Superhydrophobic patterned chips as platforms for high-content drug screening in 3D tissues produced in vitro

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Statement of Purpose: High-throughput studies in biotechnology field have been carried out mainly in 2D models, although low clinical and biological relevance is associated with 2D testing. In native tissues cells lie in a 3D environment organized in the self-secreted extracellular matrix (ECM). In such milieu, cells interact in a totally natural manner, without the interaction of foreign materials. In order to improve the relevance of the findings achieved in these areas of study, there is a demand for tests using organotypic models that can be created in vitro in the form of cell spheroids. In such micro-masses cells formed from a cell suspension the attachment of cells to any surface is not promoted. Cells tend to attach between themselves and form an organized mass. Some types of spheroids are grown in order to mimic tumor models, containing a necrotic core. The hanging drop method is one effective method to produce spheroids: the cells are pulled to the concave bottom of a hanging droplet by gravity effect, and tend to start a natural organization. We propose the use of superhydrophobic surfaces patterned with wettable regions as platforms for the affordable and scale-up production of cell spheroids by the hanging drop method. Moreover, we used the platform - whose wettable regions are transparent and whose drop has its surface totally exposed to the external media allowing its facilitated manipulation – as high-throughput screening platform for drug testing and on-chip cell response analysis by microscopy.

Methods: PVC stickers (Oracal, USA) were glued into polystyrene surfaces in the form of an array of 1x1 mm^2 squares. Polystyrene was treated by a phase-inversion method, as described elsewhere (1). The stickers were then removed from the surface, and the protected regions remained wettable and transparent. A fibroblast (L929) and an osteoblast-like cell line (SaOs-2) were used for drug screening studies. Volumes of cell suspensions were dispensed in each wettable spot of the chip. We then turned the superhydrophobic surfaces 180º. We monitored the formation of the spheroids for 24 h by transmitted light microscopy. We then added solutions of doxorubicin (Dox) to each spot in distinct concentrations. Cell viability was determined by image analysis of confocal microscopy stacks.

Results: By using superhydrophobic chips with wettable spots as platforms for high-throughput drug screening in 3D structures, we produced spherical microtissues (Figure 1) of two cell types with high reproducibility. The presence of a transparent window in the hanging drop chip allowed observing the formation of the spheroids by transmitted light microscopy, avoiding any staining step or manipulation of the chip. Moreover, the superhydrophobicity of the surrounding areas of the wettable spot where the cell suspension was dropped conferred resistance to the chip, as we could tilt, rotate and perform media exchange easily, without any damage of the whole structure. By adding distinct amounts of Dox to the spots we observed that, as expected, the tumor-derived cell line (SaOs-2) presented a concentration-dependent and high sensitivity to the drug, while the fibroblast-derived line (L929) showed more resistance to Dox. In order to see the effect of the drug in L929 viability, Dox was concentrated in the order of 10^6x.

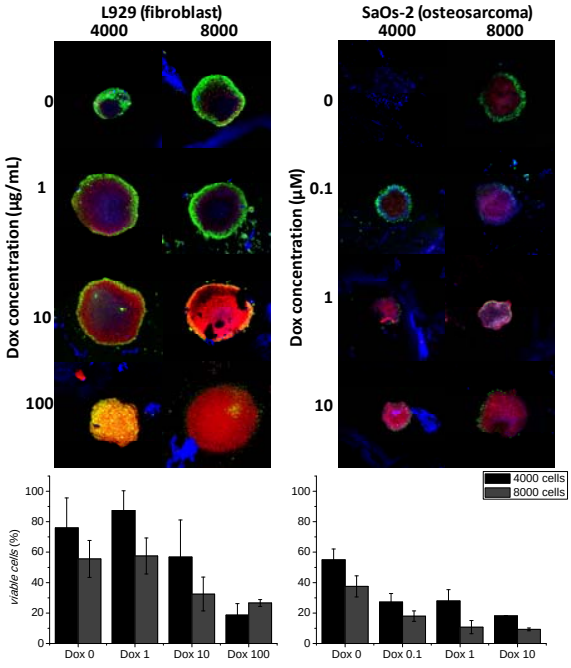

Figure 1. Confocal microscopy images of the spheroids with live/dead (Calcein/propidium iodide) and cell nuclei (DAPI) staining and respective viable cells quantification.

Conclusions: In order to establish the proof-of-concept of using superhydrophobic chips as improved and robust platforms for the hanging-drop methodology for spheroids production and drug screening, we used SaOs-2 and L929 cell lines. We tested the effect of a cytostatic drug used in clinical practice, also dispensed in a combinatorial logic in each spot of the chip. By on-chip microscopy analysis we proved the suitability of such platforms for drug screening using tumor-like models.

References: (1) Oliveira NM *et al*. Appl Phys Express. 2010;3:8.

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