects of compression on various cell types, such as neurons,²² osteoblasts,²³ chondrocytes,^{24,25} fibroblasts,⁶ and cancer cells,^{2,28} have been observed using microfluidics-based flexible microdevices. Table 2 presents studies that used microfluidic platforms to apply types of mechanical compression on living cells. Flexible microdevices can be designed and prepared to have a dynamic compression capability, while, at the same time, the compressed compartment of the device should have a good position recovery after retraction. As an example of the former, Lee et al. applied dynamic compression on chondrocytes,²⁴ however their study did not expand on the ability of their devices to retract and recover the position of the compressed unit. In contrast, the platform introduced by Onal et al. demonstrated extensive control of position recovery within the developed flexible microdevice, as well as dynamic compressions on cells.²⁸ A good position recovery shows the complete flexibility and integrity of a microdevice to switch between its static and compressed states to observe cell response at each. Furthermore, cell shape deformation and recovery post compression, which is an important phenomenon in cell mechanics, can be investigated after the position recovery of the respective compressing unit within flexible microdevices.^{27,29}

Several recent platforms have also been shown to be capable of applying compression on cells within 3D hydrogel constructs (Figure 2). Hsieh et al. developed a platform called the gradient-static-strain microfluidic chip (GSS-micro-Chip), which was able to generate a gradient of static strain internally to stimulate cell behavior within cell-laden hydrogels in a 3D microenvironment.⁶ The device was used to generate gradient forces on concentric circular cell-laden hydrogel patterns without needing an external mechanical source. The force load was supplied via the release of liquid pressure out of an initially buckled flexible PDMS membrane. Thus, the concentric circular cell-laden hydrogels stretched under the self-generated gradient strain. Fibroblast cells encapsulated within those hydrogels were cultured with compressive strains from ~65% at the center, to ~15% at the boundary of hydrogels to investigate cell alignment under compression. Due to its design, this platform allowed for the observation of cell alignment caused mainly by compressive strain. Cells elongated radially near the center and via the hydrogel geometry along the circular direction near the outside boundary. Understanding cell alignment in such miniaturized and simple to operate devices is important for the study of tissue regeneration under mechanical stimulation as existent in localized environments in the body, and independent of geometric guidance cues.⁶

To enable dynamic compression within 3D hydrogel constructs, Lee et al. developed a microfluidic cell compression device composed of PDMS balloons of different sizes, which generated multiple compression conditions on a single platform.²⁴ Pneumatically actuated PDMS balloons enabled the compression of chondrocytes in alginate hydrogel constructs at various magnitudes at the same time. The sizes of the balloons were controlled to change the amount of compression under an externally applied constant pressure of 14 kPa. High-throughput screening of the chondrocyte response to varying compression showed that the compressive strain of chondrocytes increased with PDMS balloon diameter under static compression. Furthermore, the device could be actuated to apply either static or dynamic compression. The latter was applied by using a function generator with a solenoid valve regulated with a square wave. Cell viability was high and similar between the control (non-compressed) and dynamically compressed chondrocytes, while it was as low as 58.6% under static compression, possibly due to reduced nutrition transport. The device could be dismantled for further signal transduction analysis of the relationship between compressive stress and formation of the resistant hydrated matrix in hyaline cartilage. Understanding the mechanobiology of chondrocytes in the latter is important for many moveable body joints and bone growth.²⁴

More recently, Paggi et al. reported a microfluidic platform monolithically fabricated with a vertical thin PDMS membrane adjacent to a separate channel.²⁵ The fabrication process used a single-step, which can be advantageous to the more complex multilayer device design and fabrication processes. Gradients of mechanical compression could be applied to cell-laden hydrogels. Optimized for an articular cartilage model, the device was actuated to produce 5-12% strain as the physiologically relevant compression in

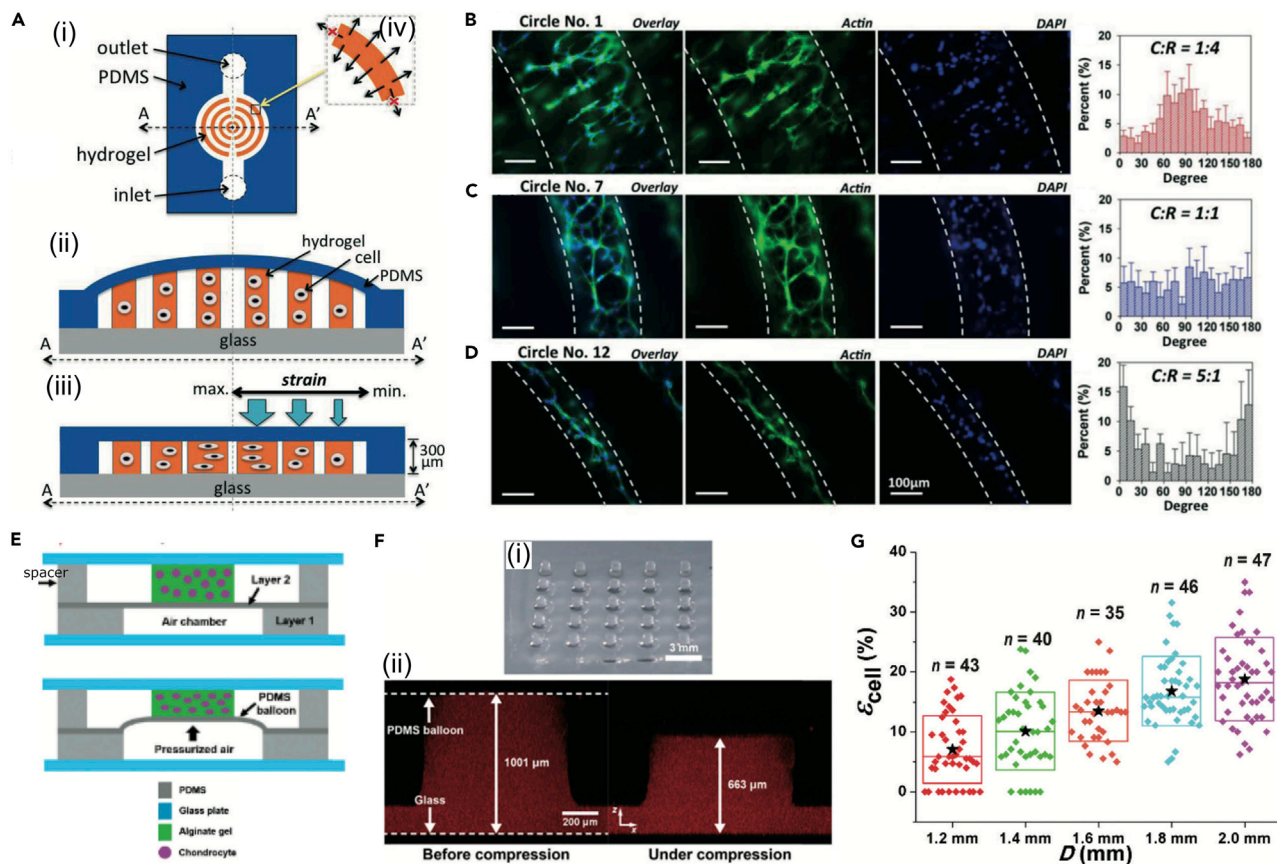


Figure 2. Examples of on-chip compression of cells within 3D hydrogel constructs

(A) Design and working principle of gradient strain hydrogels in a microfluidic chip, called GSS-micro-Chip, showing concentric hydrogel circles (i) formed with a height gradient along the radius (ii). The buckled PDMS membrane was flattened by the release of liquid pressure from the unplugged inlet and outlet, which created gradient force on cell-encapsulated circular hydrogels (iii) and strain or stress in the stretch direction (iv).

