

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

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Abstract:

Solid tumor progression is accompanied by changes in both extrinsic and intrinsic mechanical forces within the tumor microenvironment[1]. One of the defining extrinsic mechanical cues is the compressive stress imposed by the surrounding tissue on a growing tumor[1, 2]. To date, most studies investigating mechanical compression have focused primarily on its effects on tumor cell behavior, with relatively little attention given to how compression influences the ability of immune cells to infiltrate and kill tumors[1, 3]. In addition to extrinsic forces, the intrinsic mechanical properties of tumor cells have also been shown to regulate cellular behavior and influence therapeutic responses[4], highlighting the need for improved methods to characterize and quantify the physical properties of tumors. These knowledge gaps persist, in part, because there is a lack of experimental platforms that can both characterize tumor mechanics and support immune–tumor interactions within a physiologically relevant 3D environment. In this study, we developed a microfluidic microrheometer that is able to apply dynamic compression to quantify the intrinsic physical properties of tumor spheroids and investigate how mechanical compression regulates tumor–immune interactions.

Summary of Research:

Summary of Research: A previously developed microfluidic compression platform [5] is used as the foundation for quantifying tumor mechanics and investigating tumor–immune interactions (Fig. 1). The device consists of three functional layers: (1) a cell culture layer containing tumor spheroids and immune cells embedded within a three-dimensional (3D) extracellular matrix (ECM), referred to as Layer 1 (L1);

(2) a top-hat-shaped PDMS membrane that functions as a compression piston, referred to as Layer 2 (L2); and (3) a pressure control layer that uses fluid pressure to deflect the PDMS piston downward and compress the tumor spheroids, referred to as Layer 3 (L3) (Figure 1A). L2 and L3 are plasma-bonded together to form a pressure control unit, which is assembled above L1 and sealed between a Plexiglas manifold and a stainless-steel frame. Application of pressure to the control chamber deflects the PDMS piston downward by a distance, Δh , producing a precisely controlled compressive strain ($\Delta h/h$) on the tumor spheroids (Figure 1B). Figure 1C shows representative brightfield and GFP fluorescence images of a tumor spheroid before and after the application of 120 mbar pressure, illustrating the deformation induced by compression. The relationship between applied pressure and membrane displacement was established by tracking fluorescent beads attached to the underside of the membrane. Detailed calibration procedures and compressive strain calculations have been reported previously[6]. The platform is capable of applying controlled compressive strains of up to 50% to tumor spheroids 100–200 μm in diameter with Young's moduli of approximately 1250 Pa. More recently, we expanded the capabilities of this platform by incorporating a thin polyacrylamide (PAA) gel layer onto the surface of L1, enabling the device to function as a microfluidic microrheometer capable of quantifying the stiffness of intact tumor spheroids[5]. During compression, deformation of the calibrated PAA gel provides a quantitative measure of the compressive force applied to the spheroid. By combining the measured force with the applied strain, the stiffness of the tumor spheroid can be determined. A detailed description of the microrheometer design, calibration, and stiffness calculations has been reported previously[5].

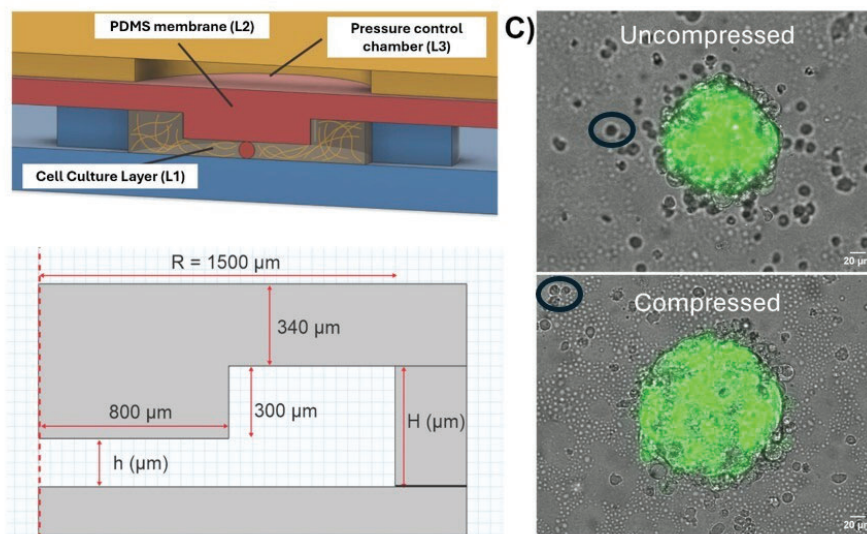


Figure 1: Three-layer PDMS microfluidic device for spheroid compression. A-B) Cross-sectional view of the three layers (initial $h = 200 \mu\text{m}$). C) Representative merged brightfield and GFP fluorescence images of a GFP-labeled MCF-7 breast tumor spheroid with NK cells embedded in 1.5 mg/mL collagen before and after the application of 120 mbar compression. Representative NK cells are indicated by the blue circles (scale = $20 \mu\text{m}$).

Conclusions and Future Steps:

The microfluidic microrheometer provides a unique platform for simultaneously characterizing the mechanical properties of intact tumor spheroids and investigating tumor-immune interactions under controlled mechanical compression. Initial studies demonstrate its ability to quantify the stiffness[5] and viscosity[6] of breast tumor spheroids. Future work will use this platform to determine how compressive stress alters NK cell migration, tumor infiltration, and cytotoxic activity, thereby providing new insights into the role of mechanics in regulating anti-tumor immune responses.

References:

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