The involvement of Nitric Oxide in breast cancer pathogenesis. Image based investigation at the resolution of single cell clusters (spheroids)

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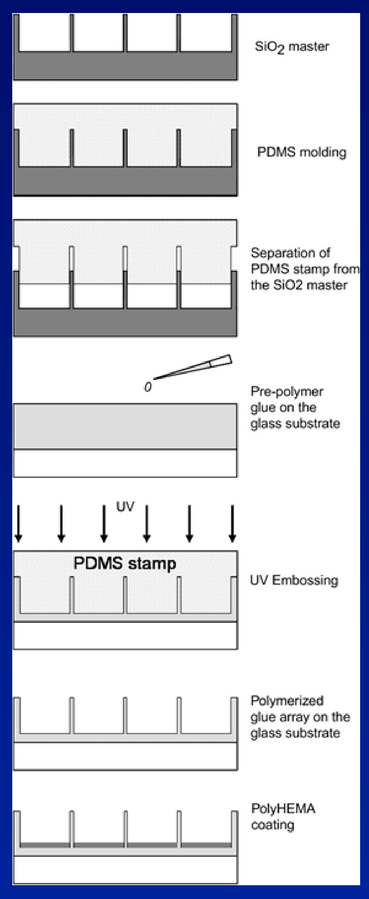
Background

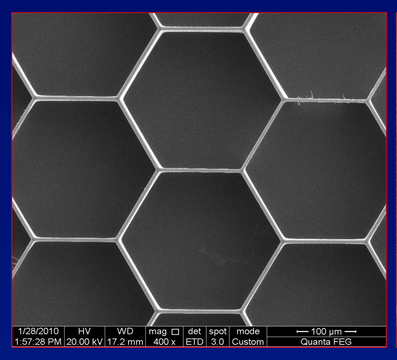
Nitric oxide (NO), a short-lived messenger, modulates a variety of physiological functions which are important for tumor survival and propagation. NO has been reported to have a dual effects on tumor depending on many factors such as tissue type, microenvironment and NO concentration. Recently NO releasing drugs have come into the focus for cancer treatment. Breast cancer is the most common cancer in women. The role of NO in breast cancer is still poorly understood. Study of NO influence on breast tumor could lead to the development of new approaches and strategies for the effective treatment of breast cancer.

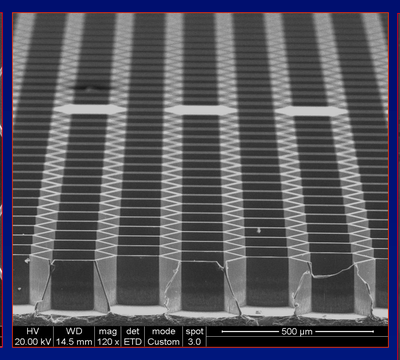
Method

The current study presents a microscopy live cell imaging technology for the kinetic, real time measurements of the formation and growth of MCF7 (estrogen receptor positive breast cancer cell line) 3D structures (spheroids) treated by different concentrations of exogenous NO. Spheroids have been recognized as an advanced model for *in vitro* cancer study as compared to monolayer cell growth. Spheroids are formed in a unique culturing device; a Petri dish equipped with a glass bottom which is embedded with an array of UV adhesive microchambers (MCs). The MCs are coated with polyHEMA in order to prevent cell adhesion to its surface.