(B–D) Alignment response of 3T3 cells stained for actin-nuclei in hydrogel circles under gradient strain, and radial-to-circular elongation ratios of the encapsulated cells analyzed for these circles, shown in the corresponding graphs.

(E) Pneumatic microfluidic cell compression device consisting of a 5×5 array of PDMS balloons with different diameters, and alginate-chondrocyte constructs located on the PDMS balloons.

(F) Fabricated alginate gel constructs (i) and the concept of alginate gel deformation under static compression (ii).

(G) Chondrocyte deformation within alginate constructs under static compression. The compressive strain of chondrocytes (ϵ_{cell}) increased with the PDMS balloon diameter (D). Reproduced, with permission, from⁶ for (A), (B), (C), and (D); from²⁴ for (E), (F), and (G).

cartilage tissue. The pressure application was extended from healthy to hyper-physiological compression with an applied pressure range of 0-1000 mbar. Gradients in cell deformation were observed as a result of the gradients in compression. Since the actuation of the membrane could be controlled by three adjacent pressurized chambers, the platform could create multi-modal cell stimulation patterns by adjusting the pressure application in different chambers. This versatility could allow a combination of normal and bulk shear strain in the knee cartilage during movement to be mimicked. As such, the platform has the potential to also be used to study the complex stimulation patterns experienced in other body tissues.²⁵

Finally, Shorr et al. developed a mesofluidic device that applies compression on *Drosophila* embryos aligned and immobilized in a single file.²⁶ The device consists of two interlaced compartments, a liquid one introducing and aligning the embryos in the microchannels, and a gas one utilizing dead-end microchannels to create pneumatic actuation on either sidewall of the liquid compartment. These gas channels control the effective width of the liquid channels to load or compress embryos. Parametric calibration including the width and height of the compression channel, thickness and rigidity of the deformable sidewalls, and the applied pressure, was achieved for *Drosophila* embryo compression. Using the optimized

parameters, compression could be tuned between 0 and 22% with the applied external pressures ranging from vacuum to 1-5 psi. The device included either 20 mm or 40 mm-long compression channels, enabling the immobilization of up to 240 *Drosophila* embryos per run. Operated without external pressure for immobilization and mild compression, the device used external pressure (1-5 psi) to apply active compression to the embryos and allowed for the recovery of live embryos for post-compression analysis. Using this device, Shorr et al. measured Young's modulus of PDMS sidewalls to be 1.88 MPa at the given parameters, and those of *Drosophila* embryos to be ~160 kPa. They also showed embryo survival and development under the effect of compression and quantified the dose- and duration-dependent mechanical induction of *twist* during early *Drosophila* development. As such, the "mesomechanics" approach developed in this study illustrates that the mechanical stimulation with high-throughput automation and precision possible with microfluidics does not have to be limited to small cellular systems, but rather can also be expanded to meso-scale multicellular organisms. Platforms like this have the potential to help map out novel mechano-sensitive pathways in meso-scale organisms such as larvae, organoids and tissue samples.²⁶

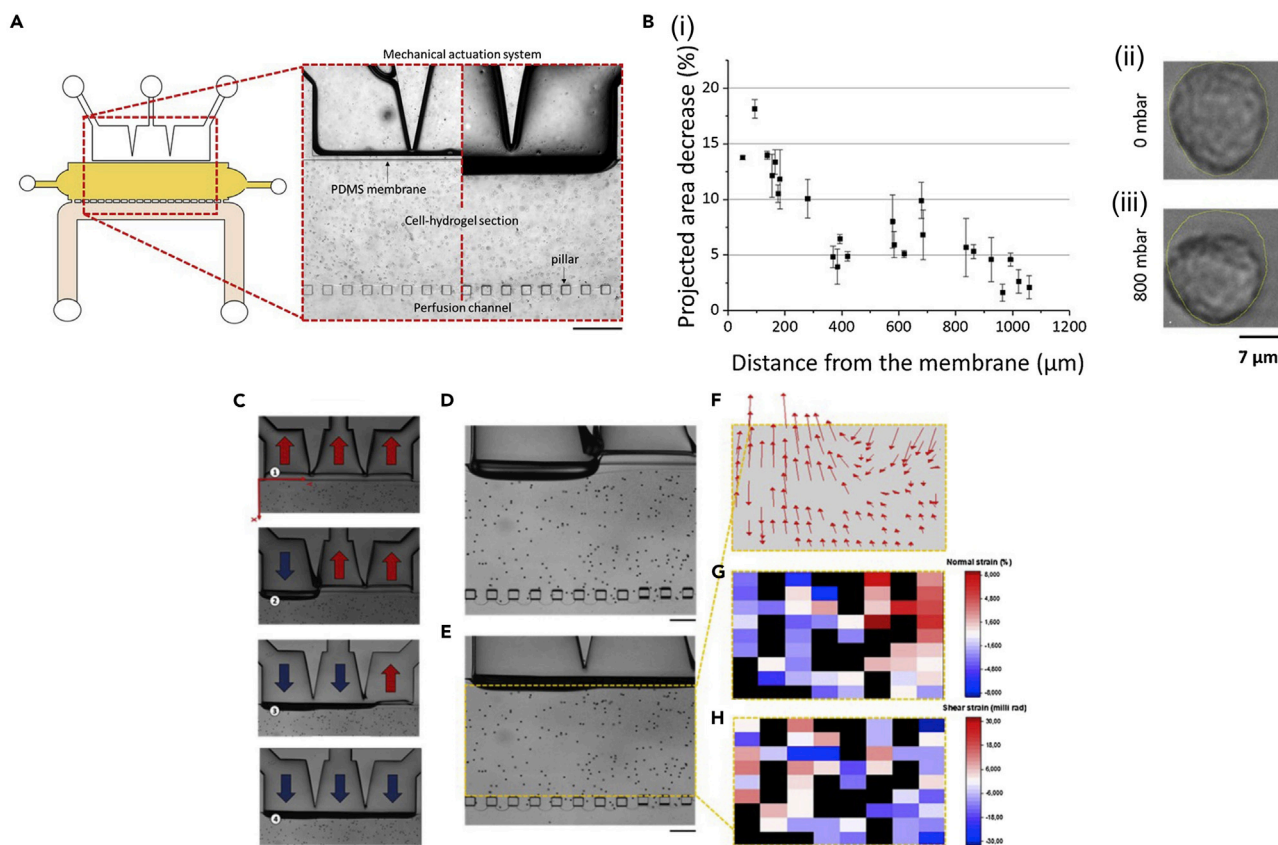
In many of the studies discussed here, cell compression devices are designed and built in a manner that layers are horizontally aligned on top of each other. Therefore, a multi-step process is required for their fabrication, which tends to be slow and laborious as it requires layer-by-layer alignment and assembly.^{2,22,24,27} Such multilayer devices also call for advanced imaging techniques, either 3D laser scanning²⁴ or further optimizations of conventional wide-field imaging to be able to observe the mechanical stimulation process and cell response to the applied forces. In contrast to other studies, Paggi et al. reported a monolithic platform composed of a thin vertical PDMS membrane located next to the cell culture chamber and actuation by a series of interconnected pressure chambers, that were fabricated all in a single-step process (Figure 3). This unique orientation of the compartments facilitated imaging of cells within the hydrogels, as the compressing unit of the device was not overlaid on the cell culture chamber.²⁵ In terms of compression device design, the mesofluidic device developed by Shorr et al.²⁶ has some similarity to the device developed by Paggi et al.²⁵ in as far as that a sidewall or vertical PDMS membrane was used to apply compression to samples in an adjacent microchannel. While Shorr et al. applied compression to multicellular organisms, in particular meso-scale embryos aligned in a microchannel,²⁶ Paggi et al. applied compression to cell-laden hydrogels in 3D for an articular cartilage model.²⁵ While both designs stand out for the single-step fabrication and facile real-time imaging, their transverse modulus of compression makes them unsuitable for use with cell monolayers or single cells.

