# 3D Cultivation of Cancer Cells in vitro

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The average cost to develop a new anticancer drug ranges around 800 million USD, which consequently increases the price of healthcare for patients. A significant part of these expenses could be suppressed by choosing a more suitable cancer model in preclinical trials. The use of 2D cultures lacking real complexity often leads to results irreproducible in vivo. Hence, our project focuses on development of a better model of cancer environment using agarose based hydrogels. After adjusting several different factors, we have found a suitable model consisting of two layers of gel: high concentration melted agar powder and mixture of agarose and medium (soft agar). This 3D layered model provides the cells an essential environment/scaffold, nutrient, and prevents cells to descend to the bottom. Cancer cells were cultivated collaterally under normoxic and hypoxic conditions and in different concentrations of soft agar (0.2-0.8 %). The attained results were evaluated after 14 days by confocal microscopy, which showed three dimensional spherical colonies. Their shape was closely related to the concentration of agarose and oxygen in the environment. Isolated proteins also showed signs of hypoxia induced by epithelial to mesenchymal transition. Our work has proven agarose-based 3D cultures to be a complex model of tumor environment, comparable to those using other commercial hydrogels but being about 30 times cheaper and directly available. Hence, the use of agarose-based scaffolding would significantly reduce the costs spends in biological labs and pharmaceutical companies worldwide.