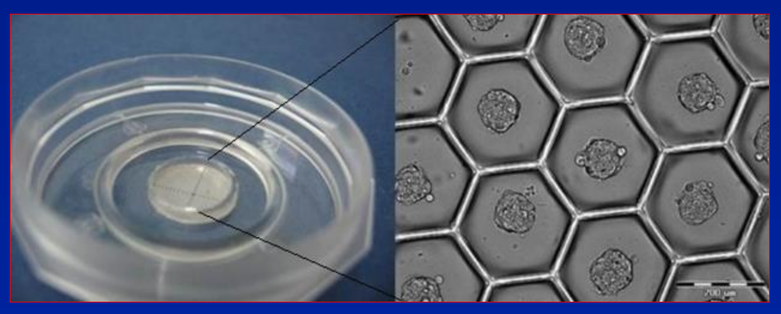


Schematic illustration of the sequence of the MC array fabrication processes

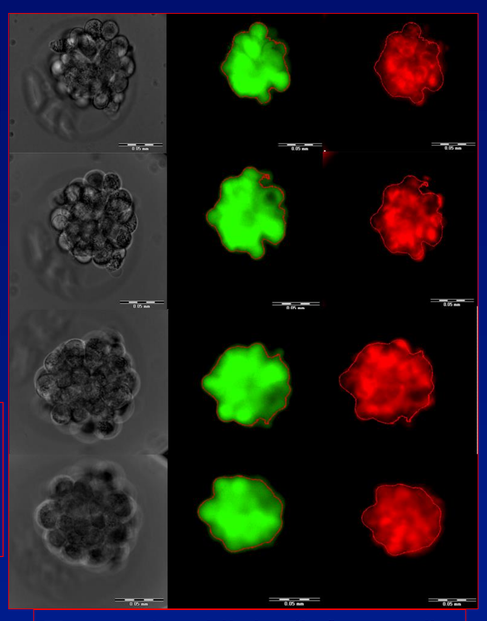




SEM micrograph of UV adhesive microchambers embossed on a glass surface. Each microchamber is designed to accommodate a single spheroid. Scale bar: left -100 µm; right – 500 µm.



MCF7 spheroids were grown in the microchambers of the Petri dish device for 72h (objective ×10)