MECHANICAL COMPRESSION ON SINGLE CELLS

Due to the scalability of microfabrication techniques, microfluidic devices in particular enable the application of mechanical compression on single cells. Figure 4 shows examples of microfluidic platforms used to apply mechanical compression at the single-cell level and the results obtained with these.

A valve-based microfluidic compression platform was developed by Hosmane et al., which they named axon injury micro-compression (AIM) platform. It was used to apply compression on single axons in the range of 0-250 kPa to achieve mild (<55 kPa), moderate (55-95 kPa) and severe levels of injury (>95 kPa).²² The device consisted of injury pads operated via compressed gas input that was modulated to apply the specified levels of injury. It allowed the mechanics of neuronal cell damage to be investigated at the single axon level. In particular, continued growth, degeneration, and regrowth of axons were studied at the specified pressure levels prior to, during, and immediately after injury. 73% of the mildly injured axons continued their growth, while the percentage of growing axons dropped to 8% under moderate injury conditions. Interestingly, while a majority of axons were transected at severe levels, 46% of those were found to regrow after injury.²²

A further single-cell microfluidic compression device to investigate the plastic deformation of cells after cyclic compression was proposed by Ho et al.²⁷ The authors of this study used microcontact printing to pattern cell spreading and an elastomeric membrane block geometry to apply compression on single cells. MCF10A cells were trapped into multiple compression chambers in a stepwise pneumatical manner to minimize the mechanical perturbation of cells. Cell spreading was confined within the single-cell trap regions by microcontact-printed fibronectin islands. Cyclic compression, applied between 10 and 15 psi at 0.5 Hz for 6 min, did not cause permanent plastic deformation of cells. Interestingly, this finding for repetitive compressive loading and unloading on cells is in contrast to similar permanent plastic deformation that can be observed in certain cells exposed to repetitive mechanical tensile loading.^{27,60}



More recently, Dannhauser et al. developed a microfluidic chip to apply tuneable compressive forces on suspended cells, resulting in in-flow deformation-dependent dynamic motion regimes, namely, rolling, tumbling or tank-treading.⁶¹ They modulated applied single-cell forces depending on the microfluidic chip geometry and rheological fluid properties. Thus, their approach generated confinement-based compressive forces acting on cells while these were passing through smaller cross-sections of the channel. In this regard, this platform is considerably different compared to flexible microdevice platforms described here, which typically apply compression in a compartment by actively deflecting or displacing part of the compartment. While the nature of their force application method currently limits its use to suspended cells, Dannhauser et al. nevertheless were able to extensively analyze cell mechanical responses under tuneable compressive forces by measuring in-flow parameters such as orientation angle, aspect ratio, cell deformation, and cell diameter. In doing so, they observed that highly invasive tumor cells (MDA-MB-231) were 6-times more deformable than healthy (MCF-10A) and low invasive (MCF-7) breast cell lines. As such, the methods used in this study has the potential to allow for label-free cell phenotyping by effectively generating a mechanical signature for each cell line based on the measured deformation parameters.⁶¹

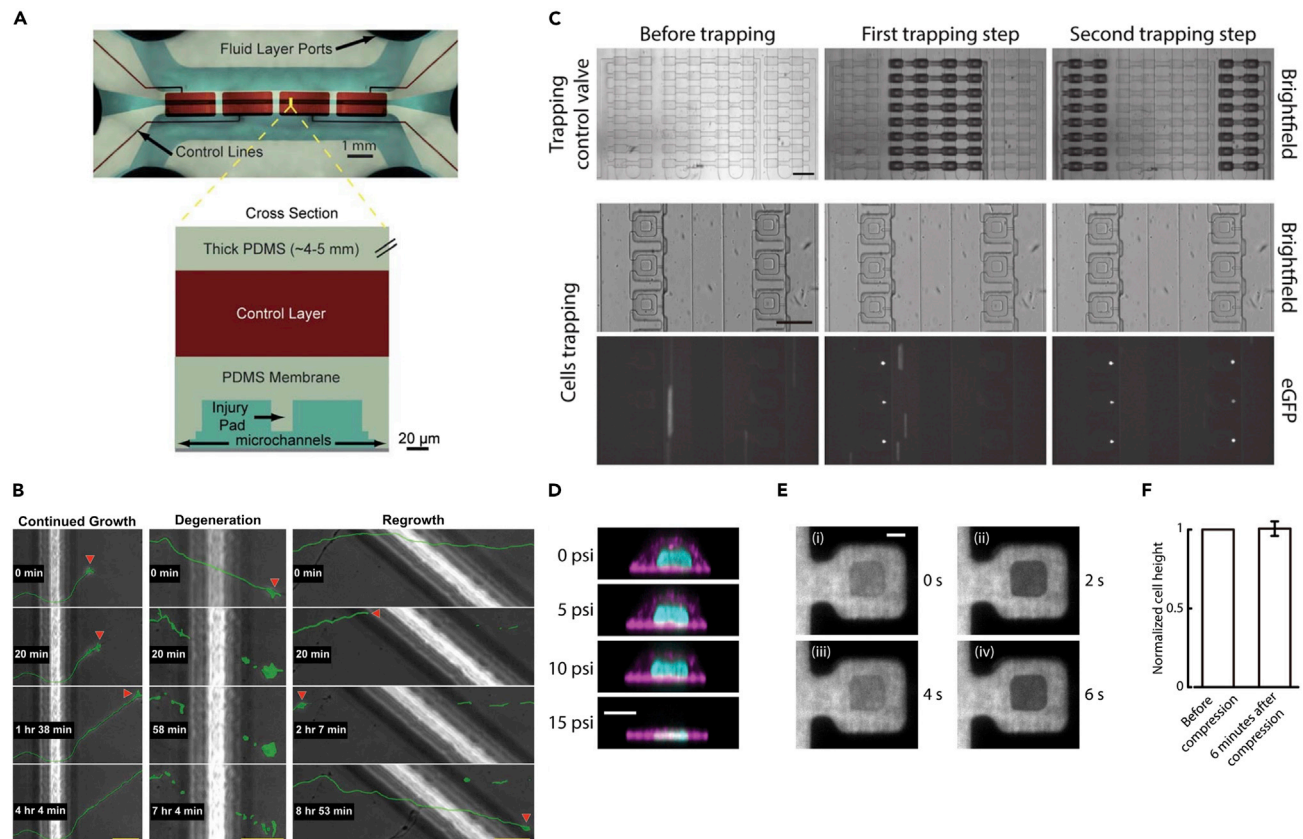


Figure 4. Examples of single-cell compression in microfluidic devices

(A) Axon injury micro-compression (AIM) device with neuronal layer (blue/green) including microchannels and injury pad fabricated from multilayer resist master, and control layer (red) fabricated from a second resist master.
 (B) Neuron response after compression in AIM device shown in representative images for continued growth, degeneration, or regrowth of axons as a function of injury level at the applied pressures.
 (C) Compression chamber of a microfluidic device for single adherent cell compression and trapped eGFP expressing MCF-10A cells.
 (D) Reconstructed side view images of DNA (cyan) and actin (magenta) of an MCF-10A cell while being compressed at different applied pressures.
 (E) Fluorescence images of the compression chamber at indicated time points during the cyclic deflection of the membrane.
 (F) Comparison of the normalized cell height before and 6 min after 6-min compression applied cyclically between 10 and 15 psi at 0.5 Hz. Reproduced, with permission, from²² for (A) and (B); from²⁷ for (C), (D), (E), and (F).

