Supplementary Figures Legends

Supplementary Fig1 Legend : Variation of cell culture medium temperature as a function of time for different voltages applied with the piezoelectric transducer. Temperature measurements were carried out in a water-filled multi-node chip in a 37°C incubator. The thermocouple was placed in the levitation zone, below the transducer. Values are averaged from 2.00 to 2.03 MHz.

Supplementary Fig2 Legend : 6240 and R633 Patiente Derived Cell Lines grown on conventional (glass) cell culture substratum. Note that 6240 cells spontaneouslu grows as spheroids of various sizes, while R633 cells attaches to the substratum and forms loose spheroids. Scale Bar 200µm

Supplementary Fig3 Legend : A - Picture of GB-PDC tumoroids trapped in acoustic levitation and surounded by BV2 cells just after BV2 injection. Scale bar 200µm. B - Overlay of the bright field and fluorescent images of a tumoroid obtained after injecting fluorescently labelled BV2 microglia cells on GB-PDC tumoroids previously structured for 2 days in acoustic levitation. C - The tumoroids were cultured in “multi-node” acoustofluidic chips for an additional 48 hours prior to be collected, fixed cryosectioned ans stained with DAPI (Blue, Nuclei) and ED-1 (Green, Microglia). Insert illustrates the 2.5D intensity plot of DAPI (nuclei, blue) and ED-1 stained microglia. Cryosection represent an equatorial slice approximately at 150 µm depth from the tumoroid surface. Scale Bar 100µm. Center image : magnification of an infiltrated ED-1 positive (green) microglial cell at the center of GBM (DAPI) tumoroid. Right : Quantification of ED1 intensity in a GBM cryostection. Individual peaks represent absolute ED1 signal intensities of each pixel over the DAPI background (ZEN software, Zeiss). Note the presence of BV2 microglia cells having infiltrated the core of the tumoroid.

Supplementary Data Movie 1 Legend

Movie 1: (a) Measured acoustic pressure with fiber optic hydrophone, as described in Material & Methods, at a frequency of 2MHz for a supplied tension of 5V. (b) Absolute value of the acoustic velocity computed from the acoustic pressure field.

Supplementary Data Movie 2 Legend

Movie 2: Twenty four hours lateral imaging of the Injection of Microglial Cell around preformed GBM tumoroids. Following injection, microglial cells are trapped as rings at the equatorial plane of the GBM tumoroids and progressively self organize around the GBM spheroids.

