A polymer microstructure array for the formation, culturing, and high-throughput drug screening of breast cancer spheroids

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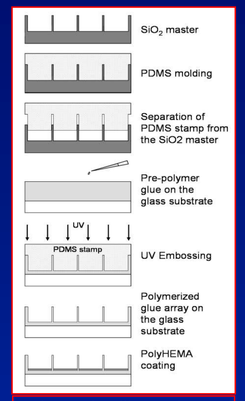
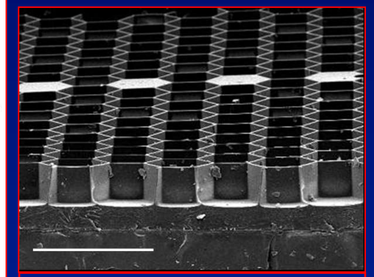
Abstract

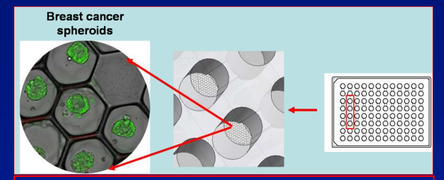
Multicellular spheroid models have been recognized as superior to monolayer cell cultures in antitumor drug screening, but their commercial adaptation in the pharmaceutical industry has been delayed, primarily due to technological limitations. The current study presents a new spheroid culture platform that addresses these technical restrictions. The new culturing device is based on a multiwell plate equipped with a glass bottom patterned with an array of UV adhesive microchambers. Each microchamber is designed to accommodate a single spheroid. The system facilitates the simultaneous creation and culturing of a large number of spheroids, as well as screening their response to antitumor drugs. The volume of the spheroids is easily controlled by seeding density. The location of each spheroid is preserved in the same microchamber throughout its growth, treatment with soluble agents, and imaging. The growth ratio parameter, a nonintrusive size analysis of the same spheroid the before and after exposure to drugs, was found to be a sensitive indicator for the reaction of MCF7 breast cancer spheroids to cytotoxic drugs. This feature helps reveal heterogeneity within the spheroid population during the formation process and their drug response, and provides an opportunity to detect specific, highly active or drug-resistant spheroid subgroups. The advantages of this spheroid-based system make it an efficient drug screening tool that may be valuable to related fields of research and clinical applications.



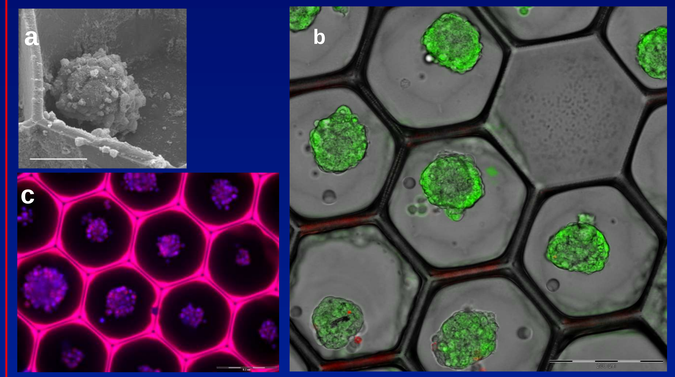
Schematic illustration of the sequence of the microstructure array fabrication processes.



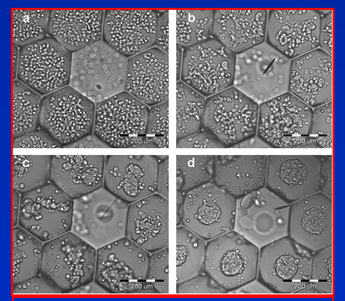
SEM micrograph of the microstructure array embossed on a glass surface. Note the dense honeycomb structure, the sharp edges between the microchambers and the filled microchambers which form built-in points of origin on the array . Scale bar: 500 µm.



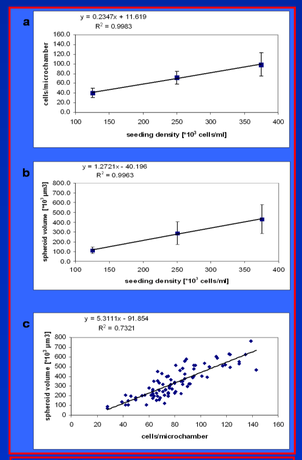
Schematic design of a multi-well plate equipped with a glass bottom patterned with microstructure array. Magnification of a representative macro-well shown at the left panel.