MECHANICAL DEFORMATION AND LYSIS OF CELLS UNDER COMPRESSION

Mechanical stimulation influences the physiological and pathological condition of an organism, and thus has a direct impact on human health and diseases. Cell deformation can emerge as a result of mechanical compression of cells and can be observed in tissue in the body at various physiological pressures, including up to lysis at hyper-physiological pressure. Thus, compressive forces can shape physiological structure and function within cell and tissue microenvironments, whether these are in healthy or diseased states. Based on the strength and mode of the mechanical loading, cells either strive to restore their shape or develop an adaptive mechanism to maintain cell integrity and prevent mechanical damage.^{29,60} The degree of cell deformation and recovery of the compressed cells to their previous state, and whether they show plastic response in form of permanent deformation and develop into a partially or completely damaged state, have been among the phenomena studied using cell compression microdevices.^{23,27,29}

In general, cells under compression display a change in cell morphology, including changes in cell height, area, circularity, and aspect ratio, due to deformation.^{24,25,27,61} Subcellular structures, such as cell nuclei and actin cytoskeleton supporting the cell body, are highly mechanoresponsive to applied forces and tend to distinctly change their morphology under the impact of compression.^{21,28,29,62} For instance, Ho et al. performed volumetric scanning of single adherent cells being flattened and observed a decrease

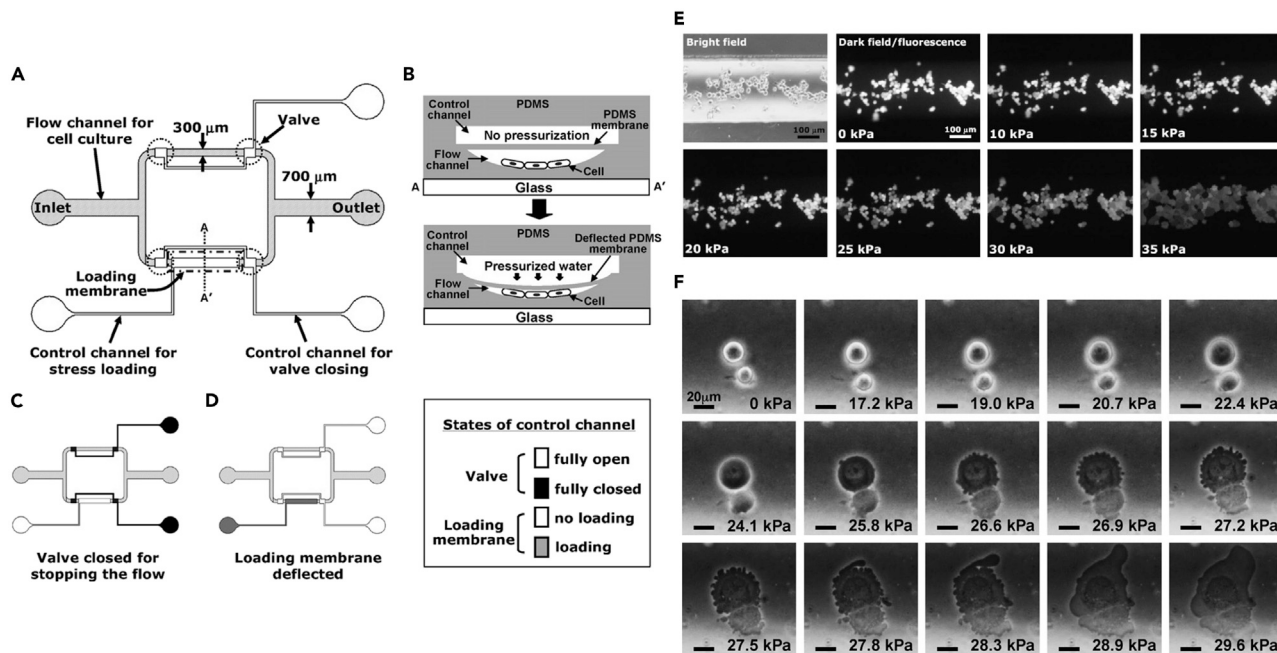


Figure 5. Microfluidic biomechanical device used for cell compression and lysis application

(A) Top view schematic of the microfluidic device. Of the two parallel channels, the lower one was used for the application of stress and the upper one for comparison as control.

(B) Schematic of pressure application through the control channel and PDMS loading membrane.

(C) Cell culture flow was stopped by the closure of four on-chip valves to facilitate cell attachment in microchannels.

(D) When the valve applying pressure through the control channel was open, the loading membrane deflected to directly contact and compress the cells.

(E) Change of fluorescence intensity of MCF7 cells stained with calcein AM recorded in response to applied compressive stress.

(F) High magnification imaging of the compression and lysis event showing the radial expansion of MCF7 cells, appearance of small bulges, and rupture of the cell membrane. Reproduced, with permission, from.²

in cell height, mainly contributed to by a change in volume of the nuclei region and cytoskeleton when increasing pressures were applied in their aforementioned microfluidic device.²⁷ Other studies have since shown the nucleus to be a mechanosensitive organelle^{62–64} which can respond to solid stress. This has further been confirmed by the investigation of nuclear deformations in ovarian cancer cells exposed to compression at increasingly higher pressures.²⁸ In general, solid stress-induced nuclear deformations are thought to impact the activity of nuclear pore complexes (NPCs) and associated proteins. Such nuclear perturbations modulate the nuclear import of transcription factors through NPCs and, in turn, alter the gene expression and induction of DNA repair programs.⁶⁵

In addition to cell deformation, mechanical compression can also induce cell lysis depending on the strength and duration of the mechanical loading.^{2,28} Mechanically induced cell lysis can naturally occur in the body during necrosis of cancer cells as briefly discussed in Section [mechanical compression on cells and tissue microenvironments](#). Application of cell deformation and lysis in a controlled manner has been achieved using microfluidics-based flexible microdevices operating at various pressures. An example of such a microfluidic biomechanical device to study the effects of compressive cell stimulation and lysis was developed by Kim et al.² Their device was composed of a fluid channel for cell culture, an on-off valve for closing said fluid channel and a control channel for valve closing, as well as another control channel and PDMS thin membrane for stress loading (Figures 5A–5D). In the device, the fluid channel itself was composed of PDMS and had a rounded cross-section. To enhance cell attachment to PDMS, the channel surface was modified with fibronectin. On-chip on-off valves were used to retain the cells and facilitate their attachment in the microchannel. The loading PDMS membrane between the fluid channel and stress loading control channel, fabricated using multilayer soft lithography, was then deflected via the control channel to apply mechanical stress on the adherent cells. As a proof of concept for the device, compressive stress was applied to mammary gland epithelial (MCF7 breast cancer) cells and their viability was tested by

the change in calcein AM fluorescence (Figure 5E). Cells were shown to deform and lyse when compressed by the deflected membrane (Figure 5F).