Fig S1

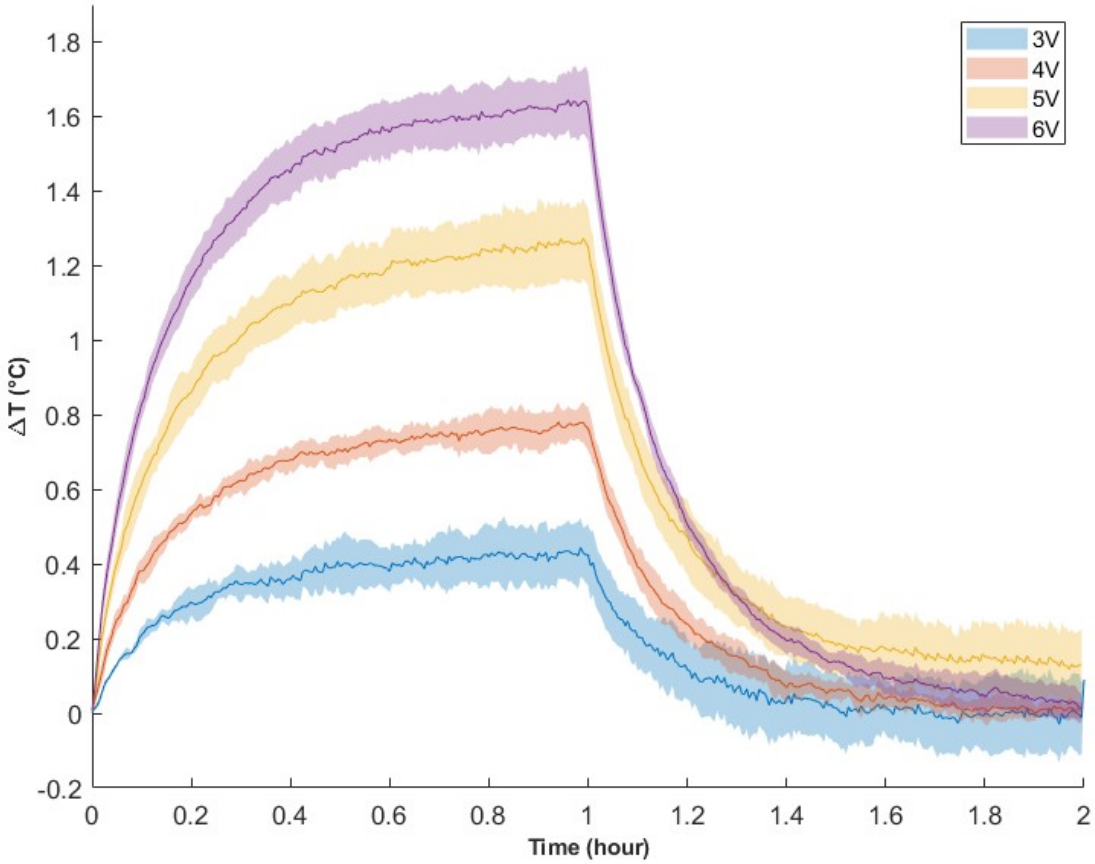
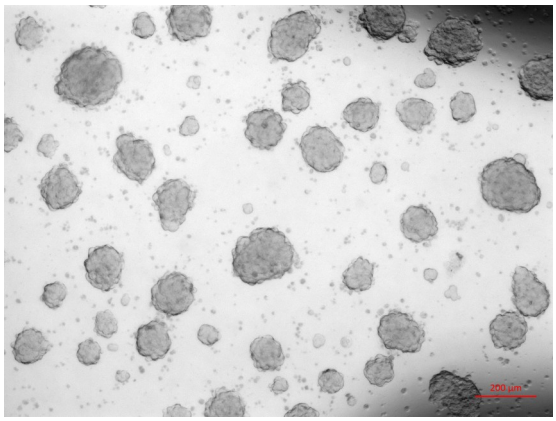

3V
4V
5V
6V
ΔT (°C)
Time (hour)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

Fig S2

6240



R633

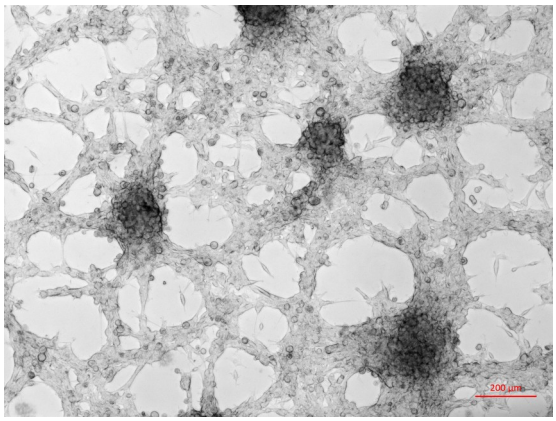
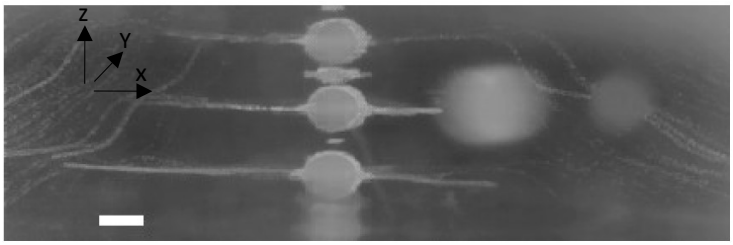
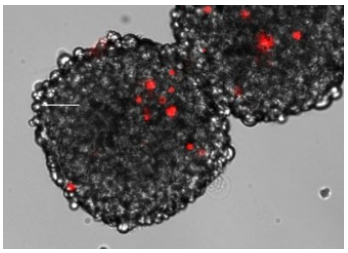


Fig S3

A



B



C