Development of a 3D Microfluidic Platform for Dynamic Compression of Tumor Spheroids

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Principal Investigator(s): Dr. Mingming Wu

User(s): Young Joon Suh

Affiliation(s): Department of Biological and Environmental Engineering, Cornell University

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Contact: mw272@cornell.edu, ys668@cornell.edu

Website: biofluidics.bee.cornell.edu

Primary CNF Tools Used: Heidelberg Mask Writer – DWL2000, ABM Contact Aligner, P7 Profilometer, MVD100, SUEX Laminator, DISCO Dicing Saw, YES EcoClean Asher, Unaxis 770 Deep Si Etcher, Plasma-Therm Deep Si Etcher, Oxford 81 Etcher, Oxford PECVD, YES Polyimide Oven, Hamatech Hot Piranha

Abstract:

Solid tumor stress caused by rapid growth of tumor cells and abnormality of vascular vessels has long been associated with a poor prognosis of cancer. However, understanding of tumor mechanics has been limited largely to single cells under static compressive loads. In this study, we have developed a high-throughput microfluidic platform providing well-controlled dynamic compression to tumor spheroids.

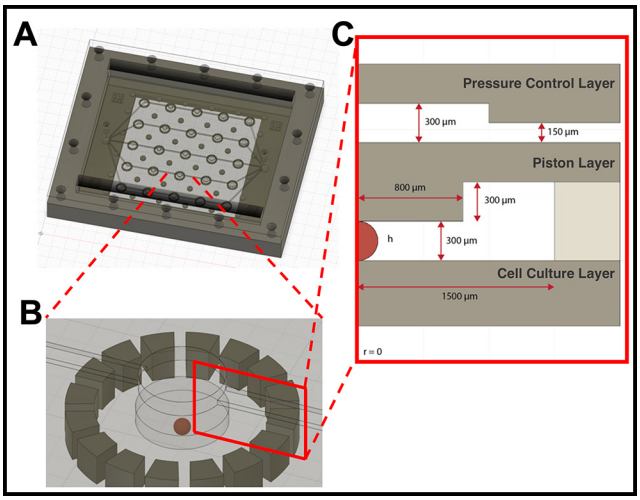

Figure 1: A 3D microfluidic platform for controlled compression of tumor spheroids. (A) An array of 5 × 5 microfluidic compression units. A schematic of an assembled device. Tumor spheroids embedded ECM is loaded into the lower cell culture layer, and pressure regulation media is introduced into the top compression layer. Each column provides five repeats of the same compression magnitude while each row provides five different compression magnitudes. (B) A close-up view of one compression unit. (C) Cross-section view of one compression unit with the dimensions. The PDMS piston is 1600 µm in diameter and the initial distance between the piston and the bottom of the well, h, is 300 µm. The diameters of the five pressure control chambers are 0, 1500, 2000, 2500 to 3000 µm.

Summary of Research:

A 5 × 5 array microfluidic compression device for tumor mechanics studies was designed (see Figure 1). The device consists of three layers: (1) cell culture layer, where the tumor spheroids embedded in extracellular matrices (ECM) are loaded; (2) PDMS piston layer, which is a PDMS membrane that has a top hat shape; and (3) a pressure control layer, which can push the PDMS piston down to apply compressive forces on the tumor spheroids. The three layers are then sandwiched between a Plexiglass® top cover and a stainless-steel frame to provide a good seal. COMSOL modeling has been used to calculate the displacement of the PDMS piston and the force applied on the tumor spheroids at pressure ranging from 0 to 7000 Pa (Figure 2). When pressure is applied in the pressure control chamber, the PDMS piston moves down a distance of Δh, applying a force on the tumor spheroids underneath, and leads to a well-controlled compressive strain, Δh/h, on the spheroids. This device can accommodate tumor spheroids of Young's modulus of about 1250 Pa, that are 100-600 µm in diameter for up to compressive strain (Δh/h) of 0.5.