In the biomechanical device by Kim et al.,² the cell culture channel was rounded deliberately to match the deflected top membrane for more consistent compression (Figure 5). This was achieved by reflow of positive photoresist into a rounded channel shape, which in turn resulted in a rounded cross-section in the PDMS channel on which the cells attached after modification by fibronectin. Although the rounded cross-sections of the cell culture channel and deflected membrane could match and provide direct contact with cells, this design is disadvantageous when wide-field microscopy is used. In addition, PDMS surface modifications were needed for different cell types, as PDMS does not fully support the attachment of mammalian cells without further modifications.⁶⁶

This is in comparison to the micro-piston device developed by Onal et al.,²⁸ in which the cell culture channel was enclosed with a flat glass surface on which cells were cultured (Figure 6). A direct contact between cells and the compressing unit was obtained by the use of micro-pistons. These had a flat surface at the free end suspended into the cell culture channel and were monolithically attached to a deflectable PDMS membrane on the other end. The micro-piston actuation, and thus compression of the cells in the channel, was externally controlled via a top microchannel and pressure supply unit. The flat glass surface supported advanced imaging and allowed various cell types to be cultured, as well as protein solutions and matrices to be coated. Localized mechanical compression and lysis of cancer cells were achieved by the micro-piston device. Dynamic application of compression on cell monolayers was demonstrated by deforming and lysing cells under the micro-pistons. Cell viability and cellular mechanobiology could be directly compared between compressed cells under the micro-piston and control (non-compressed) cells in regions adjacent to the latter within the same device (Figures 6E and 6F).

In itself, mechanical lysis has been applied to provide high throughput and higher efficiency in lysing cells compared to the other lysis methods such as chemical lysis, by lytic agents, or electrical lysis, by applied electrical fields.^{2,67} When applied in a controlled manner, mechanical lysis allows for intracellular contents to be retained in the sample and rapid cell-based assays to be run after compression.² As such, there has been a need to develop mechanical tools to control cell lysis in microenvironments. This can be achieved in LOC settings for a targeted extent and timing of mechanical lysis of cells via compression. To this end, Onal et al. demonstrated that their compression platform could be applicable for the controlled release of intracellular components, such as nucleic acids and proteins, by adjusting the degree of mechanical disruption of the cell membranes. Furthermore, cells surrounding those being lysed remained intact due to the use of a micro-piston for localized compression and lysis in their device, which is a unique functionality to mimic the partial cellular deformation and damage that can occur *in vivo* in presence of a localized mechanical loading.^{28,29} The latter in particular enables new applications compared to mechanical compression and lysis in the entirety of a microfluidic channel, such as possible with the device developed by Kim et al.²

COMPRESSION IN CANCER MICROENVIRONMENTS

The role of gene mutations in cancer has been studied extensively and cancer development has been proposed to be dependent on an accumulation of multiple mutations. Recently however, cancer has been redefined to be not only a disease of genetic mutations, as evidence has grown on that the micro- and nano-environments of cells may be essential factors in triggering tumor growth.⁶⁸ For instance, breast tumorigenesis can initiate due to dysfunctional collagen crosslinking in the extracellular matrix (ECM), which can modulate ECM stiffness, forcing focal adhesions and integrin expression, and in turn lead to breast malignancy.⁶⁹ While the surrounding microenvironment or genetic and epigenetic background of cells can inevitably induce tumor initiation,⁶⁸ metastasis remains the main cause of death in patients with cancer.⁷⁰ Recent research has revealed that mechanical forces from the changes in cell and ECM mechanics can induce tumorigenic and metastatic events.^{1,14,34,68} As alluded to in the introduction, solid stress, matrix mechanics, interstitial pressure and flow are among biomechanical forces which regulate the tumor microenvironment.³⁴ Primary tumor growth, and subsequent spread, invasion or metastasis are facilitated by altered biophysical properties and forces of cancer cells.^{1,71} All these examples point to that development and spread of cancer can be promoted by changes in the mechanobiological profile of cells and their microenvironment (a mutation-independent element).⁷² Thus, further investigations are needed to quantitatively define the nature and level of mechanical forces that influence the interactions between physical micro- and nano-environment and cancer cells.^{28,71}

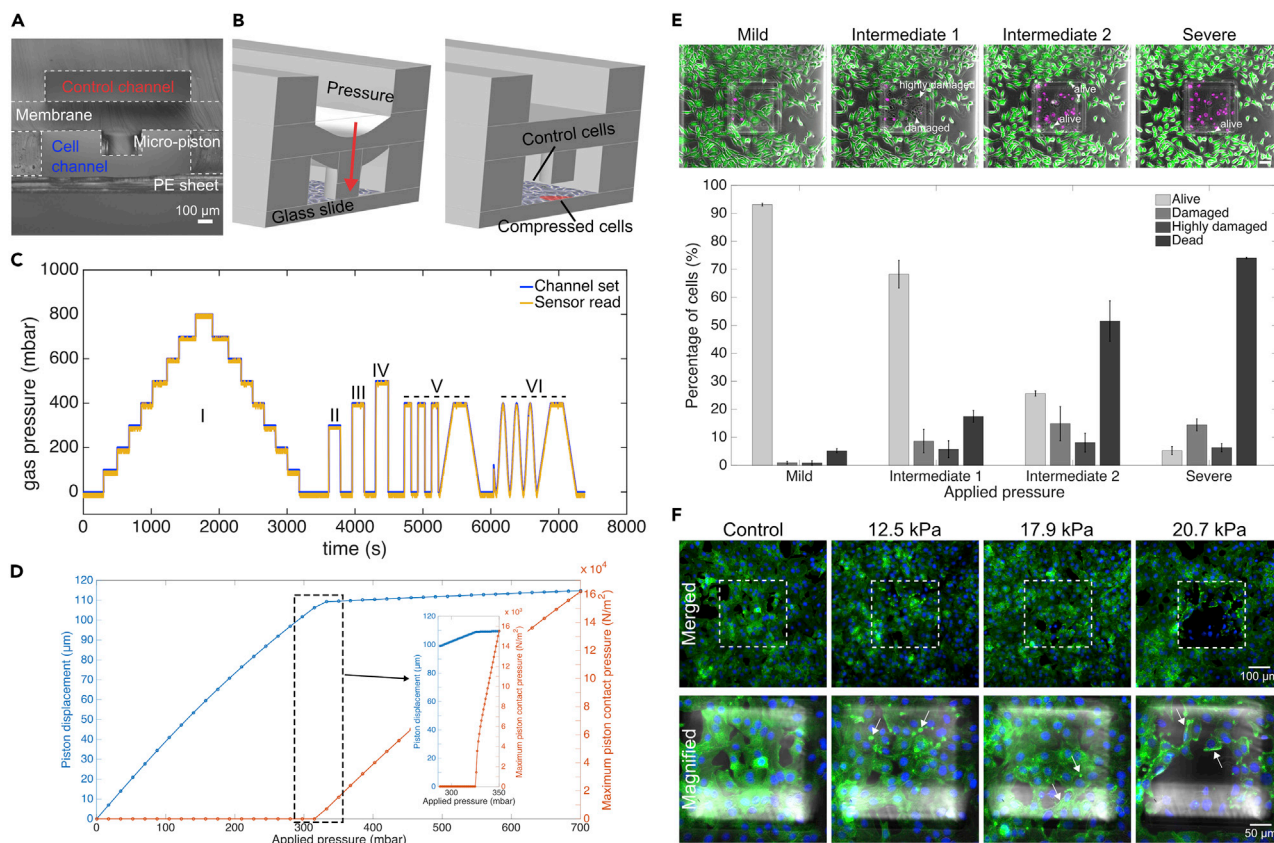


Figure 6. Device design, application of compression, and cell response in a flexible microdevice

(A) Cross-section view showing compartments of the PDMS micro-piston device (Scale 100 μm).

(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) A summary of the characterization of different compression profiles. Micro-piston actuation with various pressure magnitudes and loading profiles (I–VI) for a 215 μm membrane attached to 300 μm diameter piston, generated by a pressure controller system.

