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Optimization of *in vitro* conditions for 3D culture of rat glioma cells

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Cancer is the second leading cause of death globally, making the search for its cure one of the most important challenges of the 21st century. With ethical questions regarding animal testing and inconsistency of results of cancer drug testing in standard two-dimensional (2D) monolayer cell cultures with the results *in vivo*, there is a pressing need for better *in vitro* models of human cancers that will provide more relevant systems for cancer drug screening. Three-dimensional (3D) *in vitro* systems based on natural polymers with immobilized cancer cells that mimic cancerous tissue and bioreactors that provide relevant chemical and physical signals could close the gap between 2D *in vitro* and *in vivo* cancer models. The aim of this study was to optimize culture conditions for the rat glioma cell line C6 immobilized in alginate microfibers in perfusion bioreactors in terms of cell density and perfusion rate. In this study we investigated following sets of parameters: perfusion rate of 0.12, 0.25 and 0.30 ml min^{-1} coupled with the cell density of $4 \cdot 10^6$ cells ml^{-1}, and perfusion rate of 0.30 ml min^{-1} coupled with the cell density of $8 \cdot 10^6$ cells ml^{-1}. Microfiber cultures under static conditions in Petri dishes served as controls. The results have shown that the perfusion rate of 0.30 ml min^{-1} in combination with the cell density of $8 \cdot 10^6$ cells ml^{-1} yields higher cell viability and proliferation compared to the control static culture. These results indicate the importance of culture medium perfusion in the bioreactor for improved mass transfer of nutrients and oxygen to alginate microfibers so that the investigated system shows potentials for use as a model system in cancer research.