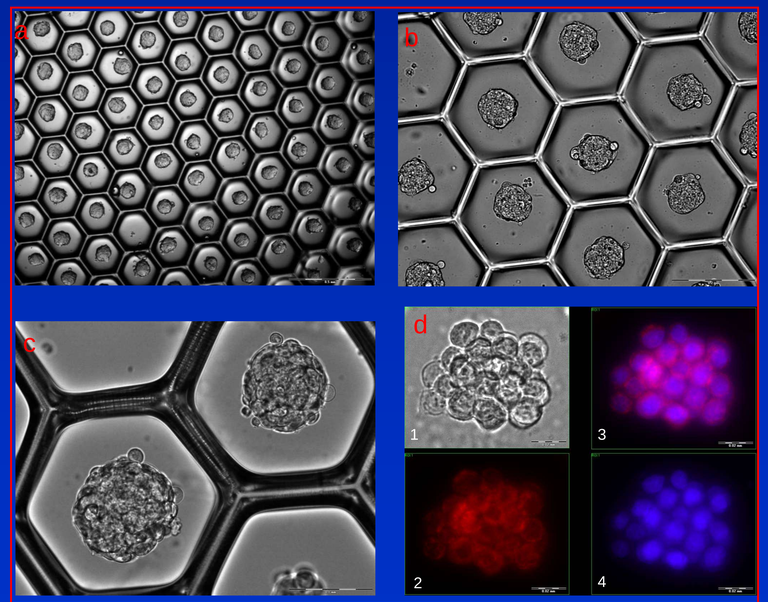
(a) SEM micrograph of one spheroid in the microchamber. (b) Structured illumination images of live-dead staining of spheroids after 72 hours, overlapped with transmission images. Green staining (FDA) indicates live cells while red (PI) indicates dead cells. Note that dead cells are rarely seen. (c) Fluorescence image of TMRM and Hoechst 33342 stained spheroids in the microchamber array.



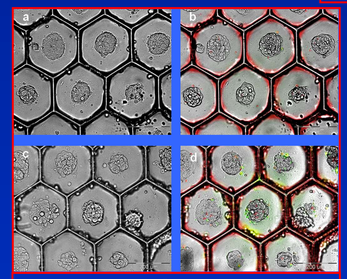
Kinetics of the assembly process of the individual cells on the microstructure. (a) Initial distribution immediately after seeding. (b) 6 hours after seeding. (c) 18 hours after seeding (note that by this time most cells in each microchamber are already arranged in one amorphous cluster. (d) Mature spheroids, 48 hours after seeding.



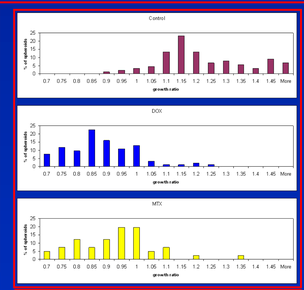
(a) Effects of the seeding density on the number of cells in each microchamber. (b) Effects of the seeding density on the volume of the spheroid 48 hours after seeding. (c) Relationship between the number of cells in each microchamber and the volume of the spheroid formed 48 hours after seeding. Note the variability between the microchambers.



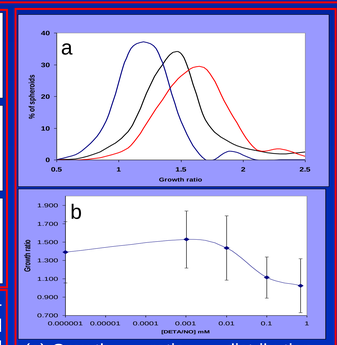
Transmitted light images of breast cancer spheroids generated within the microchambers and visualized at x4 (a) x10 (b) x20 (c). Tumor spheroid after in-situ disaggregation and cell staining. (d) Transmitted light (1) and fluorescence images (2-4) of the same multi cellular spheroid created and stained within mirochamber. Multi color staining for mitochondrial membrane potential with TMRM (2) and nucleus staining with Hoechst (4). An overlapping image of the TMRM and Hoechst fluorescence (3) Bar represents 50 µM.



Assessment of apoptosis level and cell death in untreated control. (a,b) and MTX treated spheroids (c,d). MCS were generated within the micro-chambers and imaged before (a,c-) and after 24 h of drug treatment (b,d - overlapping images of fluorescence and transmission images). Green staining (AnnexinV -FITC) indicates apoptotic cells while red (PI) indicates dead cells. Cells in late apoptosis show yellow fluorescence (green and red).



Growth ratio distribution for DOX- and MTX-treated spheroids and for the untreated control spheroids. Note the similar but down shifted distribution of the treated spheroids compared to that of the untreated spheroids. Variation between those distributions may serve as a tool for identifying drug resistance and sensitive sub-groups.



(a).Growth ratio distribution histograms of breast cancer spheroids not treated (black line) and after treatment with low (1 µM, red line) and high (1mM, blue line) of NO donor for 24 h. (b) Dose response curve of growth ratio values following treatment of MCF7 spheroids with NO donor.

Conclusions

We have developed a multiwell platform 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 various drug screening and functional tests. The system is easily manufactured, optically and biologically inert, and readily duplicable, bridging the gap between pharmaceutical industry demands and technological availability.

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