(D) 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 externally applied gas pressure (boundary load).

(E) Summary of cancer cell response under micro-piston to varying applied piston contact pressures in ascending order from Mild (15.6–15.9 kPa) to Intermediate 1 (23.8–26.8 kPa), to Intermediate 2 (37.8–51 kPa) and Severe (127.8–140 kPa) out of cyclic compression experiments using micro-piston devices operated in a continuous manner.

(F) Representative fluorescent microscopy images and analysis for actin and nuclei of cancer cells that experienced 1 h-long cyclic compressions in the micro-piston device. Control and compressed cell groups stained for actin (green) and nuclei (blue). Dashed areas are under micro-pistons, while the surrounding is the control region. Representative arrows (white) show distinct actin deformations indicated by the increased fluorescence signals at the edges of the cells in the compressed groups under the micro-piston. Reproduced, with permission, from.²⁸

Biomechanics is well-studied for cells in tissues that have explicit biomechanical properties, such as vasculature, muscle, and cartilage. Less is known about cancer cells even though these also respond to mechanical forces just like every cell type in the body.³⁴ Among the mechanobiological aspects of cancer, the influence of compressive forces in particular has been largely neglected until very recently. Mechanical compression has been known to contribute to shaping physiological structure and function within cell and tissue microenvironments.^{34,35} As such, there is a significant need to investigate the impact of external compressive forces on cancer cells. Whilst bulk compression systems have provided invaluable insights, a novel and comprehensive way for the field would be to use flexible microdevices to better mimic the physiological physical microenvironment and force values.

Helmlinger et al. first revealed the role of solid stress in inhibiting the growth of tumor spheroids cultured in increasing concentrations of the agarose gels.⁷³ The stress-induced growth inhibition of tumor spheroids

was measured as a function of increasing initial stiffness of the embedding matrix. The growth-inhibiting stress was found to be 45 to 120 mmHg.⁷³ It was further shown in human-tumor xenografts grown in mice that such solid stresses can compress and collapse intratumor vessels *in vivo*. This, in turn, can cause impaired function of the blood and lymphatic vessels, and subsequently reduced efficacy of drug delivery inside the tumor. Indeed, reducing compressive forces and decompressing vessels proved to be a promising strategy to enhance the delivery of the therapeutics to intratumor sites.⁷⁴ In this context, better understanding of compression events in the body and the tumor microenvironment could help to develop therapeutic strategies to alleviate stress on vessels, as well as tumor and stromal tissue.

To date, compression has been applied to the models of various cancer types, such as breast,^{14,44–46} brain,⁴⁰ pancreatic,¹⁵ and ovarian^{21,47} cancers. These compression applications on cancer cells have generally resulted in more invasive and metastatic forms. Conversely, Ricca et al. showed that single malignant breast cells in laminin-rich ECM (lrECM) formed acinar-like structures after being stimulated with brief transient compression, a phenomenon called “mechanical reversion” which occurred above a threshold determined as 15% of compressive strain in their study.⁷⁵ This reversion was mediated by compression-induced nitric oxide production in malignant cells. It has since been proposed that external forces enable cells to revert their cell-lrECM engagement and signaling which are lost during malignancy, and to re-establish normal-like tissue architecture.⁷⁵ The methodological approach used by Ricca et al. included gel embedding of cells in custom-made PDMS wells that were pre-stretched using laser-cut acrylic frames and stainless steel dowel pins. The applied strain ranged from 0% to 23% compression depending on the amount of initial stretch, which was varied by the dimensions of the custom-made acrylic frames.⁷⁵ Thus, their method works in off-chip settings.

While the findings so far are indicative that compression plays an important role in cancer metastasis, the effect of compressive mechanical stimuli in different cancer types continues to require further investigation. For instance, ovarian cancer cells are exposed to compressive stress mainly by tumor growth, native tissue and hydrostatic pressure from the ascites.⁷⁶ This chronic stress induces ovarian cancer dissemination via direct extension of cells and multicellular aggregates from the primary tumor into the peritoneal cavity, adhering to and retracting peritoneal mesothelium and proliferating in the submesothelial matrix to form metastatic lesions.¹⁹ As such, it is crucial to gain better understanding of the impact of compression in epithelial ovarian cancer with intraperitoneal metastatic dissemination. This may be why the first studies exposing ovarian cancer cells to chronic mechanical forces have recently appeared.^{47,76} For example, Novak et al. applied cyclic compression on ovarian cancer cells using a bioreactor to simulate the chronic stress cells experience during ovarian cancer metastasis.⁴⁷ This work can be further compared to Asem et al.²¹ and Klymenko et al.,¹⁹ who applied static compression at ~3 kPa and 3.18–3.53 kPa, respectively, to ovarian cancer models in off-chip settings using the aforementioned BioPress compression culture plates. As such, the work by Novak et al. may be the first study to date that used a 3D bioreactor device (Figure 7), albeit at a millimeter scale, to apply compressive stress to cancer cell-laden hydrogels and in turn investigate the effect of said compression in ovarian cancer.⁴⁷ The dynamic *in vitro* platform could stimulate cells with confined cyclic or static compressive stresses, for 24 and 72 h, at 3.9–6.5 kPa cyclic loading regimes or 5.2 kPa static compression. Such a study of compression in ovarian cancer is important, as it is thought that cyclic compression applied on ovarian cancer cells can simulate the chronic stress the cells are experiencing in metastasis.^{47,76} Using this platform, it was observed that the aspect ratio of ovarian cancer cells increased when cells were exposed to compressive stimulation. This change in cell elongation indicated invasive cellular morphology and ovarian cancer metastasis. ki67 and caspase-3 staining of the cells showed increased proliferation and reduced cell death in compressed cultures compared to control. Chemoresistance to standard cancer drug treatment with paclitaxel and carboplatin also increased in an upregulated CDC42-dependent manner under compression. When CDC42 was inhibited with the CDC42 inhibitor ML141, these cellular responses under compression were inverse such that cell proliferation was significantly reduced and cell death was increased. Overall, findings with this dynamic *in vitro* 3D compression platform point to the need to incorporate compressive stimulation in cancer biology and therapeutic development studies.⁴⁷ More recently, applied cyclic pressures were further expanded on from those used by Novak et al.⁴⁷ to a more physiologically relevant range of 3.7–18.9 kPa, and higher, as predicted to occur in human tumors,^{42,76} by Onal et al. using a flexible microdevice-based sequential cyclic compression method.^{28,29,77} The improved understanding of the impact of compressive forces on cells brought about by such studies may in the future contribute to the development of pharmaceuticals for signal transduction mechanisms associated with mechanical stimulation^{15,47} and mechanical treatment^{17,42} in form of so-called “mechanoceuticals.”

