Glioblastoma and nitric oxide: three-dimension model *in vitro*

Afrimzon E, Zurgil N, Shafran Y, Sobolev M, Deutsch M.

The Biophysical Interdisciplinary Schottenstein Center for the Research and Technology of the Cellome,

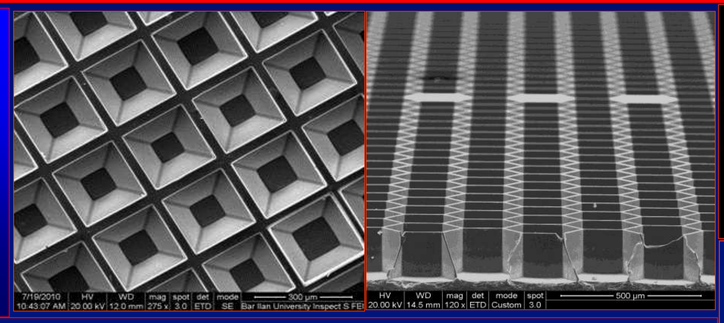
Bar-Ilan University, Ramat Gan, Israel

Abstract

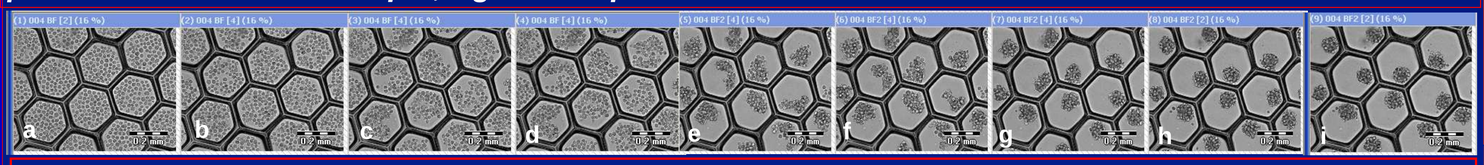
Glioblastoma is a very aggressive brain tumor that responds poorly to treatment and has a lethal outcome of less than 15 months life expectancy. Pathological features of glioblastoma include an active invasiveness, strong angiogenesis, necrosis and high resistance to radio- and chemotherapy. Successful tumor progression and expansion requires the supportive conditions created by both tumor cells and tumor microenvironment, via direct contact as well as remotely, by active molecules. One of the widespread and most important molecules is nitric oxide (NO), an apparent neurotransmitter and which, depending on its level, mediates normal synaptic transmission or neurotoxicity. A better understanding of the role of NO in glioblastoma pathogenesis can provide additional perspectives on the nature of lesions and possible therapeutic approaches.

Spheroid size and volume were easily controlled by the initial number of cells in each microchamber. Spheroid viability evaluated by the morphology change and vital dye staining demonstrated good survival for at least 7 days. DAF FI within 3D spheroids doubled after PMA introduction, in comparison to non-stimulated control (22% in contrast to 9% ,*P*< 0.001) and indicates an intracellular NO level increase accompanied by a change in MMP as well.

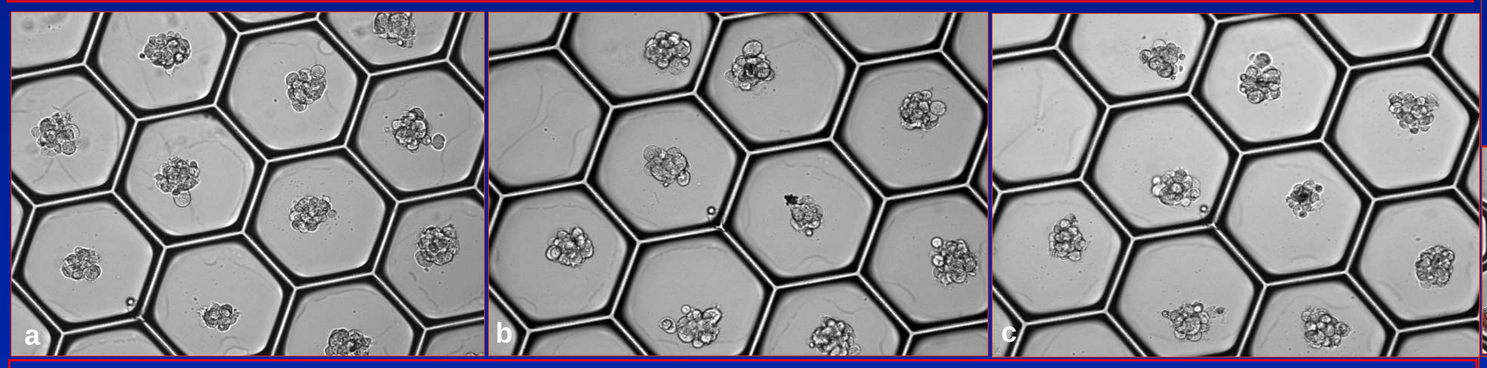
This study proposes a new methodology designed to culture 3D glioblastoma spheroids for in vitro detection of intracellular levels of NO in tumors. Human glioblastoma A-172 cells were grown initially as a monolayer in a standard Petri dish, and then detached. Cell suspension was loaded onto a 250 µm picowell array and then, set aside to allow A-172 spheroid formation in each microchamber.