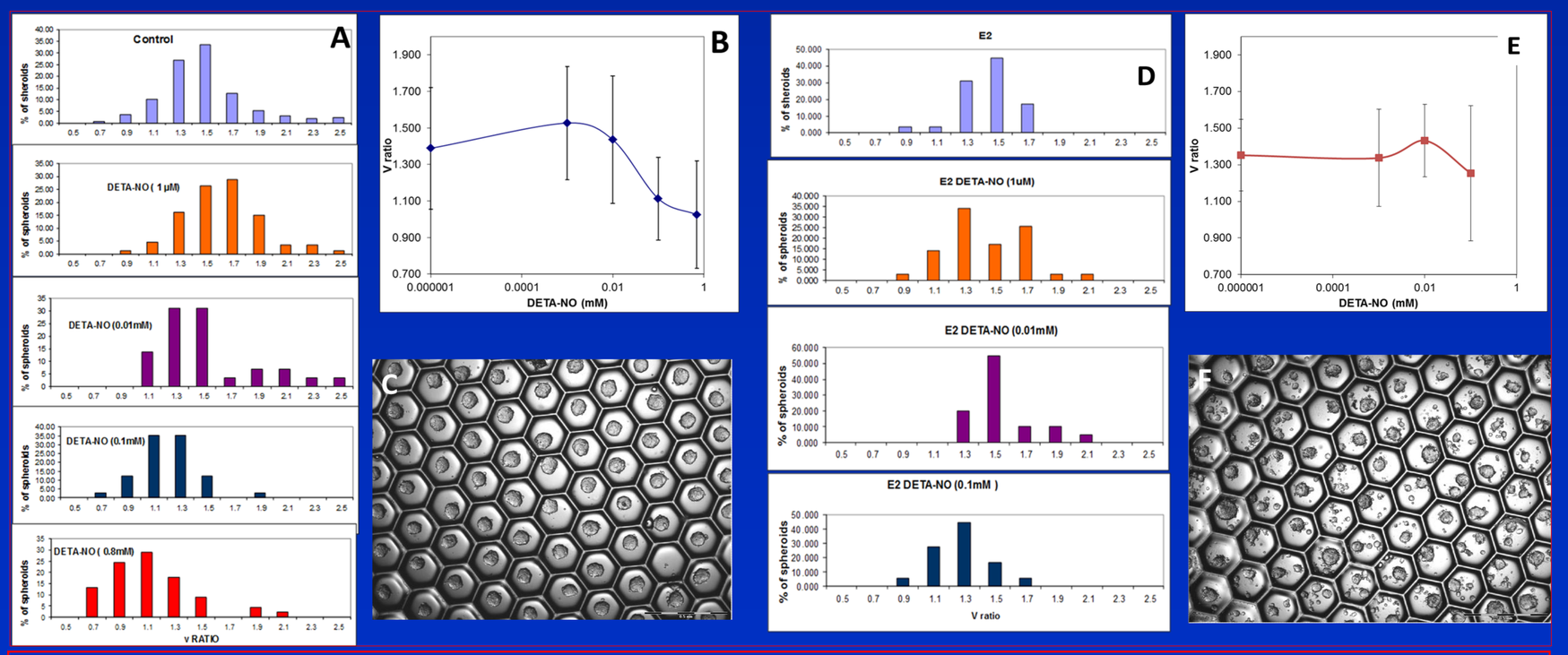


Transmitted light (left panel) and fluorescent z-stack images from the same spheroid stained by DAF2 (green) and TMRM (red). The fluorescence signal was detected across the whole spheroid volume, demonstrating the feasibility of using fluorescence based assays

Results

After cell loading the spheroid assembly from the individual cells was monitored. The location of each spheroid is preserved in the same MC throughout its growth, NO-donor treatment and imaging.

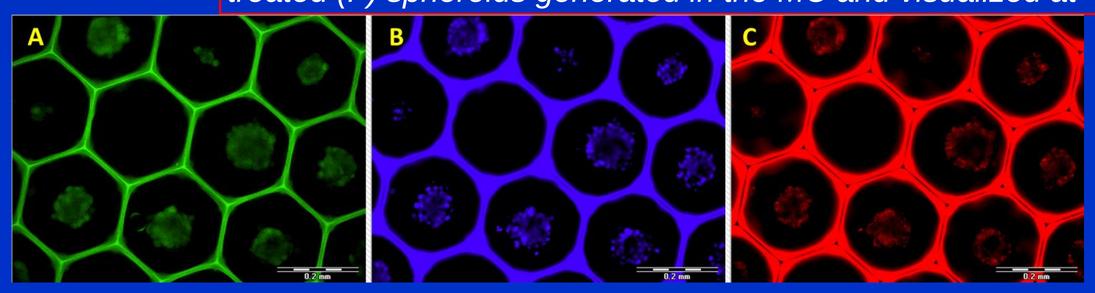
Kinetic probe free image analysis of spheroid formation and growth showed a significant difference in formation rate and morphology of the spheroids cultured in the presence of estrogen (E2) as compared to its absence. Moreover, different NO concentrations showed biphasic effect. The ratio between individual spheroid volume after and before treatment (the growth rate) was found to be major parameter which reflects the spheroid biophysical change.



Growth ratio distribution (A,D) and dose response curve (B,E) of control (A,B) and E2 treated (D,E) breast cancer spheroids exposed to different NO-donor concentrations. Transmitted light images of MCF7 untreated (C) and E2 treated (F) spheroids generated in the MC and visualized at ×4 objective.

High NO concentration (0.1 µM) inhibits growth as compared to the control spheroids (P<0.001), while low exogenous NO level (1 nM) causes enhanced growth (P=0.001). No influence of NO on spheroid growth ratio was observed when E2 was present in the culture media.

Fluorescence microscopy of apoptotic, mitochondria membrane potential, and NO markers showed that high NO concentration promotes apoptosis of the E2 deprived spheroids.



Triple fluorescence staining of MCF7 spheroids: A- DAf2 indicates endogenous NO level, B- shows clear Hoechst staining of the cells' nuclei and C- mitochondrial membrane potential evaluated by TMRM.

Conclusions

We have developed a MCs device suitable for both probe free and fluorescent microscopy study of cancer spheroids formation, culturing and treatment. The ability to measure the growth rate of the individual spheroid eliminates the noise Caused by the heterogeneity of the spheroid population. MC methodology could help to understand the mechanisms of NO action on breast cancer and to design novel therapeutic approaches.

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