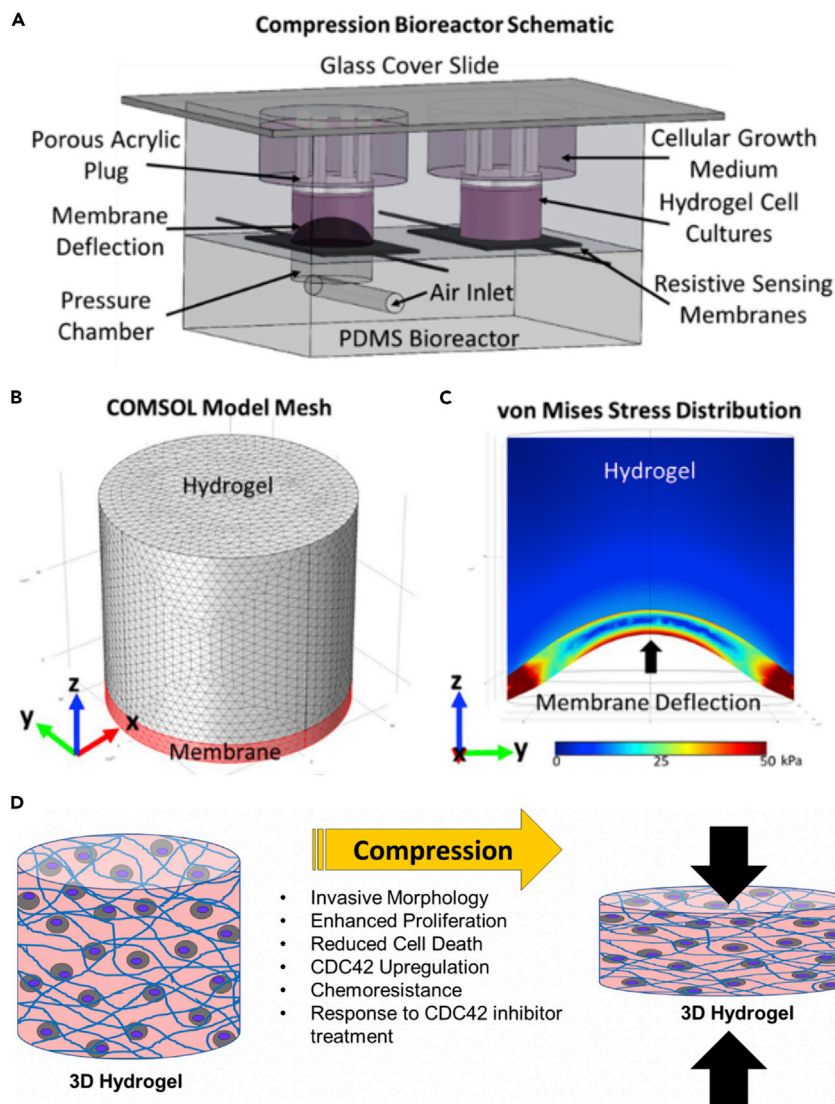


Figure 7. Example of 3D compression of cancer cells

(A) Schematic of ovarian cancer compression bioreactor. The pressure chamber underneath the hydrogel cell cultures was operated by pumping air to deflect the membrane and compress cancer laden interpenetrating hydrogel. A porous acrylic plug held the 3D cellular hydrogel while supplying the cellular growth medium from the top of the chamber. (B) COMSOL model mesh of the hydrogel and deflectable membrane, representing the setup in the bioreactor and used for computational modeling of cell compression in 3D. (C) Sample output of COMSOL analysis for the deformation of the membrane and hydrogel with an applied pressure of 20 kPa. Compressive stress within the deformed hydrogel was 5.2 kPa on average. (D) Ovarian cancer cells exposed to the compressive stimulus within 3D hydrogel showed invasive morphology, enhanced proliferation, and reduced cell death. In addition, CDC42 was upregulated and chemoresistance to standard ovarian cancer drug treatments increased, while treatment with the CDC42 inhibitor facilitated chemotherapeutic response. Reproduced, with permission, from.⁴⁷

Force application on 2D vs. 3D cancer models

Compressive force application to cancer, epithelial and endothelial cell monolayers have significantly advanced our understanding of fundamental cell behavior. However, in physiological conditions, cancer cells are surrounded by and interact with extracellular matrix and stromal cell types. Such interactions can be only partially mimicked in 2D cell culture monolayers to achieve physiological relevance. Studies elaborating on the multidimensionality of cancer have shown that cellular phenotype, cell invasion and migration, cell signaling, and cell response to drugs are different between 2D and 3D cell culture models even when consisting of the

same cell types.^{68,78,79} These cellular events are organized by the physical forces in the microenvironment⁷¹ and provide a feedback to externally applied forces. Such differences in cell response are also expected to emerge between 2D and 3D cell culture models under external force application.

Although cancer cells can create 2.5D or 3D cultures by forming their own matrix in long-term cultures,^{80–82} microdevices need to be specially modified to run fully 3D cancer cell culture compression. Such a 3D microfluidic compression setup would need to adapt a design for continuous media supply, to maintain a culture when hydrogels, such as Matrigel, are used in high concentrations with the embedded cells. This is typically not necessary for hydrogels used at low concentrations for surface coatings or for spheroids. In itself, the principle of applying compression on 3D models with microdevices might not necessitate significant changes. However, it can be expected that cells may not exhibit the same response to the same applied pressures in 2D versus 3D. Hydrogels and other cell layers that affect the response of cells in 3D cultures are typically far from and thus less affected by the compression source. Thus, if cancer cell death and lysis need to be induced, the amount of pressure that one needs to apply in a 3D setting might differ from that in the 2D culture environment. Currently, the limited number of studies published on 3D cancer cell compression means that it is difficult to compare these to 2D. Of the examples that do exist, Novak et al. applied a low-pressure amount that was calculated to be on average 5.2 kPa of compressive stress within hydrogels containing OVCAR3 and OVSAHO ovarian cancer cells over 24–72 h. Using this method, reduced cell death and significantly increased proliferation were observed.⁴⁷ In contrast, Klymenko et al. applied a comparatively lower pressure of 22.1 mmHg (~3 kPa) on ovarian cancer multicellular aggregates (MCAs) in hydrogel carriers over 24–96 h. They observed changes in the expression of genes related to epithelial-mesenchymal transition (EMT) and in the dispersal of compressed MCAs on collagen gels.¹⁹ The latter can be considered to suggest that compression facilitates ovarian cancer metastasis by altering cell-cell and cell-matrix adhesions rather than proliferative signaling pathways. Asem et al. also applied a lower pressure of ~3 kPa on OVCAR5 or OVCAR8 cells added atop the mesothelial surface of murine peritoneal explants *ex vivo* for 24 h in a bulk compression system.²¹ As a result, they observed enhanced interaction between peritoneal mesothelial cells and cancer cells via the induction of tunneling nanotubes (TNT), which later lead to metastatic ovarian cancer progression. While this setup did not correspond to a fully 3D cancer model, Asem et al. also indicated the presence of a cell monolayer in the peritoneum where the metastasizing ovarian cancer cells exist.²¹ Thus, the investigation of compression effects on ovarian cancer cells, as well as mesothelial and endothelial cells in 2D, remains of significant interest and crucial to the advancement of the field. Functional microdevices enabling compression applications to be performed in a controlled and dynamic manner in microfluidic settings continue to significantly contribute to the cell compression field. Based on the work to date, the effects of higher compressive stresses on ovarian cancer models in 3D should be tested further.

Comparison of the effect of applied compression on various cancer cell types

The effects of applied compression on cancer cells can vary depending on several parameters such as type of compression setup, amount of applied pressure, mode and duration of compression, and cancer cell type. For instance, Takao et al. applied stresses of 5.1, 9.3, 12.9, and 18.7 kPa on breast cancer cells in dynamic compression with a low frequency (0.1–30 Hz) and for short durations (30–300 s).¹⁷ As a result, they observed a mixed mode of apoptosis and necrosis dominant with necrotic cell death, which was called mechanical stress-induced cell death (MSICD). In this study in which increased apoptosis and necrosis under compression were observed, cells were cultured on plates and agarose cushions were placed between the stress load and cells.¹⁷

This is in contrast to Novak et al., who observed that applied compression enhanced the proliferation of ovarian cancer cells when they were compressed for 24 to 72 h at 5.2 ± 1.6 kPa in cyclic loading mode with a frequency of 0.5 Hz and at 5.2 kPa in static mode.⁴⁷ Both cyclic and static compression were performed using cancer cell-laden 3D hydrogel components in their bioreactor device. The study illustrated that, under a physiological compressive stimulus, the proliferation capacity of cells increased while apoptosis decreased.