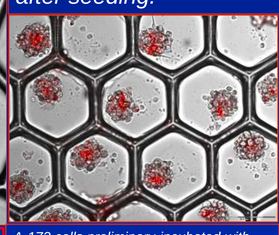
SEM micrograph of the UV adhesive microchambers embossed on a glass surface. Each of the microchamber is designed to accommodate a single spheroid. The microchambers with 130 µm depth (upper view) are presented in the left panel and with 170 µm depth (side view) in the right panel. Scale bar: left -300 µm; right – 500 µm.



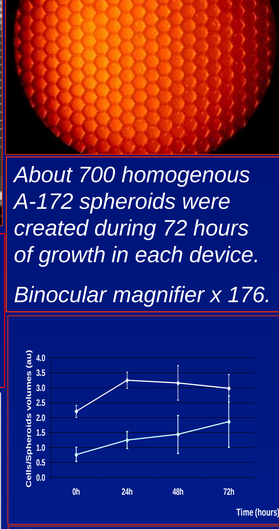
The process of individual spheroid forming. The beginning steps of A172 spheroids forming: (a) cells loaded into microchambers; (b) 2h 30 min cell closing in; (c- d) 5 h -7 h cell initial clustering; (e–f) 12 h- 20 h cell aggregation; (g –h) 24 h cell packing; (i) 32 h early spheroids. Scale bar: 200 µm



The process of the individual spheroid forming (continuation): (a) 24 hours after seeding (note that by this time most cells in each microchamber are already arranged in one light aggregate; (c) Early spheroids 48 hours after seeding. (d) Mature spheroids, 72 hours after seeding.



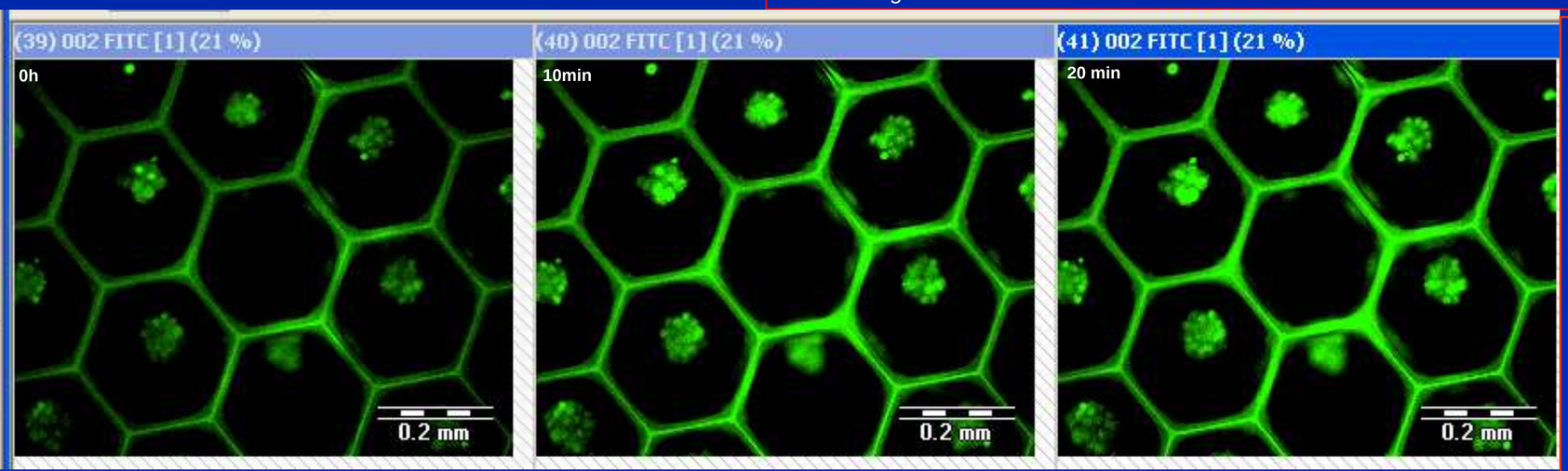
A-172 cells preliminary incubated with HSA- Fe_3O_4 nanoparticles created 3D spheroids. The transmitted light and corresponding fluorescent images are presented as combined image. Scale bar 300 µm.

