MORPHOLOGICAL CHARACTERIZATION OF TUMOR SPHEROIDS BY MICROSCOPY AND IMAGE ANALYSIS TECHNIQUES

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Tumor spheroids are a widely studied *in vitro* model of solid tumors. Thanks to their 3D structure, spheroids can simulate tumor conditions *in vivo* with higher accuracy as compared to 2D cultures, in particular concerning cell morphology and organization, cell heterogeneity, growth and proliferation, cell-cell and cell-matrix interactions, and presence of biochemical concentration gradients. The presence of these gradients causes the formation of three different zones along the spheroid radius: a necrotic core, a quiescent zone and a proliferation zone [1, 2].

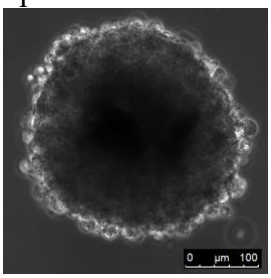

Figure 1: NIH-3T3 spheroid

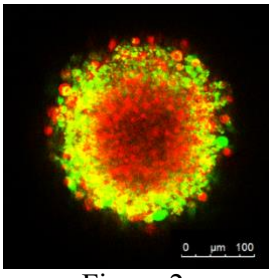

Figure 2: fluorescently labelled spheroid

In this work, the morphology of NIH-3T3 murine cells spheroids were analyzed using microscopy and image analysis techniques. Spheroid formation, starting from cells suspended at different concentrations, was followed by time-lapse microscopy with an inverted optical microscopy workstation. The spheroids were grown in 48-well multiwell plates, which were placed in a microscope stage incubator to control environmental conditions. A typical bright field image is shown in Figure 1.

The morphology of the spheroids was characterized by image analysis techniques based on the measurement of radial light intensity profiles over the spheroid. The method allows to identify the presence of the above mentioned three zones by exploiting changes in local scattering and absorbance due to the different biological structures corresponding to proliferating, quiescent and necrotic cells.

Control test were run by staining cells to distinguish the living cells from the dead ones. *Calcein AM* (green) was used to label living cells, while *Ethidium homodimer-1 (EthD-1)* was used to label dead cells (red) [3]. A confocal image of a fluorescently labelled spheroid is shown in Figure 2. The 3D reconstruction of an image z-stack was in agreement with the results obtained by using the intensity profiles in bright field imaging.

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