Regarding the effect of compression on cell death, other studies exist which show that compression can induce apoptosis in spheroids, thus decreasing the proliferation in breast cancer cells.¹³ In general, it is thought that spheroids constitute a cancer model that mimics the tumor in the body well. Investigating this, Cheng et al. used an *in vitro* compression device and observed increased apoptosis in murine mammary

carcinoma cells with increasing stress levels ranging from 0 to 60 mmHg (0-8 kPa) in monolayers of cells compressed for 17 h and in spheroids embedded in 1% agarose gel compressed for up to 7 h.¹³ The compression time for spheroids was deliberately shortened to ensure that apoptosis levels were due to externally applied compression and not a potential impact of nutrient/growth factor/oxygen limitations in the 3D culture.

In other work, Alessandri et al. developed a microfluidic technique with which they grew multicellular spheroids (MCSs) of CT26 mouse colon carcinoma cells in permeable elastic capsules.⁸³ This encapsulation controlled the size of the MCSs by creating confined conditions. Although confinement is not an applied dynamic compression, it can be considered a type of force application that can create solid stress on tumor models. In this study, confined spheroids exhibited necrotic cores compared to unconfined spheroids, while peripheral cells of the MCSs were more proliferative and migratory. This result proposes cell invasion out of a growing tumor to be triggered by the external stress from the surrounding microenvironment. Similarly, Desmaison et al. showed the effect of mechanical confinement of growing spheroids of HCT 116 human colon carcinoma cells, which translates into solid stress, on tumor growth and cancer cell mitosis by developing a channel-shape PDMS device. Their device included reservoirs that allowed spheroids to access nutrients homogeneously.⁸⁴ Cells within the body region of spheroids became arrested at mitosis by the negative effect of mechanical confinement on the bipolar spindle assembly. This impaired mitotic progression varied cell proliferation between the body and tips of confined spheroids. The presence of proliferative cells in the innermost cell layers was confirmed by the absence of hypoxia in spheroids with mechanically confined growth conditions. Thus, it could be concluded that such confinement created modifications in both proliferation gradients and hypoxia.⁸⁴

Apart from compression produced by a mechanical loading unit, such as a piston, and confinement through physical constraints, indirect restriction of the volume of multicellular spheroids (MCSs) can also be used to apply compression on cells. To achieve this, Delarue et al. cultured MCSs of various cell lines including colon carcinoma, breast cancer, and sarcoma cells and supplemented the culture medium of MCSs with dextran which is a biopolymer that does not penetrate single cells.⁸⁵ Dextran addition into media exerted moderate osmotic stress directly on the outermost layer of cells in spheroids. This osmotic stress was transmitted to the inner cells of spheroids as mechanical compressive stress, which in turn reduced the overall volume of the MCSs. Compressive stress applied through such volume limitation inhibited cell proliferation in tumor MCSs. A similar setup was constructed by interposing a dialysis membrane between the MCSs and media with dextran, upon which the MCSs of CT26 mouse colon carcinoma cells were mechanically compressed by stress transmitted from the dialysis membrane to inner cells via osmotic stress at the outermost layer of the cells.^{86,87} In either method, with or without dialysis membrane, the volume of the MCS was reduced by the applied stress. Although this osmotic effect by dextran did not originate from high osmolarity, such as produced by salts, compressive stress in these setups emerged as network stress by an osmotic origin, which impacted the tumor growth rate and MCS volume. The resultant compressive stress was estimated to be 5 kPa or 10 kPa based on the concentration of dextran added to the culture medium. Thus, it can be said that the compressive stress transmission on the cells was not the product of a contacting physical surface, for example, a flexible solid polymer (e.g. PDMS). Furthermore, this type of compression was also statically applied in bulk in 48-well plates, unlike the controlled and dynamic manner that can be achieved in microfluidic devices.

As exemplified, particular differences observed with the different compression platforms and force application strategies employed in each study have the potential to translate into differences in biological results. Thus, although not directly comparable, the combined study results illustrate that, similar to in the body, applied compression can have varying effects on cancer cells, especially if compressive stress is applied in physiological values. Indeed, compression effects have been proposed to vary among patients for the same cancer type. For instance, hydrostatic pressure from excess fluid and ascites translates into compressive forces of different magnitudes depending on the volume of ascitic fluid in individual patients with ovarian cancer.⁷⁶

In summary, we have presented a number of studies that show that the impact of compression on cancer cells can vary based on the respective force application method. Examples of this are illustrated by the variations in the proliferation and apoptosis response of cancer cells observed after being compressed at various settings in the studies summarized in this section.^{13,17,47,83-87} The varying impacts compression can have on cancer, such as through metastasis or mechanical reversion of malignancy, have also been

highlighted in the previous sections.^{14,15,19,21,40,44–47,75} Additional information about *in vitro* and *in vivo* studies investigating the effect of solid stress in tumor progression can be found in the review by Kalli et al.⁸⁸ While their review does not feature microfluidic platforms for applying compression on-chip on cancer cells and other living cells, the authors do discuss microfluidic methods related to mechanical confinement through either encapsulation and growth of cancer cells inside permeable and elastic microspheres⁸³ or spatial restriction of spheroid growth within PDMS channels,⁸⁴ thus creating static solid stress on tumors. Finally, we emphasize that although current cancer cell compression studies have improved our understanding in the field, more advanced microdevices may well be needed to reap the full benefits of applying mechanical compression in controlled, dynamic, and high-throughput manner, all while mimicking physiological conditions for various cell types and thus improving study comparability.

CONCLUSIONS AND PERSPECTIVES

As this review has highlighted, a growing number of microfluidics-based flexible microdevices are being developed and used for novel and functional cell compression applications on living cells. These systems promise process automation, readability through pressure sensors, and portability of the pressure application. Live compression of cells in real-time can readily be achieved. In the future, using well-defined pressure supply and sensing methods for force feedback should further increase the uptake of flexible microdevice-based cell compression platforms. As discussed, compression studies in the bulk setting of conventional cell-culture methods have produced valuable insights into biomechanics of the living tissues; however, these do not always fully recreate forces present in the microenvironments of living cells and tissues. Microfluidics, on the other hand, enable the design of LOC systems that mimic *in vivo* microenvironments in *in vitro* culture systems to spatially and temporally control the application of force. In discussing these systems, this review also emphasizes that the triggering mechanism of cancer is influenced by the mechanical forces which are present and applied in the microenvironment surrounding the cells. Better understanding of the role of compressive forces in cancer can aid in developing more effective methods to prevent cancer progression. Based on current studies and future perspectives, it can be concluded that microfluidic platforms can provide controlled mechanical tools to drive the study of the direct effects of compressive forces on living cells. In particular, this is illustrated by the recent studies on cancer cell compression discussed here, which have begun to uncover the role compressive forces play in shaping cancer mechanobiology. The presented comparison of cell compression studies and their results should act as an encouragement for the development of even more advanced flexible microdevices to further cell compression applications. Specifically, this will help to better understand the influence of compression in cancer and to progress applications of compressive stimulation in cancer mechanobiology studies. Defining the impact of compression in healthy and diseased states may contribute to the development of therapeutics to target mechanotransduction pathways with pharmaceuticals alone or their synergistic impact in combination with mechanical treatment, also called mechanocuticals.

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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