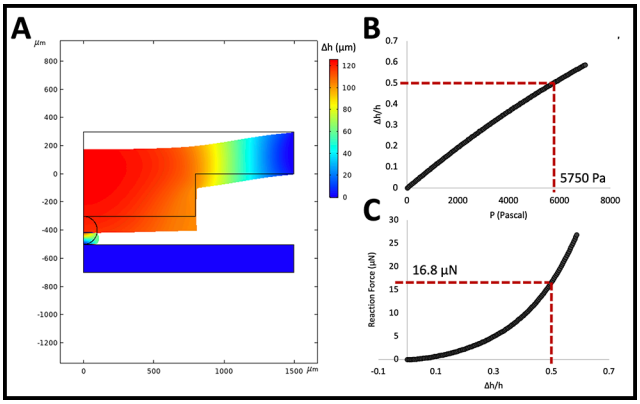

Figure 2: COMSOL computation of a microfluidic compression unit with a tumor spheroid. (A) A colored rendition of COMSOL calculation result of Δh at 7000 Pa of pressure applied. Here, Δh/h = 0.5 at the location of spheroids. The Young's modulus of spheroid and PDMS is 1250 Pa and 2 MPa, respectively. (B) Compressive strain versus applied pressure with spheroid in place. (C) Compression force on spheroid versus compression strain, Δh/h.

Fabrication:

Three layers of the device were fabricated separately. *The cell culture layer* consists of SU-8 wells 600 μm in depth on a 500 μm thick glass (Borofloat®). To fabricate this layer, SU-8 100 was spun on a Borofloat wafer at 475 rpm and soft baked at 95°C for 30 hours. The SU-8 was then exposed to 2310mJ/cm^2 of UV light through a 365 nm filter using an ABM contact aligner. The resist was then post-exposure-baked and developed in the SU-8 developer, followed by a hard bake at 200°C.

The main challenge was to fabricate the height of the wells uniform at 600 μm across the wafer. Keeping the wafer leveled at all steps was found to be crucial.

The piston layer consists of PDMS pistons that are 300 μm in height and 1600 μm in diameter and the PDMS membrane is 300 μm thick. To fabricate the master for this layer, 300 μm wells were etched into a Si wafer. Briefly, 4.5 μm of SPR-220-4.5 was spun on an Si wafer. The resist was then baked at 115°C for 2 mins on a proximity hot plate. Then, it was exposed to the pattern of the pistons at 120 mJ/cm^2 on the ABM contact aligner. After leaving it in room temperature for 30 mins for the post exposure reaction, it was baked at 115°C for 2 mins on a proximity hot plate for the post exposure bake. It was then developed in 726MIF for 120 sec. Then, a mild descum procedure was completed using the Oxford 81 for 90 sec.

Finally, the Si wafer was loaded on the Unaxis 770 Deep Si etcher and a total of 567 loops (200 + 200 + 167) of Bosch process were performed to etch 300 μm into the Si wafer. To remove any excess resist, the wafer was exposed to a strong plasma in a EcoClean Asher. The wafer was then coated with FOTS using the MVD-100 to make the surface hydrophobic. The depth of the piston wells was then measured using the P-7 profilometer.

The pressure control layer is a PDMS membrane with five parallel channels of 200 μm depth. The master is fabricated in a similar way as that for cell culture layer, except that a Si wafer is used instead of a Borofloat wafer.

A 10:1 PDMS was poured and cured on the master molds of the piston and the pressure control layer. After curing the PDMS in a 65°C oven overnight, these two layers were bonded together after plasma treatment and placed in a 90°C oven for 20 mins. Then, these two layers were placed on top of the cell culture layer and sandwiched between a metal frame and a Plexiglass top and connected to a pressure controller.

The compression (Δh) was measured using the defocused particle imaging velocimetry, which was originally developed in our lab [1]. Tumor spheroids were embedded in collagen, which was then introduced into the cell culture chamber. The pressure control chamber is pressurized with a pressure controller. We were able to precisely control the tumor compression with a precision of 1 μm.

References:

[1] Wu, et al., Three-dimensional fluorescent particle tracking at micron-scale using a single camera, Experiments in Fluids, 38, 461 (2005).