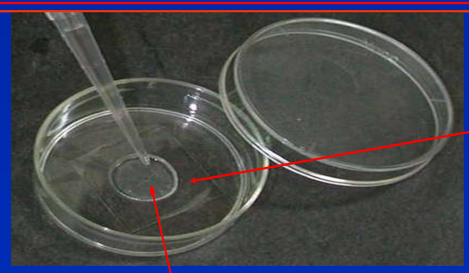


About 700 homogenous A-172 spheroids were created during 72 hours of growth in each device.

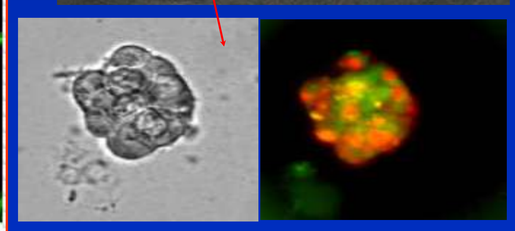
Binocular magnifier x 176.

Effects of the initial number of cells in each microchamber on spheroids' volume. Relationship between the total volume of cells in each microchamber (0 h) and the change of the spheroid volume formed for 24, 48 and 72 hours after seeding.





Endothelial b.End3 cells stained by AO

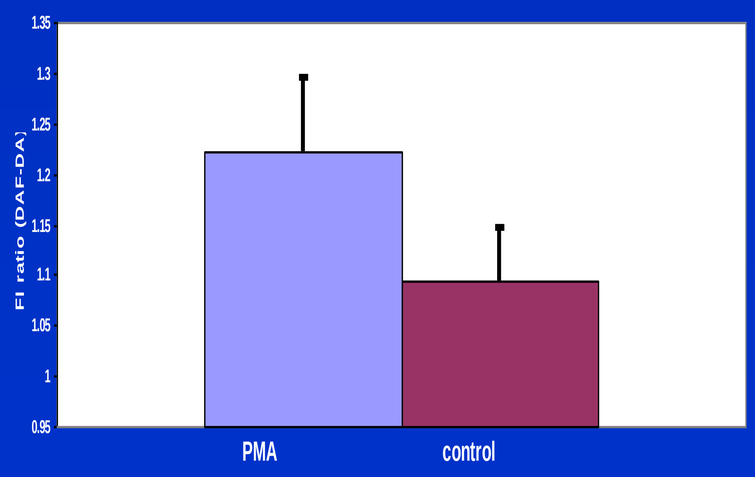


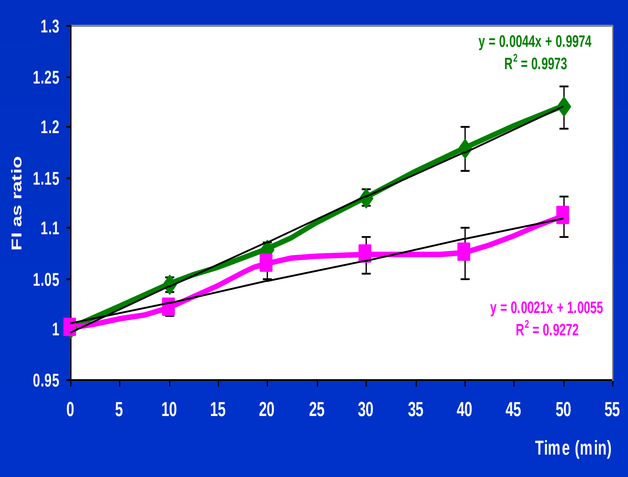
Glioblastoma A172 spheroid stained by acridine orange (AO):

left panel –transmitted light image; right panel – combined image of green and red fluorescence.

Side by- side co-culturing of the glioblastoma 3D spheroids and the stromal cells.

Live-cell imaging device was designed to the separately maintaining of the cells with the common medium in order to monitoring the intercellular distant signaling.





The A-172 3D spheroids were double stained with diaminofluorescein (DAF-DA) dye, and with tetramethylrhodamine methyl ester perchlorate (TMRM) After first image acquisition, phorbol 12-myristate 13-acetate (PMA) was introduced into the device in order to stimulate the tumor spheroid to NO production. The change in fluorescent signals within each individual 3D spheroid was measured by imaging system.

Conclusions

We have developed a new easy used culturing device with a glass-bottomed patterned array of UV adhesive microchambers. Our platform facilitates the creation, culturing, single-spheroid level monitoring, and analyses of large spheroid populations for functional tests and various drug screening. Retrieval of specific spheroids is essential for further analysis and is an efficient screening tool that may be valuable to related fields of research and clinical applications. Preliminary results of the NO screening within individual 3D spheroids demonstrate the ability to study active molecules which are to tumor pathogenesis.

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